Effects of estradiol on cortisol response, working memory, and emotional memory during stress in young and post-menopausal women

By

Alexandra E. Ycaza

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Dedication

This dissertation is dedicated to my parents, Maria and Tim Noone, for teaching me that anything worth doing requires hard work and dedication. Thank you for your unwavering support in all I do. Also to my fiancé, Alex Herrera. Your work ethic and dedication inspire me every day, and remind me that everything I strive for is within my reach.

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Abstract

Estradiol and the class of stress hormones called glucocorticoids exert contrasting effects on various systems throughout the body, including neural tissue and cognition. Evidence also exists showing that estradiol can mitigate the damaging effects of excessive glucocorticoid levels on neural tissue. Given the sharp decline in estradiol levels that characterize the menopausal transition in human females, it is important to understand if the loss of estradiol leaves post-menopausal women at a higher risk of the negative effects of stress on neural tissue, and by extension the negative effects of stress on cognition.

Studies do show that estradiol treatment after menopause can dampen the physiological stress response to a stressful event; however, it is less clear whether estradiol can dampen the effects of stress on cognition. The general aim of this dissertation was to examine whether, and to what extent, estradiol could blunt the stress response and the effects of stress on working memory and emotional memory in women. This was examined in a population of post-menopausal women taking estradiol or placebo through the ELITE trial (Study 1) and in a population of young, spontaneously cycling, women during the low-estradiol and high-estradiol phases of the menstrual cycle (Study 2).

Study 1 investigated the effects of estradiol treatment after menopause on the cortisol response to the cold pressor task and the effects of stress on working memory and emotional memory. It was revealed that higher estradiol levels, as a result of estradiol treatment after menopause, were associated with a blunted cortisol response to ice water exposure and protection against stress-induced impairment of working memory. Although no effects of stress, estradiol, or interactions were found for emotional memory.

Study 2 investigated the effects of estradiol fluctuations during the menstrual cycle in young, spontaneously cycling women on the cortisol response to the cold pressor task and the effects of stress on working memory and emotional memory. It was revealed that the late follicular, higher estradiol, phase of the menstrual cycle was associated with a larger cortisol response to ice water exposure, but still provided protection against stress-induced impairment of working memory. Although no effects of stress, estradiol, or interactions were found for emotional memory. The potential mechanisms involved leading to the different patterns of results are discussed.

Overall, this dissertation provides evidence that estradiol treatment after menopause can mitigate the effects of stress on cortisol release and working memory, and that the pattern of estradiol effects on stress may differ in young premenopausal women. The mechanisms that contribute to the differences may be related to the different physiological relationships between the stress response system and the estradiol system across the two age groups, and how this may influence the cortisol response and how estradiol and cortisol interact. These results can further inform the medical field on the effects of estradiol treatment after menopause, as well as help women understand their vulnerabilities to stress depending on their internal hormone milieu.

CHAPTER 1

Estrogen and Stress. Interactions and implications for postmenopausal women.

1.1 Introduction

Estrogen and stress, particularly the class of stress hormones known as glucocorticoids (e.g. corticosterone in rodents and cortisol in humans), are two hormone systems that may not be considered to influence one another. However, cross-communication between these two systems is well documented. Yet, despite this documentation, little work examines the vastly contrasting effects the two systems individually exert on the body, brain, and cognition. First, stress hormones are associated with numerous maladaptive effects. For example, long-term stress hormone exposure is linked to development of the metabolic syndrome (Pasquali, Vicennati, Cacciari, & Pagotto, 2006; Rosmond, 2005), unhealthy alterations in fat distribution (Rebuffe-Scrive, Walsh, McEwen, & Rodin, 1992), promotion of hyperglycemia and hyperinsulinemia (McGuinness et al., 1993; Rebuffe-Scrive et al., 1992), promotion of bone resorption (O'Brien et al., 2004), and maintenance of bone degrading osteoclasts (Jia, O'Brien, Stewart, Manolagas, & Weinstein, 2006). In contrast, the primary estrogen in women, estradiol, is linked to less unhealthy fat distribution (Green et al., 2004; Musatov et al., 2007), lesser occurrence of hyperglycemia and hyperinsulinemia (Krotkiewski, Bjorntorp, Sjostrom, & Smith, 1983; Musatov et al., 2007), and promotion and maintenance of bone mineral density (Delmas et al., 1997; Felson et al., 1993; Sowers et al., 1998). Further, chronic or extreme stress results in cell damage or death and impairments in cognitive performance, while estradiol promotes neural growth and protection, and improvement of cognitive function.

Because stress and estradiol are present in the everyday lives of women, it is important to consider the dramatically different effects they have on neural tissue and cognition. Stress, or any

experience that is emotionally or physiologically challenging (McEwen, 2007), is present throughout the lifespan, while estradiol presence changes across the lifespan. Women experience monthly fluctuations of estradiol during their reproductive years, until they reach menopause, at which time the ovaries stop producing estradiol. Given that the stress and estradiol hormone systems interact, the dramatic decrease in estradiol levels during menopause may alter functioning of the stress system. This alteration may leave post-menopause women more vulnerable to the above-described negative effects of stress exposure.

In the following sections, I will briefly review the stress and estradiol hormone systems, then discuss the effects of glucocorticoids, stress, and estrogen on neural tissue and cognition, and how these two hormone systems interact. I will show that these factors exert dramatically different effects in both the brain and cognition, and the interaction of the two hormone systems may have important day-to-day implications for both pre- and post-menopausal women.

1.2 Overviews of the Hypothalamic-Pituitary-Adrenal and Hypothalamic-Pituitary-Gonadal Axes and their Interactions

The hormone systems governing the stress response and reproductive function are complicated and include many different components. The following sections will provide brief overviews of the major components of each system. These brief overviews will provide the basic understanding of the systems needed to review this work, but are by no means comprehensive discussions of the many intricacies of these two systems or their interactions.

1.2.1 The Hypothalamic-Pituitary-Adrenal Axis: The Stress System (Figure 1.1)

The hypothalamic-pituitary-adrenal (HPA) axis is the primary response system to a stressor. When an emotional or physiological challenge is detected, the paraventricular nucleus of the hypothalamus releases corticotropin-releasing hormone into the portal system (CRH; also

known as corticotropin-releasing factor) where the neuropeptide is carried to, and acts on, the anterior pituitary. Delivery of CRH to the anterior pituitary causes release of andrenocorticotropic hormone (ACTH). ACTH then enters the bloodstream and travels to the adrenal glands, which sit atop the kidneys. ACTH causes the adrenal cortex (comprised of the outer layers of the adrenal gland) to release glucocorticoids and the adrenal medulla (the most medial layer of the adrenal gland) to the release the catecholamines epinephrine and norepinephrine (Lupien, McEwen, Gunnar, & Heim, 2009).

This work is interested in the effects of glucocorticoids on the system. In humans, the primary glucocorticoid released during the stress response is cortisol. Cortisol provides access to immediate energy by tapping into energy stores, which prepares the individual for a flight-or-flight response. For instance, cortisol helps break down protein for conversion to glucose, assists in converting fat to usable energy, increases blood to skeletal muscles for energy, and decreases blood flow to immediately nonessential systems, like the gut (Carlson, 2010).

Importantly, cortisol also is responsible for terminating the HPA-axis-initiated stress response via negative feedback. In addition to exerting inhibitory action on the hypothalamus and pituitary gland, cortisol also acts on brain regions outside of the HPA axis to shut down the stress response. Of the regions containing glucocorticoid receptors capable of initiating inhibition, the hippocampus contains the highest concentrations and is considered to be the major source of HPA axis inhibition.

1.2.2 The Hypothalamic-Pituitary-Gonadal Axis: The Estradiol System (Figure 1.2)

Unlike the HPA axis, which is anatomically and mechanistically the same regardless of sex, the hypothalamic-pituitary-gonadal (HPG) axis differs for males and females, with the final

target of the axis being the testes or ovaries, respectively. Because this work is concerned with females, only the female HPG axis will be discussed.

The HPA and HPG axes follow the same general pathway before reaching their terminal tissues (i.e., the adrenal gland or gonads, respectively). The HPG axis also begins in the hypothalamus, where neurons that produce and release gonadotropin-releasing hormone (GnRH) reside. However, these neurons are located in multiple subnuclei, including the arcuate nucleus and the sexually dimorphic preoptic area, rather than just the paraventricular nucleus. GnRH-producing neurons project to the median eminence where GnRH is released into the portal system and carried to the anterior pituitary. Delivery of GnRH to the anterior pituitary causes release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which travel through the bloodstream and act on the ovaries to cause release of estrogens and progestins. There are three major forms of estrogens: estrone, estradiol, and estriol. Estradiol (E2) is the primary estrogen and is predominantly responsible for the estrogenic effects observed in the brain (Purves et al., 2004).

Estrogens, particularly E2, exert both stimulating and inhibiting effects on the HPG axis (Herbison, 1998; Jones, 2009). Estradiol levels maintained during most of the menstrual cycle exert a constant inhibitory effect on the hypothalamus, limiting elevations in LH and FSH. However, large increases in estradiol lasting for 2 or more days, such as the pre-ovulatory estradiol increase observed during the menstrual cycle, increases the sensitivity of LH and FSH neurons to GnRH leading to LH and FSH release. Reductions in E2 after this surge return GnRH, LH, and FSH to more common moderate-to-low levels (Jones, 2009).

1.2.3 HPA and HPG Cross-talk: Basis for Stress and Estradiol Interactions

Cross-talk between the HPA and HPG axes is well documented. The most commonly discussed functional cross-communication between the two systems is the ability of stress to interfere with normal reproductive function (Selye, 1939). Particularly, glucocorticoids appear to be necessary for HPG interference (Breen & Karsch, 2006), to reduce sensitivity of the ovaries to LH, suppress estradiol release by the ovaries (Carlson, 2010), and block ovulation (Rivier & Rivest, 1991).

Activation of the HPA axis is capable of interfering with the HPG axis at every level, particularly at the level of the hypothalamus and pituitary (Tilbrook, Turner, & Clarke, 2000), but also the ovaries (Tetsuka, 2007) where glucocorticoid exposure can stop follicle maturation. Further, HPA interference differs depending on whether stress exposure is acute or chronic. In rodents, acute exposure to a synthetic glucocorticoid decreased FSH levels, but not GnRH mRNA, suggesting acute stress hormone exposure reduces sensitivity of the pituitary to GnRH rather than suppress GnRH secretion (Gore, Attardi, & DeFranco, 2006). However, multiple days of glucocorticoid exposure resulted in decreased GnRH mRNA, suggesting longer exposure to stress hormones may act on the hypothalamus to suppress GnRH secretion thereby dampening overall HPG axis activity (Gore et al., 2006).

Cross-talk between the axes is not unidirectional, however. Estradiol can indirectly limit glucocorticoid availability (Brien, 1981). A large percentage of circulating glucocorticoid is often bound to a binding globulin, making only 5-10% of the hormone available to enter and act on cells (Burke & Roulet, 1970). Estradiol can upregulate corticosteroid binding globulin, thereby decreasing levels of biologically available glucocorticoids (Brien, 1981). That HPA-HPG interactions are bidirectional, it is important to understand whether, and how, these

interactions may change when one system is dramatically altered, such as when women go though the menopause transition. The decrease in estradiol levels accompanying menopause may alter how HPG activation affects HPA function. For instance, the decreased estradiol levels may lead to increased stress responses as a result of no longer limiting the level of unbound, biologically active cortisol.

This work is focused on investigating how changes in estradiol levels may increase HPA activity in response to stress and how such an increase may facilitate or attenuate effects of stress on different memory processes. In the following sections I will review the effects of estradiol, stress, and stress hormones on neural morphology, function, and cognition.

1.3 The Competing Effects of Stress and Estrogens on Neuronal Morphology and Function

1.3.1 Glucocorticoids Are Necessary For Normal Neuronal Function

Exposure to stress and glucocorticoids (GC) are documented to negatively impact the brain, however, GCs also are necessary for normal brain functioning (Doi, Miyahara, & Hori, 1991; Nadeau & Rivest, 2003; Sapolsky, Stein-Behrens, & Armanini, 1991). In rodents, loss of glucocorticoids by adrenalectomy (removal of the adrenal gland) decreases hippocampal CA1 excitability relative to CA1 excitability from adrenal-intact control animals; while corticosterone treatment (the primary rodent GC) returned reactivity to the normal amplitude (Doi et al., 1991). Further, rats receiving a GC antagonist (i.e. blocking the effects of GC) experienced potentiated inflammatory responses following lipopolysaccharide infusion resulting in more prominent neural damage compared with control animals (Nadeau & Rivest, 2003). Results such as these suggest GC is necessary for proper stimulation of neurons in the hippocampus, and likely elsewhere in the brain. Necessity of GC for proper neuronal stimulation is likely part and parcel to the necessity of GC for long-term potentiation (Pavlides, Watanabe, Magariños, & McEwen,

1995). Additionally, GC also is necessary for shutting down the innate inflammatory response that follows insults to tissue, and protecting the central nervous system from increased damage as a result of prolonged inflammation. Yet, despite GC necessity for normal function, the hormone can impede the system when simultaneously present with other insults (e.g. beta-amyloid), or when present in extreme levels or for prolonged periods (i.e. extreme or chronic stress).

1.3.2 Exposure to Glucocorticoids Exacerbates Damage by Other Insults

The most deleterious effects of GC exposure occur in the hippocampal formation, a region containing a high concentration of glucocorticoid receptors (Gerlach & McEwen, 1972; McEwen, Weiss, & Schwartz, 1968). One such effect is the ability of GC to exacerbate damage induced by other neuronal insults (Behl, 1998; Sapolsky, 1990, 1999, 2000). For instance, neuron cultures pretreated with dexamethasone (a synthetic GC) or corticosterone for 24 hours experienced greater rates of cell death from either beta-amyloid (Aβ) or glutamate exposure than cultures not pretreated with GC (Behl et al., 1997). Corticosterone preexposure has the same effects in cultures exposed to hypoxic or hypoglycemic environments (Tombaugh, Yang, Swanson, & Sapolsky, 1992) and kainic acid (Stein-Behrens et al., 1992). The ability of GC to increase glutamate and aspartate levels (Stein-Behrens et al., 1992) and concomitantly inhibit excess glutamate uptake from the synaptic cleft by astrocytes (Virgin et al., 1991) may be one mechanism for the heightened damage induced by GC. Increased glutamate levels coupled with a decrease in glutamate clearance from the cleft would leave neurons susceptible to a level of excitotoxic cell death beyond what is observed when exposed to the other neuronal insults alone.

1.3.3 Excess Exposure to Glucocorticoids Alone is Dangerous to Neural Tissue

Chronic glucocorticoid exposure also damages hippocampal neurons in the absence of other toxic insults. Illustrating this are reports countering the anti-inflammatory effects

mentioned above (Nadeau & Rivest, 2003). First, cell cultures chronically exposed to GC showed *up*regulation of the immune factors interlukin-1-beta and tumor necrosis factor-alpha[1] suggesting chronic GC exposure is *pro*-inflammatory, not *anti*-inflammatory (MacPherson, Dinkel, & Sapolsky, 2005). Second, chronic restraint stress in rats increased the presence of microglia markers in areas involved in the immune response, such as the medial prefrontal cortex, anterior cingulate, nucleus accumbens, dorsal bed nucleus of stria terminalis, CA3 region of the hippocampus, and the lateral periaqueductal gray (Tynan et al., 2010). Increases in inflammatory and microglia markers are indicative of an increased immune response, the combination of which suggests chronic exposure to GC induces a neuroinflammatory response, rather than an anti-inflammatory response.

Chronic GC and stress exposure also affect neuronal morphology and function in the brain. Twenty-one days of corticosterone injections decreased dendrite length and branch points in the CA3 region of rats compared with injections of a control substance (Woolley, Gould, & McEwen, 1990), while dexamethasone administration induced apoptosis and cell damage in the dentate gyrus, CA1, and CA3 regions of the rat hippocampus and striatum (Haynes, Lendon, Barber, & Mitchell, 2003). Further, prolonged exposure or exposure to high levels of corticosterone or a GC agonist has the potential to interfere with processing and learning by decreasing long-term potentiation (Pavlides et al., 1995; Pavlides, Watanabe, & McEwen, 1993).

Induction of GC release by stress exposure results in a similar pattern of neuronal damage as administration of exogenous GC. Three weeks of daily restraint stress decreased apical dendrite length and branch points in CA3 pyramidal neurons of rats (McLaughlin et al., 2010; Watanabe, Gould, & McEwen, 1992), as well as in vervet monkeys exposed to social stress.

[1] Interlukin-1-beta and tumor necrosis factor-alpha are cytokines involved in the inflammatory response of the immune system. Both cytokines have involvement in apoptosis.

Chronic social stress in these primates was associated with a reduction of CA3 hippocampal pyramidal cells and atrophy of dendritic branches of the surviving cells (Uno, Tarara, Else, Suleman, & Sapolsky, 1989). The same effects have been observed in layers II and III of the prefrontal cortex, where repeated acute and chronic restraint stress decreased apical dendrite branch number and length in male rats (Brown, Henning, & Wellman, 2005; Cook & Wellman, 2004; Wellman, 2001). Thus, GC exposure damages prominent brain regions, whether studied *in vitro* or *in vivo* or as a result of physical, psychological, or social stress.

1.3.4 The Effects of Estrogens On Neurogenesis and Neural Maintenance

Analogous to experiments examining the effects of stress hormones on neuronal viability, many experiments have examined if, and to what extent, estrogen might affect neuronal integrity. In contrast to the damaging effects of GC and stress on neuronal integrity, estrogens, particularly estradiol, show neurogenic properties. First, fetal primary hippocampal neurons exposed to conjugated-equine estrogens (Brinton et al., 2000) or 17β-estradiol (Chen, Nilsen, & Brinton, 2006) showed increased neurite number and length, as well as increased secondary branching and branch length when compared with cultures free of any estrogenic compounds. The growth promoting effect of conjugated-equine estrogens (CEE) was most robust in hippocampal neurons, but also promoted neurite outgrowth in basal forebrain and cortical tissue (Brinton et al., 2000). Estradiol also protected oligodendrocytes, the glial cells responsible for myelinating axons within the central nervous system, from hyperoxic insults *in vitro*, indicating estradiol may promote proper myelination later in development (Gerstner et al., 2007).

Estradiol also promotes neurogenesis in mature animals. Treatment with E2 increased cell proliferation and decreased cell death in the dentate gyrus of middle-aged males (Saravia, Beauquis, Pietranera, & De Nicola, 2007), essentially slowing down the rate of age-related

declines in cell proliferation of this hippocampal region. In adult animals, mature granule cells appear to only respond to specific stimuli or stimuli ranges, while new granule cells have a lower threshold for response to a wider range of stimuli (Marin-Burgin, Mongiat, Pardi, & Schinder, 2012) possibly allowing them to form new connections as learning occurs. Thus, estrogenic protection of this process should aid maintenance of hippocampal-dependent learning and memory processes.

1.3.5 Estrogens as a Neuroprotectant

In addition to promoting neurogenesis and maintaining neural integrity, estradiol also acts as a neuroprotectant. In *in vitro* studies, E2 (Chen et al., 2006) or CEE (Brinton et al., 2000) pretreatment of hippocampal neurons blocked Aβ-induced neurodegeneration, decreased the degree of apoptosis, and attenuated the Aβ-induced decrease in ATP levels. Estradiol exerted a similar protective effect against hydrogen peroxide challenge and glutamate excitotoxicity (Brinton et al., 2000). These results extend the pattern of *in vitro* promotion of neural growth and development, to *in vitro* protection against deleterious agents.

In vivo experiments also report estrogenic neuroprotection. Ovariectomy (removal of the ovaries) followed by cyclic estradiol replacement increased dendrite spine density in layer III of dorsolateral prefrontal cortex in young-adult rhesus monkeys and restored density in aged monkeys, compared with rhesus monkeys not treated with estradiol (Hao et al., 2007). Estradiol also protects against the excitotoxicity typically accompanying severe seizure activity. Ovariectomized (OVX) female rats injected with kainic acid, an animal model for seizures, showed marked dose-dependent neuron loss in the entorhinal cortex and hippocampus; an effect blocked by E2 pretreatment (Hoffman, Moore, Fiksum, & Murphy, 2003). Estradiol pretreatment also protected male mice from the stereotypical motor deficits accompanying methylmercury

exposure and reduced the amount of lipid peroxidation in the cerebellum (Malagutti et al., 2009), suggested to be a result of E2 protection of the antioxidant glutathione (Malagutti et al., 2009). These *in vivo* studies suggest estrogenic protection is partially achieved by reducing excitatory ion influx and subsequent excitotoxicity, and by promoting other endogenous neuroprotective factors, such as antioxidant systems.

1.3.6 Estrogens and Neurodegenerative Diseases

Estrogens also protect against neurodegenerative diseases. In animal models, endogenous E2 levels were associated with delayed progression of neuropathology in a transgenic rat model of Huntington's disease (Bode et al., 2008), and E2 treatment prevented the progression of motor dysfunction in a rodent model of amyotrophic lateral sclerosis (Choi et al., 2008). In these cases, estradiol attenuated degeneration in the brain regions most targeted by these diseases. Estradiol also exerts protective effects over the brain regions most affected by the Aβ plaques characteristic of Alzheimer's disease (Brinton, 2001, 2008; Pike, Carroll, Rosario, & Barron, 2009). *In vitro,* pretreatment with CEE (Brinton et al., 2000) or E2 decreased cell death (Chen et al., 2006; Hosoda, Nakajima, & Honjo, 2001; Pike, 1999), attenuated the accompanying rise in intracellular calcium concentrations (Chen et al., 2006; Hosoda et al., 2001), and decreased presence of apoptotic markers in cells exposed to Aβ (Brinton et al., 2000; Hosoda et al., 2001; Sharma & Mehra, 2008). Estradiol pretreatment also significantly increased the level of Bcl-x_L and Bcl-2, anti-apoptotic proteins, in cultured fetal hippocampal neurons (Pike, 1999) and in OVX adult female rats (Sharma & Mehra, 2008), suggesting estradiol's neuroprotective effects also may include a combination of inhibiting proteins that promote apoptosis while simultaneously upregulating proteins that defend against apoptosis, potentially offering dual protection from the cell death typical of neurodegenerative diseases.

1.4 The Competing Effects of Stress and Estrogens on Cognition

1.4.1 Stress Can Benefit Cognitive Function

The pattern of glucocorticoid exposure on neural tissue extends to cognitive functioning, such that GC is required for optimal functioning, but is deleterious in excess levels or over prolonged exposure. In their classic 1908 work, Yerkes and Dodson reported that learning of a shock-motivated avoidance task followed an inverted-U shaped function, such that insufficient arousal (too weak a shock) led to low learning rates, as did excess arousal (too strong a shock). However, moderate stimulation resulted in the best learning rates (Yerkes & Dodson, 1908). Given that shock elicits a strong corticosterone response in rats (S. B. Friedman, Ader, Grota, & Larson, 1967), the results indicate that some amount of stress is required for peak performance to be attained, and deviations from this optimal level result in impaired learning and memory. More contemporary works suggest a similar inverted-U shaped pattern for stress-cognition interactions in different domains, including the radial arm water maze (Salehi, Cordero, & Sandi, 2010), spatial learning and associative learning (Mateo, 2008), and corticosterone-primed burst potentiation interactions (Diamond, Bennett, Fleshner, & Rose, 1992). Importantly, the inverted-U shaped function appears to vary by task difficulty, with moderate arousal beneficial for difficult tasks and stronger arousal beneficial for easier tasks (Dodson, 1915). In this work, Dodson (1915) showed kittens learned an avoidance task faster with moderate shock when visual discrimination of the shock and no-shock chambers was difficult, but that stronger shock was required to aid learning rates as visual discrimination between the chambers became easier. This suggests that as tasks become increasingly easy, more stress is required for more rapid learning. However, Dodson utilized the same “strong” shock for all three difficulties of discrimination, therefore, the inverted-U shape could still hold, but the “strong” shock has now become the

optimal “moderate” shock required to aid learning. In this case had the “strong” shock been made stronger on the easier versions of the task, the kittens’ learning may have been impaired, again showing an inverted-U function. Such a shift of the inverted U with task difficulty has been observed and supported more recently (Anderson, 1994; Salehi et al., 2010; for a brief discussion see Shors, 2004).

While stress may generally benefit learning, stress reactivity may actually be necessary for the type of avoidance learning discussed above, and others. In the conditioned taste aversion literature, learning is based on the introduction of a physical stressor (the illness agent), such that animals must be made ill in order to learn to avoid the recently consumed novel substance (Hintiryan, Foster, & Chambers, 2009; Miele, Rosellini, & Svare, 1988; Nachman & Ashe, 1973). Arguably, this would be the case for most types of avoidance learning. In these instances, activation of the stress response would signal to the animal that the stimulus most temporally and spatially contingent to stress onset should be avoided.

The ability of these stimuli to elicit physiological responses likely makes them easy to remember and contributes to the relative ease of avoidance learning. If the innate ability to elicit physiological responses contributes to learning and memory for these stimuli, then stress application prior to exposure of these negative stimuli should enhance encoding as a function of the stress response plus the unconditioned response being interpreted as a stronger unconditioned response to the stimulus. This seems to be the case with memory for emotional items. For instance, exposure to stress 30 minutes *before encoding* impaired recall of neutral words, but had no effect on the recall accuracy of emotional words, relative to controls (Smeets, Jelicic, & Merckelbach, 2006). Stress experience also can *enhance* memory for emotional items. Stress applied *before encoding* resulted in *better* memory for an emotional story and *worse* memory for

a neutral story after a one-week delay interval, compared with comparable memory for both stories in control subjects (Payne et al., 2007). Similar enhancement has been reported in pharmacological studies, where administration of 20mg hydrocortisone resulted in better cued recall of highly arousing emotional stimuli than cued recall after placebo administration (Buchanan and Lovallo, 2001). However, stress can impair recall of emotional and neutral words when the stressor is applied *after encoding* and before retrieval (Kuhlmann, Piel, & Wolf, 2005), versus only prior to encoding as in the aforementioned studies. Together, the literature on avoidance learning and emotional memory suggest stress benefits learning, and may be necessary for certain types of learning, just as GC is necessary for proper neural function. However, as reviewed in the section on glucocorticoid effects on neural tissue, although stress hormones are required and beneficial for some forms of learning and memory, excess or prolonged exposure negatively impacts many other cognitive domains.

1.4.2 Associations Between Stressful Life Events and Cognition in Humans

Experiencing highly stressful events or a greater number of life stressors is associated with cognitive impairment. Individuals residing within 5 miles of the Three Mile Island nuclear meltdown had higher levels of epinephrine and norepinephrine in their urine, more somatic complaints, higher anxiety and isolation, and worse performance on an embedded figure and proofreading task, 17 months after the nuclear meltdown, compared with residents near an undamaged nuclear plant, a coal-burning plant, or no plant (Baum, Gatchel, & Schaeffer, 1983). Likewise, individuals scoring higher on the Life Experiences Scale (e.g. more stressful events) displayed impaired word recall and greater intrusion of irrelevant thoughts during an operation span task assessing working memory (Klein & Boals, 2001). However, these non-experimental studies cannot conclude that the cognitive deficits are a result of the stressors measured. In the

case of the Baum et al. (1983) study, participants may have suffered minor radiation exposure contributing to the differences in physiology and cognition, and the impaired performance of college students reporting a higher number of life stressors could result from distraction due to these stressors rather than the effects of cortisol. However, experimental studies in animals and humans report similar relationships between stress and cognitive performance.

1.4.3 Negative Effects of Glucocorticoids on Cognition in Animals

The animal literature is rife with reports of stress-induced cognitive impairment. Discussion of these works, however, first requires a brief description of the tasks and apparatus commonly employed. One such apparatus is the radial arm maze. Radial arm mazes can have a variable number of arms radiating out from a central starting box. Some arms are baited with food, and the animal is trained to traverse each baited arm only once and never enter the never-baited arms. A similar procedure is used in the Y-maze and T-maze, except these mazes consist of one starting alley and two arms shaped like a "Y" or a "T", respectively. These tasks measure working memory in different ways. The radial arm maze assesses working memory because the animal must remember which arms contain food, which arms have already been entered, and which arms still need to be entered. The Y-maze and T-maze can measure short-term memory, long-term memory, and working memory, but specific means of doing so vary depending on the task employed within the maze.

Chronic GC administration impairs cognition in these animal models of working memory. Nine weeks of corticosterone administration increased the number of working memory errors in a radial arm maze (Arbel, Kadar, Silberman, & Levy, 1994), and eight weeks and three weeks of corticosterone injections resulted in increased working memory and reference memory errors in a Y-maze, respectively (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson,

2003). These pharmacological experiments extend the detrimental effects of GC exposure on neural tissue to detrimental effects in animal models of working memory.

1.4.4 Negative Effects of Stress Application on Cognition in Animals

Importantly, chronic stress results in the same cognitive deficits as chronic administration of exogenous GCs. Subordinate male tree shrews, whose stress hormone levels were elevated compared with dominant males, were unable to remember which holes contained and did not contain a food reward in a holeboard task, suggesting stress exposure leads to greater reference memory errors (Ohl & Fuchs, 1999). In another social stressor, rats chronically exposed to a cat showed impaired working memory on a radial arm water maze – a radial arm water maze requires animals to swim to a platform that allows them to rest rather than running to a food reward as seen in a standard radial arm maze (Park, Campbell, & Diamond, 2001). Similarly, repeated exposure to a novel environment or the Morris water maze resulted in more working memory errors in a radial arm maze compared with animals in the control condition (Diamond, Fleshner, Ingersoll, & Rose, 1996; Diamond & Rose, 1994), and rats exposed to chronic restraint stress showed worse retention for a familiar versus novel arm in a Y-maze (Kleen, Sitomer, Killeen, & Conrad, 2006). Thus, stress induces declines in cognitive function, whether the stress treatment involves exposure to exogenous stress hormones or to various physical or social stressors.

1.4.5 Negative Effects of Controlled Non-Laboratory Stressors on Cognition in Humans

Overall, the animal literature suggests that chronic glucocorticoid and stress exposure negatively affect cognitive processes, and working memory in particular. This pattern implies humans should show impaired cognitive function in the face of various stressors. In order to examine the effects of extreme stress on cognition, researchers made use of a unique situation:

Survival School. Survival School trains and prepares soldiers for becoming trapped behind enemy lines, pursued by enemy soldiers, and captured and interrogated as prisoners of war. Soldiers tested using the Rey Osterrieth Complex Figure drawing task showed atypical strategy for copying the figure and poor recall of figure details when tested immediately after an interrogation session, compared with soldiers tested prior to commencement of Survival School or after release from the mock prisoner of war camp (Morgan, Doran, Steffian, Hazlett, & Southwick, 2006). Furthermore, soldiers provided with sources of misinformation, such as being shown a photograph of another person during their interrogation, were more likely to falsely identify their interrogator than soldiers not provided with potential sources of misinformation (Morgan, Southwick, Steffian, Hazlett, & Loftus, 2013). These results suggest that even individuals trained to withstand stressful situations are susceptible to the negative effects of highly stressful experiences. These studies also provide a more plausible relationship between stress exposure in the world and declines in cognitive performance in humans than did Baum et al. (1983) and Klein and Boals (2001), although they still fail to provide measurement of the stress response and the relation between stress hormone levels and cognitive performance.

1.4.6 Negative Effects of Laboratory Stressors on Cognition in Humans

The discussion thus far provides a connection between *chronic* stress and cognitive interference; however, most laboratory research with humans focuses on the effects of *acute* stress on cognitive function. As shown by Yerkes and Dodson (1908) and Dodson (1915) such acute manipulations may lead to mixed results depending on how much arousal is induced by the stressor or how difficult the target task is relative to the amount of stress induced. One frequently used acute stressor is the Trier Social Stress Test (TSST). The TSST reliably elicits a stress response from participants by requiring them to give a speech and perform mental arithmetic in

front of an audience (Oei, Everaerd, Elzinga, van Well, & Bermond, 2006; Schoofs, Preuß, & Wolf, 2008; Wolf, Minnebusch, & Daum, 2009). Performance of this task prior to testing resulted in increased reaction times and working memory error rates in the Sternberg item recognition task (Oei et al., 2006), increased reaction times and decreased accuracy in the N-back task (Schoofs et al., 2008), and interference with acquisition of a classically conditioned eye-blink task (Wolf et al., 2009).

Another commonly employed acute laboratory stressor is the cold pressor task (CPT). The CPT reliably induces a stress response by requiring participants to hold one of their hands in ice water (Bullinger et al., 1984; Edelson & Robertson, 1986; Lighthall, Mather, & Gorlick, 2009; Lighthall et al., 2011; Mather, Lighthall, Nga, & Gorlick, 2010). Like the TSST, completion of the CPT can impair cognitive function. CPT exposure impaired performance on working memory tasks such as the digit span backward (Schoofs, Wolf, & Smeets, 2009) and the Sternberg item recognition task (Duncko, Johnson, Merikangas, & Grillon, 2009). Thus, although the effects of acute stress might lead to variable effects on cognition, these stressors must have been sufficient to induce interference in working memory. Based on the above-discussed works for Yerkes and Dodson, these stressors either induced a level of stress that surpassed the optimal level of arousal for optimal performance and/or the difficulty of working memory tasks makes this type of cognition relatively sensitive to the effects of stress. In either case, the literature shows that stress exerts a parallel pattern in neural tissue and cognition, namely, that overexposure of stress detrimentally affects both systems.

1.4.7 How Are Glucocorticoids and Stress Affecting Cognition?

Thus far the evidence demonstrates that exposure to stress or stress hormones can enhance memory for emotional items, but also negatively affects neuronal health and LTP, both

essential components in learning and memory. The different pattern of stress effects on emotional versus non-emotional memory is likely a result of different neural involvement – with the amygdala more involved in emotional memory and the prefrontal cortex and hippocampus more involved in non-emotional memory – and the ways stress effects these regions. In humans acute stress is associated with reduced activity in the dorsolateral prefrontal cortex (Cabeza, Dolcos, Graham, & Nyberg, 2002) during performance of a non-emotional working memory task (Qin, Hermans, van Marle, Luo, & Fernández, 2009). Furthermore, the most deleterious effects of glucocorticoids and stress on neural tissue are observed in the hippocampus and prefrontal cortex, regions involved in the cognitive domains impaired by stress and its hormones, like short-term memory (Alonso et al., 2002; Cabeza et al., 2002), long-term memory (Alonso et al., 2002), and working memory (Cabeza et al., 2002; Curtis & D'Esposito, 2003; Laroche, Davis, & Jay, 2000).

The neural underpinnings for learning and memory *aided* by stress application are different, and mainly rely on the amygdala. The amygdala is considered of utmost importance in recognition and processing of innately arousing stimuli (Zald, 2003) as well as in stimuli that were once neutral but have since obtained a negative association (Gallo, Roldan, & Bures, 1992; Lamprecht & Dudai, 1996; Navarro, Spray, Cubero, Thiele, & Bernstein, 2000; Roldan & Bures, 1994). Importantly, glucocorticoids can modulate amygdalar activation during memory formation of emotional items. Glucocorticoid application resulted in better retention of a one-trial avoidance learning task after a 48-hour delay when infused into the basolateral nucleus of the amygdala immediately after training (Roozendaal & McGaugh, 1997). Inactivation of GC resulted in the opposite effect, however; 24-hour retention of a contextual fear response was impaired when a glucocorticoid antagonist was infused into the basolateral amygdala 10 minutes

prior to training (Donley, Schulkin, & Rosen, 2005). That glucocorticoid or stress exposure doesn't interfere with amygdala-dependent learning could be a result of the structure's imperviousness to glucocorticoid damage (Morales-Medina, Sanchez, Flores, Dumont, & Quirion, 2009), while stress-induced enhancement of memory consolidation may be a result of GC ability to increase kinase activity related to learning (pErk1/2) when infused in the basolateral amygdala (Roozendaal et al., 2009).

1.4.8 Estrogen Can Impair Cognitive Function

Similar to the effects of glucocorticoids on cognition, estrogen is associated with both negative and positive effects on cognitive function. The variability is largely observed in studies of estrogen effects on post-menopausal women, with some reporting positive effects (Baker et al., 2012; Duff & Hampson, 2000; Maki et al., 2011; Maki, Zonderman, & Resnick, 2001; Miller, Conney, Rasgon, Fairbanks, & Small, 2002; Smith, Giordani, Lajiness-O'Neill, & Zubieta, 2001; Wolf & Kirschbaum, 2002; Wolf et al., 1999), no effect (Maki et al., 2001; Pefanco et al., 2007; Yaffe et al., 2006), and negative effects (Espeland et al., 2004; Mulnard et al., 2000; S. R. Rapp et al., 2003; Resnick et al., 2006; Shumaker et al., 2003).

The Women's Health Initiative (WHI) studies comprise the most widely reported negative findings. At the time of inception, the Women's Health Initiative Memory Study (WHIMS) and Women's Health Initiative Study of Cognitive Aging (WHISCA) were the largest randomized studies examining prospective observations that post-menopausal hormone treatment maintained cognitive function otherwise observed to decline after menopause. Rather than supporting these observations of estrogenic protection, the WHISCA and WHIMS studies found that CEEs negatively affected performance on various cognitive measures, such as the California Verbal Learning Test (Resnick et al., 2006) and the Modified Mini Mental State Exam (a

measure of global cognitive function) whether administered unopposed (Espeland et al., 2004), or opposed with medroxyprogesterone acetate (S. R. Rapp et al., 2003), as well as an increased risk of developing dementia (Shumaker et al., 2003) and a faster rate of Alzheimer's progression in older women with preexisting Alzheimer's disease (Mulnard et al., 2000).

The results were surprising because of the reports of estrogenic protection of cognitive function (Baker et al., 2012; Duff & Hampson, 2000; Maki et al., 2001; Miller et al., 2002; Smith et al., 2001; Wolf & Kirschbaum, 2002; Wolf et al., 1999) and the basic science reports of estrogenic fortification of the central nervous system (Brinton et al., 2000; Chen et al., 2006; Hosoda et al., 2001; Pike, 1999). To account for the disparity between basic science experiments and human studies, Brinton (2005) proposed a healthy cell bias of estrogen action, stating estrogen would be advantageous to a population of healthy cells, but would be detrimental to a population of declining or already injured cells. To test this hypothesis, Brinton and colleagues compared treatment and prevention models *in vitro*. In treatment models, estradiol is applied to neurons either at the time of, or after, application of the deleterious agent; this differs from the prevention model, which pretreats cells with estradiol *before* exposure to the agent. Consistent with the hypothesis, neurons simultaneously treated with estradiol and Aβ protein did not receive any estrogenic protection, nor did neurons exposed to the toxic protein for 1 to 2 days prior to estradiol exposure. However, introduction of estradiol to the cultures after 5 days of Aβ exposure resulted in greater cell death than the Aβ-alone cultures (Chen et al., 2006). Given that the women in the WHI studies were over 65 years of age, the negative findings may have been a result of estrogenic exacerbation of preexisting age-related tissue damage, particularly in the those women with preexisting Alzheimer's disease.

Emotional memory is another cognitive domain negatively associated with estrogen. A recent review (Sakaki & Mather, 2012) proposed that decreased neural activation of the amygdala during *high* estradiol phases of the menstrual cycle, relative to low estradiol phases, should translate into worse memory for negative stimuli during the high estrogen phases; a prediction supported in a study examining the effects of menstrual cycle phase on memory for emotionally valenced pictures. The study found free recall for emotional photographs was worse during the high estradiol phase of the menstrual cycle than during the low estradiol phase of the cycle (Mather, Cowell, Whiteside, Ledger, & Mangold, under review). The same pattern was reported in an emotional working memory task. Performance of a delayed-match-to-sample task using "disgust" and "sadness" as the target facial expressions to remember was worse in women tested later in the follicular phase of the menstrual cycle (higher estradiol) than in women tested during menses when estradiol levels are lowest (Gasbarri et al., 2008). In contrast to this, however, are reports of better reward learning when estradiol levels are high (Sakaki & Mather, 2012). Blunted responses to negative imagery and increased proclivity for learning positive associations occurring together during the late follicular phase (i.e. preovulatory) may have an evolutionary underpinning. A female less sensitive to detecting negative stimuli and more susceptible to recognizing positive associations during the time-sensitive ovulatory phase may be more receptive to copulating with available mates, increasing the likelihood of passing on her genes.

Although the estradiol findings discussed here are opposite the effects on the central nervous system, these negative effects are limited to specific circumstances, such as time of initiation of post-menopausal hormone treatment, and specific cognitive domains during certain

limited days on the menstrual cycle. When considering broader effects of estrogens on cognition, however, the literature largely supports a beneficial role for the hormone.

1.4.9 Positive Effects of Estradiol on Cognition in Young-adult Female Animals

Although estradiol is associated with impaired performance in some domains, the evidence largely supports a beneficial effect of the hormone. Ovariectomized animals exhibited fewer working memory errors (Bimonte & Denenberg, 1999), a higher rate of correct choices (Daniel, Fader, Spencer, & Dohanich, 1997; Fader, Johnson, & Dohanich, 1999), and a greater number of consecutive correct choices (Wilson, Puolivali, Heikkinen, & Riekkinen Jr., 1999) if treated with estradiol after ovariectomy compared with animals not treated with estradiol. Similar results have also been reported in other paradigms. Ovariectomized animals treated with estradiol benzoate displayed better retention for which arm to enter in a delayed match-to-sample task across 10-, 30-, and 100-minute delay intervals than did OVX animals not receiving estradiol treatment (Sandstrom & Williams, 2001). Estradiol treated animals also required less time to learn a delayed *non*-match-to-sample task (learning the reward was in the non-baited arm during training) than the non-estradiol treated OVX animals (Gibbs, 1999). The same pattern was observed in an object recognition task. In this task animals were exposed to a total of 4 novel objects. Animals first explored a pair of 2 novel objects, after some delay they were exposed to another 2 objects (one object from pair #1 and the third novel object); after a second delay period the animals were exposed to 2 additional objects (one being the second object from pair #1 and the fourth novel object). The animal is said to recognize an object if it spends less time exploring that object compared with time spent exploring the novel object. Ovariectomy followed by 21 days of E2 treatment enhanced retention for the first-tested object after a 3-hour delay and of the second-tested object after a 6-hour delay, compared with non-estradiol treated

mice (Vaucher et al., 2002). Thus, estradiol aids in maintaining or enhancing working memory, short-term memory, and long-term memory in young-adult female animals.

1.4.10 Effects of Menstrual Cycle on Cognition in Young-adult Human Females

The effect of estradiol on cognition in young-adult human females is not as consistent as the reports in young-adult female animals. The examination of estradiol effects typically looks for differences in cognition between the different menstrual cycle phases, when estradiol levels are low versus high, but have failed to find consistent effects across the hormone fluctuations of the menstrual cycle. The preovulatory phase (high estrogen and low progesterone) has been associated with increased creativity and decreased motor perseveration, compared with menses (low estrogen and low progesterone) and the midluteal phase when estradiol levels are moderate and progesterone levels are high (Rosemarie Krug, Stamm, Pietrowsky, Fehm, & Born, 1994). However, no effect of cycle phase has been observed in other cognitive domains. Females did not differ in their reaction times or accuracy in a semantic decision task asking participants to press a button when shown a pair of synonyms or when two strings of consonants were identical, when tested during menses or the midluteal phase (Fernandez et al., 2003). Correlation analysis also failed to find a relationship between estradiol levels during the whole follicular phase (consistently low progesterone and rising estradiol levels) and recognition of previously seen words mixed in a list of previously seen and never seen words (Craig et al., 2008). Still others reported no differences in performance of the Iowa Card Task or the Weather Task between menses and the midluteal phase (Reavis & Overman, 2001). Similar mixed results were also reported in a study that administered estradiol to young spontaneously cycling females; while estradiol enhanced accuracy on a 1-back task, a measure of working memory, treatment had no

effect on declarative learning and memory, verbal fluency, attention, cognitive flexibility, or psychomotor function and information processing (Bartholomeusz et al., 2008).

Failure of the young-adult human female literature to be as consistent as the animal literature is not surprising. Animal studies allow for the complete control of all gonadal hormones by ovariectomy and hormone administration. In humans, however, studies must search for differences between the naturally fluctuating hormone levels of reproductively intact females. Thus, when searching for the true effects of estrogen on cognition in humans we may need to focus on populations of women who no longer endogenously produce estrogen, such as postmenopausal women. In this regard, much work has been completed investigating the effects of estrogen on cognition in aged animal and human females.

1.4.11 Positive Effects of Estrogen on Cognition in Aging Female Animals

Aging female rodents do not experience menopause, instead they experience "estropause". This phase of the reproductive lifecycle is characterized by irregular cycling until a stable constant state of moderate estradiol is reached coinciding with reproductive senescence. In the rat, the average age the estrous cycle becomes irregular ranges from 10 to 12 months, slowly progressing to a state of anestrous at approximately 19 months of age (Lu, Hopper, Vargo, & Yen, 1979; Matt, Sarver, & Lu, 1987). Little work has focused on the effects of estradiol administration in intact middle-aged or aged female rodents, although studies that have find beneficial effects of the hormone. For instance, intact aged mice reached asymptotic performance of the Morris water maze task faster if treated with a moderate daily dose of estradiol benzoate, compared with animals receiving a lower dose or no estradiol treatments (Frick, Fernandez, & Bulinski, 2002), suggesting that moderate levels of estradiol aid in the recovery of working memory capabilities of aged animals.

Like studies using younger female animals, however, studies of estradiol effects on cognition in older females typically employ ovariectomy and find estradiol replacement beneficial to cognitive function. Aged animals ovariectomized 8- to 12-months prior to testing displayed lower error rates on a delayed match-to-sample task if treated with estradiol either immediately or starting 3 months after ovariectomy, compared with animals not treated with estradiol (Gibbs, 2000). Similarly, animals ovariectomized in middle age performed better on a delayed non-match-to-sample task if estradiol administration mimicked the estrous cycle compared with animals not treated with estradiol (Markowska & Savonenko, 2002). Similar results have been reported in primates. Twenty-year-old OVX rhesus monkeys learned a delayed response task and delayed non-match-to-sample task faster when treated with estradiol than non-estradiol-treated primates, and performed comparably to young, intact, females (Rapp, Morrison, & Roberts, 2003). Together, the literature indicates that estrogen replacement exerts beneficial effects on working memory processes of aging female animals.

1.4.12 Positive Effects of Estrogens on Cognition in Aging Human Females

Aging human females experience menopause, a state of reproductive senescence accompanied by dramatic declines in estradiol production and estradiol levels. Few studies have examined the cognitive effects of naturally declining estradiol levels in the absence of hormone replacement. One such study found endogenous levels of estradiol in postmenopausal women *not* taking any hormone supplements was positively correlated with performance on a verbal fluency task (Wolf & Kirschbaum, 2002). A comparable pattern was also observed in females receiving 2 weeks of estradiol treatment; those who experienced higher endogenous levels of estrogen in response to the dosage performed better on the immediate and delayed recall portion of a verbal memory test compared with women experiencing lower endogenous estradiol levels in response

to the dosage (Wolf et al., 1999). In accordance with the reviewed animal literature, the human literature to this point supports the putative beneficial effects of estradiol on cognition.

In addition to exerting beneficial effects on verbal memory and executive function, other prospective studies have found estrogen therapy to benefit cognition. Women on a hormone replacement regimen made fewer errors on a non-spatial working memory task, spatial working memory task, and had a longer memory span during the digit span backward task (Duff & Hampson, 2000), performed better on measures of semantic fluency, attention, and working memory (Miller et al., 2002), and displayed better performance on a non-verbal memory task (Smith et al., 2001) compared with women not taking any hormone supplements. The difference in results between these studies and the negative results on the WHI studies may be that, on average, the women in these studies began treatment closer to the age of menopause than the women in the WHI studies. For example, estrogen-associated enhancements in working memory and spatial working memory were observed in women who began hormone treatment during peri-menopause or soon after menopause (Duff & Hampson, 2000), those showing benefits in semantic fluency, attention, and working memory were on average 63 years old but had been taking estrogen supplements for an average of 12 years (Miller et al., 2002), and those displaying better performance on a non-verbal memory task initiated estrogen replacement within two years of menopause (Smith et al., 2001).

This pattern of estrogenic benefit accords with the basic science findings suggesting estradiol must be present before damaging agents are introduced in order to prove protective or beneficial, otherwise the hormone will prove detrimental (Chen et al., 2006). Taken together the results indicate that initiating estradiol treatment during the peri-menopausal phase and

prolonging estradiol exposure may prove beneficial to the maintenance of neural function and cognitive capabilities in postmenopausal females.

1.4.13 How is Estradiol Affecting Cognition?

Estrogenic effects on cognition in younger adult females may occur as neural concentrations of E2 fluctuate, coinciding with the hormone fluctuations of the menstrual cycle. Neural levels of estradiol have been linked to circulating levels of the hormone. Rodents either left intact or gonadectomized followed by estradiol replacement or vehicle showed treatment-dependent levels of estradiol in the hippocampus (Barker & Galea, 2009). Given that the hippocampus produces estradiol locally (Mukai et al., 2006), the Barker and Galea (2009) results suggest that local production of estradiol in the hippocampus may depend on circulating plasma levels of the hormone. Further, hippocampal dendrite spine density (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley, Gould, Frankfurt, & McEwen, 1990; Woolley & McEwen, 1993) and synapse density (Woolley & McEwen, 1992) are modulated by estradiol levels, as a function of NMDA receptor activation (Woolley & McEwen, 1994), suggesting that the ability of the hippocampus to locally produce estradiol may affect cognition by modulating the ability of the hippocampus to exhibit long-term potentiation.

Older females may be affected through the same mechanism only with more drastic effects. If the hippocampus locally produces estradiol relative to the circulating plasma levels, then older females would produce very little estradiol in the structure as result of the low plasma estradiol levels after menopause. Combined with the above-reviewed protective effects of the hormone on the hippocampus, absence of circulating and locally produced estradiol in the hippocampus may make the structure more vulnerable to degeneration, resulting in cognitive decline. Application of estradiol close to the time of menopause, however, may help maintain

estradiol production in the hippocampus, thereby prolonging the integrity of the structure and aiding in maintenance of cognitive function.

As for the inhibitory effect of estrogen on emotional memory, estrogenic modulation of the amygdala may be responsible. Unlike in the hippocampus, estradiol *decreases* excitability in the amygdala (Womble, Andrew, & Crook, 2002) by decreasing the amplitude of excitatory post-synaptic potentials (EPSPs). Decreasing EPSPs in the amygdala could lead to failure of an emotional stimulus to elicit enough EPSPs to reach threshold and cause activation of a cell. The failure to activate neurons in response to the stimulus would then leave the nucleus unable to communicate with other brain regions that there is a stimulus present requiring attention, thereby impairing learning about the stimulus and memory formation for such events.

1.5 Estradiol-Stress Interactions

1.5.1 Estrogenic Protection Against Glucocorticoid Insults

In addition to the countervailing effects of estradiol and glucocorticoids on neural tissue and cognition, estrogen can directly protect neural tissue from glucocorticoid exposure. Administration of dexamethasone (DEX) to male rats increased apoptosis and cell damage in the striatum and hippocampus, an effect attenuated by estradiol pretreatment (Haynes et al., 2003), and hippocampal slices from males exposed to a stressor prior to termination and tissue collection displayed greater long-term potentiation when bathed in a medium containing E2 than slices bathed in artificial cerebrospinal fluid (Foy, Baudry, Foy, & Thompson, 2008). Similar patterns are observed between OVX and intact female rodents. Chronic restraint stress decreased apical dendrite complexity in the CA3 region of the hippocampus (McLaughlin et al., 2010) and layers II and III of the medial prefrontal cortex (Garrett & Wellman, 2009) in OVX females,

effects prevented by E2 replacement after OVX (Garrett & Wellman, 2009; McLaughlin et al., 2010).

A possible mechanism for estradiol protection against glucocorticoid overexposure is estrogenic modulation of the GC receptor. Typically, chronic stress or stress hormone administration results in downregulation of the GC receptor (Ferrini, Lima, & De Nicola, 1995; Herman, Patel, Akil, & Watson, 1989; Sapolsky, Krey, & McEwen, 1984; Seckl & Olsson, 1995). Such downregulation likely acts as a protective factor. Reducing the number of receptors should minimize the number of cells vulnerable to damage from glucocorticoid overexposure. However, the nature of a chronic stressor is *continual* presence and continued release of GC. The reduced number of receptors induced by GC presence would already be filled to capacity and unable to detect the continually elevated glucocorticoid levels, hindering the effectiveness of the HPA axis' negative feedback system. However, estrogen administration upregulates glucocorticoid receptors whether chronic stress is simulated by drug administration (Ferrini et al., 1995) or directly applied (Ferrini & De Nicola, 1991). Upregulation of GC receptors by estrogen would allow the brain to better detect lower levels of the stress hormone. The increased sensitivity to GC detection would make the negative feedback system of the HPA axis more responsive leading to more rapid shut down the stress response. These basic science findings indicate estradiol provides physical protection from the damaging effects of glucocorticoids and may directly modulate HPA axis response to a stressor by modulating GC receptor density.

1.5.2 Estrogenic Modulation of the Stress Response

Sex differences provide one line of evidence for estrogenic influence on HPA axis activation. Young-adult females show lower adrenocorticotropic hormone (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka et al., 1998) and biologically active

free cortisol (Davis & Emory, 1995; Kirschbaum et al., 1999; Kirschbaum, Wust, & Hellhammer, 1992; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Kudielka et al., 1998) responses compared with young-adult males. Likewise, although brain activation while viewing negative visual stimuli did not differ between young men and young women in the low estradiol phase of the menstrual cycle, women tested during the high estradiol phase of the menstrual cycle showed lower stress-related activation of the anterior cingulate gyrus, orbitofrontal cortex, medial and ventromedial prefrontal cortex, amygdala, and hippocampus than men (Goldstein, Jerram, Abbs, Whitfield-Gabrieli, & Makris, 2010), with similar activational differences in women tested during the low estradiol versus higher estradiol phases of the menstrual cycle (Goldstein et al., 2005). Further, 1-day estradiol treatment in young-adult males resulted in blunted systolic blood pressure, pulse rate, epinephrine, and norepinephrine responses to a mental stressor (Del Rio et al., 1994).

Similar effects have been observed in post-menopausal women on estrogen treatment. Transdermal or oral E2 interventions spanning 1 day (Del Rio et al., 1998), 3 weeks (Ceresini et al., 2000), 1 month (Puder, Freda, Goland, & Wardlaw, 2001), 6 weeks (Lindheim et al., 1992), and 8 weeks (Komesaroff, Elser, & Sudhir, 1999) reduced the epinephrine (Ceresini et al., 2000; Del Rio et al., 1998), norepinephrine (Ceresini et al., 2000), diastolic blood pressure (Ceresini et al., 2000; Komesaroff et al., 1999; Lindheim et al., 1992), systolic blood pressure (Komesaroff et al., 1999; Lindheim et al., 1992), ACTH (Puder et al., 2001), and cortisol (Puder et al., 2001) responses to various stressors, such as mental stressors (Ceresini et al., 2000; Del Rio et al., 1998; Lindheim et al., 1992), the cold pressor task (Lindheim et al., 1992), and an endotoxin challenge (Puder et al., 2001).

In all, these findings indicate that estradiol blunts the HPA axis response. When considered with the above-discussed effects of stress on cognition, this pattern of attenuated HPA axis reactivity suggest that: 1) the high estradiol phase of the menstrual cycle should be less susceptible to stress-induced alterations in cognitive function and 2) post-menopausal women using hormone supplements should be less likely to experience stress-induced alterations in cognitive function.

1.5.3 Implications for Young-Adult Women

Based on the findings thus far, younger women should show reduced HPA reactivity to stressors during the high estradiol phase of the menstrual cycle compared with the low estradiol phase. However, the literature examining the effects of cycle phase on stress reactivity is not so clear-cut. In fact, many studies fail to find any difference in stress reactivity across the different hormone phases of the cycle (Galliven et al., 1997; Morimoto et al., 2008), or report greater reactivity during higher estradiol phases (Altemus et al., 1997; Altemus, Roca, Galliven, Romanos, & Deuster, 2001; Andreano, Arjomandi, & Cahill, 2008; Kirschbaum et al., 1999; Roca et al., 2003).

This counterintuitive pattern of results may be related to in which phases young-adult women undergo testing. In order to maximize the difference in hormone profile, and ensure cycle phase accuracy, most studies examine females during the early follicular phase when estradiol and progesterone levels are at the lowest concentrations, and the luteal phase when both estradiol and progesterone are at significantly higher concentrations. Thus, it is important to note that the findings reported in these studies are examining the *combined* effects of estradiol and progesterone on the stress response, *not* the effects of estradiol on the stress response. The inclusion of progesterone is important since this hormone counteracts some of estrogen's

beneficial effects (Woolley & McEwen, 1993). This may also be the reason for the lack of clear differences in cognitive performance across the menstrual cycle. Recall that most of the studies investigating cognition across the menstrual cycle tested women during menses and the mid-luteal phase. If the inconsistent reports of menstrual cycle effects on cognition and the stress response are a result of progesterone presence, it may still be the case that the estradiol-only fluctuations characteristic of the first half of the menstrual cycle can independently affect cognition and the stress response, thereby modulating stress effects on cognition.

1.5.4 Implications for Aging Women

Estrogenic protection against glucocorticoid-induced damage and the putative attenuation of stress reactivity by estradiol have important implications for the aging female. Based on the evidence reviewed herein, the loss of estradiol after menopause, and therefore loss of the hormone's protective effects, may lead to potentiated age-related changes in HPA function.

Typical aging is accompanied by a host of changes in the body. Included in these changes are alterations in the function of the stress response system, particularly the HPA axis. Animal studies have found aged rodents show smaller DEX-induced corticosterone suppression to a stressor, a treatment that significantly reduces the corticosterone response in young-adult animals (Ferrini et al., 1999; Riegle & Hess, 1972), because of activation of the system's negative feedback mechanism. This has also been observed in baboons and rhesus monkeys subjected to CRH, ACTH, and DEX tests (Goncharova & Lapin, 2002) and in a CRH test performed in dogs (Reul, Rothuizen, & de Kloet, 1991). Aged rats also displayed prolonged corticosterone secretion after termination of an acute stressor compared with young animals (Ferrini et al., 1999; Sapolsky, Krey, & McEwen, 1983); with similar results observed in rodents exposed to chronic stress (Riegle, 1973; Sapolsky et al., 1983).

Additionally, aged animals experience significant downregulation of glucocorticoid receptors, particularly in the hippocampus (Ferrini et al., 1999; Mizoguchi et al., 2009; Sapolsky, Krey, & McEwen, 1986). Loss of glucocorticoid receptors in the aged hippocampus leads to a failure to accurately detect circulating glucocorticoid levels and thus failure to terminate HPA axis reactivity. The resulting excess GC exposure leads to hippocampal damage and subsequent loss of more GC receptors. This cycle of receptor loss, tissue damage, and HPA hyperactivity was first presented as the Glucocorticoid Cascade Hypothesis (Sapolsky et al., 1986) and is a possible model to account for the hippocampal and cognitive decline observed in aging.

Consistent with the glucocorticoid-cascade hypothesis, some report that older human adults also exhibit higher basal cortisol, potentiated and prolonged cortisol secretion to stress (Almela et al., 2011; Dodt et al., 1991), and reduced levels of GC receptor mRNA in plasma compared with younger adults (Grasso, Lodi, Lupo, & Muscettola, 1997). Reduced levels of GC receptor mRNA in plasma may indicate that receptor density in the brain also declines, leading to failures to downregulate the HPA axis after a brief stressful event.

The previously described estradiol-related protection against glucocorticoid-induced damage, and the putative attenuation of stress reactivity by estradiol, have important implications for the aging female. The loss of estradiol, and its protective effects, during the post-menopause period is likely to lead to potentiated age-related changes in HPA axis function, whereas prolonging estradiol exposure past menopause is likely to maintain proper HPA axis function. For instance, recall that estradiol administration upregulated GC receptors in the hippocampus of older male rats, compared with older animals not treated with estradiol (Ferrini et al., 1999). This type of estrogenic upregulation of GC receptors in the hippocampus could help reverse the age-

related hyperactivity of the HPA axis as more receptors would be available to detect circulating GC levels and spare the hippocampus from further damage.

Importantly for human females, estradiol has been shown to counter each of the age-related dysfunctions (e.g., hippocampal damage, receptor downregulation, and HPA axis hyperactivity) described above. As such, estradiol supplementation past menopause should mitigate the extent or postpone the onset of HPA axis malfunctions. Insofar as estradiol protects the hippocampus and consequently the negative feedback system of the HPA axis, then extension of estradiol exposure past the time of menopause may be a possible mechanism for the relatively enhanced cognitive function observed in post-menopausal women who initiated hormone treatment close to the time of menopause.

1.6 Conclusions

Estrogens, glucocorticoids, and stress each impact daily female functioning. Depending on the circumstances surrounding their presence and action in the female body, each factor can exert positive or negative effects on the system. Furthermore, estradiol can alter how glucocorticoids and stress affect the brain and cognition. Understanding how these factors influence the adult female can be useful in determining how one should handle certain life events; for example, scheduling stressful events to fall on high estradiol phases of the menstrual cycle, or initiating estradiol replacement close to the time of menopause to maximize benefits and minimize risks.

It is important, however, not to speak in absolutes when discussing the effects of these factors, as each are associated with positives and negatives. For instance, glucocorticoids are necessary for normal neural stimulation (Doi et al., 1991) and can be necessary or beneficial for some types of learning, like avoidance learning (Yerkes & Dodson, 1908) and emotional

memory (Buchanan & Lovallo, 2001; Payne et al., 2007; Smeets et al., 2006). This is in stark contrast to the general trend of neural damage and cognitive interference resulting from excessive or chronic glucocorticoid application (Behl et al., 1997; Coburn-Litvak et al., 2003; Haynes et al., 2003; MacPherson et al., 2005; Stein-Behrens et al., 1992; Tombaugh et al., 1992; Virgin et al., 1991; Woolley, Gould, & McEwen, 1990) or stress exposure (Baum et al., 1983; Behl et al., 1997; Brown et al., 2005; Cook & Wellman, 2004; Diamond et al., 1996; Diamond & Rose, 1994; Haynes et al., 2003; Klein & Boals, 2001; McLaughlin et al., 2010; Ohl & Fuchs, 1999; Park et al., 2001; Tynan et al., 2010; Uno et al., 1989; Watanabe et al., 1992; Wellman, 2001). This same pattern is observed with estradiol. While most research suggests the hormone is beneficial to neural tissue (Brinton et al., 2000; Chen et al., 2006; Gerstner et al., 2007; Hao et al., 2007; Hoffman et al., 2003; Hosoda et al., 2001; Malagutti et al., 2009; Pike, 1999; Saravia et al., 2007; Sharma & Mehra, 2008) and cognition (Duff & Hampson, 2000; Maki et al., 2001; Miller et al., 2002; Smith et al., 2001; Wolf & Kirschbaum, 2002; Wolf et al., 1999), estradiol can be detrimental in other regards – such as when administered long after menopause (Espeland et al., 2004; Mulnard et al., 2000; S. R. Rapp et al., 2003; Resnick et al., 2006; Shumaker et al., 2003) and for emotional memory (Gasbarri et al., 2008; Mather et al., under review; Sakaki & Mather, 2012). Thus while each of these factors can be associated with a typical outcome, they each have exceptions where they exert an influence opposite to what is considered within their range of effects. It also is important that these factors work in concert to affect females. Not only do estrogen and stress result in dramatically different effects in the brain and cognition, but estrogen can act directly on the stress response to modulate the magnitude of response to a stressor (Ceresini et al., 2000; Del Rio et al., 1998; Komesaroff et al., 1999; Lindheim et al., 1992; Puder et al., 2001).

The cortisol and estradiol hormone systems show opposing effects both in isolation and in their interactions. We see that chronic stress exposure can be dangerous, estradiol can be protective, and estradiol can act to make stress less detrimental. Each point is interesting on its face, but the real value of the existing research is in putting these puzzle pieces together in order to understand how such an estradiol-stress interaction affects behavior and health. In particular, this interaction affects women in their daily lives via its influences on cognitive function. The dampening effect of estradiol on the stress response can affect the day-to-day cognitive functions of peri- and post-menopausal women who experience elevated cortisol levels in response to perceived family and work stressors. For instance, women who feel under pressure to complete family oriented tasks, and those who feel unrewarded for the amount of effort given to tasks, experience higher cortisol levels throughout the day (Eller, Netterstrøm, & Hansen, 2006), while those who feel work pressures are generally too high due to time pressures at work and having too many projects to simultaneously attend to also show elevated cortisol levels (Evolahti, Hultcrantz, & Collins, 2006). Given the potentially negative effects of these types of on-going stress in the everyday lives of women, it is critical to further investigate the possibility of estrogenic protection against stress-induced cognitive interference and its underlying mechanisms. The studies presented and discussed here aim to determine if estradiol does in fact protect women from the deleterious effects of stress on higher order cognitive functions, such as emotional memory and working memory.

CHAPTER 2

Study 1: Influence of estradiol on the stress response, and stress effects on working memory and emotional memory.

2.1 Introduction

Although the HPA and HPG systems are known to communicate and influence one another, less attention is paid to countervailing effects the two systems exert on the body, brain, and cognition. For example, long-term glucocorticoid exposure is linked to development of the metabolic syndrome (Pasquali et al., 2006; Rosmond, 2005), unhealthy alterations in fat distribution (Rebuffe-Scrive et al., 1992), promotion of hyperglycemia and hyperinsulinemia (McGuinness et al., 1993; Rebuffe-Scrive et al., 1992), promotion of bone resorption (O'Brien et al., 2004), and maintenance of bone degrading osteoclasts (Jia et al., 2006). In contrast, estrogens, the primary female gonadal hormone, are linked to less unhealthy fat distribution (Green et al., 2004; Musatov et al., 2007), lesser occurrence of hyperglycemia and hyperinsulinemia (Krotkiewski et al., 1983; Musatov et al., 2007), and promotion and maintenance of bone mineral density (Delmas et al., 1997; Felson et al., 1993; Sowers et al., 1998).

Similar contrasting effects of glucocorticoids and estrogens occur in neural tissue and cognition. For instance, while glucocorticoids are necessary for optimal neuronal functioning (Doi et al., 1991; Nadeau & Rivest, 2003; Sapolsky et al., 1991), beneficial to memory for emotional stimuli (Buchanan & Lovallo, 2001; Payne et al., 2007; Smeets et al., 2006), and possibly necessary for avoidance and aversion learning (Garrido, De Blas, Giné, Santos, & Mora, 2011; Hintiryan et al., 2009; Miele et al., 1988; Nachman & Ashe, 1973; Yerkes & Dodson, 1908), chronic or extreme glucocorticoid exposure results in cell damage or death (Behl et al.,

1997; Gerlach & McEwen, 1972; MacPherson et al., 2005; McEwen et al., 1968; Stein-Behrens et al., 1992; Tombaugh et al., 1992; Tynan et al., 2010; Woolley, Gould, & McEwen, 1990) and impairments in cognitive performance. Contrary to this pattern, estrogens, particularly estradiol, can be detrimental to already compromised neural tissue (Chen et al., 2006) and is reported to interfere with memory for emotional items (Gasbarri et al., 2008; Mather et al., under review; Sakaki & Mather, 2012), however, most research finds that estradiol promotes neural growth and protection (Brinton et al., 2000; Chen et al., 2006; Gerstner et al., 2007; Hao et al., 2007; Saravia et al., 2007; Ter Horst, Wichmann, Gerrits, Westenbroek, & Lin, 2009) and can improve cognitive function (Baker et al., 2012; Duff & Hampson, 2000; R. Krug, Born, & Rasch, 2006; Maki et al., 2001; Miller et al., 2002; Smith et al., 2001; Wolf & Kirschbaum, 2002; Wolf et al., 1999). This pattern of opposing effects on the brain and cognition has important implications for women throughout the reproductive and post-reproductive lifespan, but particularly for aging post-menopausal women.

Aging is accompanied by a host of changes, including changes in the ability to respond, or shut down response, to a stressor (Almela et al., 2011; Dodt et al., 1991; Wilkinson et al., 2001). Given the deleterious effects of stress exposure on the body (McGuinness et al., 1993; O'Brien et al., 2004; Pasquali et al., 2006; Rebuffe-Scrive et al., 1992; Rosmond, 2005), brain (Behl et al., 1997; Gerlach & McEwen, 1972; MacPherson et al., 2005; McEwen et al., 1968; Stein-Behrens et al., 1992; Tombaugh et al., 1992; Tynan et al., 2010; Woolley, Gould, & McEwen, 1990), and cognition (Alexander, Hillier, Smith, Tivarus, & Beversdorf, 2007; Duncko et al., 2009; Elzinga & Roelofs, 2005; Luethi, Meier, & Sandi, 2009; Oei et al., 2006; Schoofs et al., 2008; Schoofs et al., 2009; Young, Sahakian, Robbins, & Cowen, 1999), and the already increased incidence of illness in the aged, the inability to effectively shut down the stress

response may make aging persons more susceptible to neural and cognitive decline. Aging women, however, may be able to stave off the decline in effective stress reactivity by maintaining estradiol levels beyond menopause, as suggested by evidence showing short-term estradiol interventions, ranging in time from 1 day to 8 weeks, can attenuate the stress response (Ceresini et al., 2000; Del Rio et al., 1998; Komesaroff et al., 1999; Lindheim et al., 1992; Puder et al., 2001).

While various benefits of estradiol treatment have been studied, there has not been much attention paid to the possible benefits of the hormone for attenuating stress reactivity. Protection of this type may result in protection against declines in neural integrity and therefore cognition, given that animal studies show that stress hormones target and damage areas important for cognition, such as the hippocampus and prefrontal cortex (MacPherson et al., 2005; McEwen & Morrison, 2013; Tynan et al., 2010). Thus, the rapid and dramatic decrease in estradiol production and circulation during and after menopause may leave middle-aged and older women more vulnerable to the detrimental effects of stress hormone exposure on neural and cognitive integrity. However, maintenance of estradiol past the menopause transition may attenuate some of these age-related changes in the stress response and stress-induced declines in the brain and cognition.

With this study we aimed to address what effects estradiol might have on the physiological and behavioral effects of stress. Specifically, we tested three hypotheses regarding the effects of estradiol treatment after menopause. First we hypothesized that estradiol treatment after menopause would be associated with attenuated cortisol release in response to a stressor, the second hypothesis stated women taking estradiol would show less stress-induced interference

in working memory performance, and the third hypothesis stated that the women taking estradiol would display decreased stress-induced enhancement of emotional memory.

2.2 Methods

2.2.1 Participants

Forty-two post-menopausal women (54-87 years) were recruited from the double-blind, placebo-controlled, Early versus Late Intervention Trail with Estradiol (ELITE) clinical trial. Average time of enrollment in the ELITE trial, prior to participation in this estradiol-and-stress study, was 4.9 years. Women began their participation in the ELITE trial either within 6 years of their last menses (early initiation) or beyond 10 years of their last menses (late initiation). Women in the early and late initiation groups were randomly assigned to take either 1mg oral estradiol daily, or a placebo, creating 4 groups: early initiation-estradiol, early initiation-placebo, late initiation-estradiol, and late initiation-placebo. Participants had normal or corrected vision, were fluent in English, free of cognitive impairment, and free of conditions or medications that would enhance risks associated with the stressor or compromise data validity of this study or the ELITE trial.

2.2.2 Inclusionary and Exclusionary Criteria

Post-menopausal participants were required to be enrolled in the ELITE clinical trial and be free from the following conditions and medications: heart disease, peripheral vascular disease, diabetes, Reynaud's phenomenon, cryoglobulinemia, vasculitis, lupus, tingling or numbness in the hands and/or feet, or any other serious chronic illness. Subjects also were nonsmokers, were not taking beta-blocker medications, corticosteroid-based medications, or psychoactive drugs. Subjects also were free of any cognitive impairment, were fluent in English, and had normal or corrected vision.

2.2.3 Sessions

Participants came for 2 sessions, one stress and one control session, order counterbalanced. Women were given the option of being tested at the main USC campus (UPC) or at the USC Health Sciences Campus (HSC). The majority of women elected to be tested at HSC, where they were seen for their ELITE trial-related medical visits.

Sessions lasted 50-90 minutes, depending of factors such as time needed to produce sufficient saliva samples, time taken to read and sign informed consent, and time taken to complete questionnaires at the beginning the session.

2.2.4 Hormone Sampling

Three saliva samples were collected to assess cortisol, estradiol, and progesterone levels. Samples were collected before the stress manipulation, prior to starting the behavioral tasks (on average, 15 minutes after stressor onset), and after completion of the behavioral tasks (on average, 40 minutes after stressor onset). In order to minimize variations in hormone levels, participants were asked to refrain from exercise and food/drink (except water) within one hour, sleep within two hours, and caffeine and alcohol within three hours of their session start time. Participants were then asked to drink 8oz of water upon arrival to the lab in order to ensure proper hydration for saliva production and collection of a clean saliva sample. The first saliva sample was not taken until a minimum of 10 minutes had elapsed since the participant finished the 8oz water bottle.

Salivary samples are a reliable source for determining biologically available, unbound, levels of hormones (Tunn, Mollmann, Barth, Derendorf, & Krieg, 1992; Vining, McGinley, & Symons, 1983). Saliva sample 1 was used to assess baseline cortisol and sex hormone levels; to collect a large enough sample, participants passively drooled 1.25mL of saliva into a collection

tube. The remaining 2 samples only assessed cortisol and were collected using two sponge sorbettes. Samples were labeled with a barcode containing no personal information and then stored at 0°C until all data collection was completed. Once all samples had been collected they were packaged and transported frozen to CLIA-certified analytical laboratories for immunoassay (Salimetrics, LLC, State College, PA).

2.2.5 Stress Manipulation

To induce a stress response, participants completed the Cold Pressor Task (Hines Jr. & Brown, 1936; Lovallo, 1975), which has been shown to reliably induce cortisol secretion (Bullinger et al., 1984; Edelson & Robertson, 1986; Lighthall et al., 2009; Lighthall et al., 2011; Mather et al., 2010). In the Cold Pressor Task (CPT) participants submerge their non-dominant hand, up to the wrist, in ice water (0-5°C; average temperature in this study was 2.8°C or 37.04°F) for one to three minutes. The control condition uses warm water (37-40°C; average temperature in this study was 38.3°C or 100.94°F).

2.2.6 Psychological Measures

Participants completed questionnaires for demographics, emotional state, mood, and daily stress. Some measures were completed upon arrival at the lab and prior to the CPT, while others were completed after the CPT and before the behavioral tasks.

The measures used were: 1) Health and Demographics form, to determine sleep habits, stress, education level, and income; 2) the Daily Stress Inventory (Almeida, Wethington, & Kessler, 2002) to assess the current level of stress, 3) the Positive and Negative Affective Scale (Watson, Clark, & Tellegen, 1988) to assess emotional state; and 4) the Center for Epidemiological Studies Depression Scale (Radloff, 1977) to determine mood. Subjects also were screened for intellectual capabilities using the Wechsler Test of Adult Reading (Wechsler,

1981) as a measure of verbal intelligence. Lastly, immediately before and after the stress manipulation subjects completed a visual analog scale indicating 1) the amount of pain and stress they felt at the moment before stress and 2) the peak amount of pain and stress they felt *during* the stress manipulation. Participants were screened for cognitive impairment using the Telephone Interview for Cognitive Status – modified (Brandt, Spencer, & Folstein, 1988; Welsh, Breitner, & Magruder-Habib, 1993) prior to participating in the study.

2.2.7 Behavioral Tasks

Behavioral tasks commenced after collection of Saliva Sample 2, or approximately 18 minutes after stressor onset. The delay between stressor onset and commencement of behavioral tasks was utilized to ensure participants were experiencing peak cortisol responses during the tasks. Other studies have shown that peak cortisol responses are observed between 15-45 minutes after stressor onset (Kern et al., 2008; Kirschbaum et al., 1999; Kirschbaum et al., 1992; Kudielka, Buske-Kirschbaum, et al., 2004; Kudielka et al., 1998; Kudielka & Kirschbaum, 2005; Kudielka, Schmidt-Reinwald, Hellhammer, & Kirschbaum, 1999; Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). If time remained between completing the second round of questionnaires (CES-D and WTAR) and the second sample, participants completed a word search as a filler task until 14 minutes had passed since stressor onset.

2.2.8 Emotional Memory Task: Encoding Phase

We used the same task used in Mather et al. (under review) to assess whether emotional items were remembered differently depending on estradiol levels and cortisol levels. Participants viewed pictures of positive, negative, and neutral valence shown in different locations on a computer screen during an encoding phase. After viewing the pictures participants completed a working memory task, followed by a free recall test for the pictures they viewed in the encoding

phase and were then tested for their memory of for where pictures were presented during the encoding phase.

Participants viewed pictures of negative, positive, or neutral valence on a computer screen. They viewed 24 pictures in total, 12 emotional and 12 neutral. Of the 12 emotional pictures, 6 were negative and 6 were positive. Of the 12 neutral pictures, 6 were neutral versions of negative photos, and 6 were neutral versions of positive pictures. Each participant only saw either the negative, positive, or neutral version of each picture. Four versions of the task were created and each participant viewed two versions. Of the two versions each participant viewed, none of the negative and neutral or positive and neutral photo pairs overlapped. Photos were presented on different locations of the screen. Each photo was presented for 2000ms. Between each individual photograph, subjects were shown a yellow or green dot and indicated the color of the dot by key press on a keyboard. The dot task helped ensure participants were paying attention to the screen throughout the duration of the task. Participants were tested for their recall of the photos as well as for photo-location associations after completing the working memory task. On average, this portion of the task began approximately 18 minutes after stressor onset and took approximately 2 minutes to complete. Participants viewed different pictures at each session. The versions of the task viewed during session 1 and session 2 were counterbalanced.

2.2.9 Working Memory Task

The sentence span task was used to assess working memory. In this task, sentences were shown one at a time on the computer screen and participants were asked to make their best judgment as to whether the sentence "makes sense" or was "nonsense" **and** to remember the last word of every sentence presented within a given block. "Makes sense" and "Nonsense"

judgments were recorded by key press on a computer keyboard, while word recall was recorded on a paper scoring sheet by the experimenter.

Sentences were collected from various sources and have been used in other reading or sentence span tasks (Copeland & Radvansky, 2001; Daneman & Carpenter, 1980; N. P. Friedman & Miyake, 2004). Nonsense sentences were created as done in Turner and Engle (1989), "[Semantically and Syntactically] 'Incorrect' sentences were made nonsense by reversing the order of the last four…preterminal words e.g. 'The grades for our finals will be posted outside the classroom door' to 'The grades for our finals will be classroom the outside posted door'". Sentences were presented on the center of the screen with the last word in all capital letters (e.g. The boy said HELLO), for a duration of 5 seconds. At the end of the 5-second sentence presentation, the screen changed and displayed "Makes Sense" and "Nonsense", on the left and right side of screen, respectively. Subjects made a key press indicating whether they thought the just-viewed sentence made sense or did not. This was followed by a 500ms inter-trial interval, and then presentation of the next sentence. Participants completed 13 blocks in total. Blocks 1 and 2 were practice blocks and consisted of a 1-sentence load and 2-sentence load, respectively. At the end of each load the subject was prompted to tell the experimenter the last word of the 1 or 2 sentences they just viewed. The main portion of the task included 4 blocks of 2-sentence loads, 3 blocks of 3-sentence loads, and 2 blocks each of 4-, 5-, and 6-sentence loads. At the end of each block participants were prompted to tell the experimenter the last word of the 2, 3, 4, 5, or 6 sentences they just viewed, in the order they were presented in.

A lenient scoring criterion was used for this task; women were given 1 point for each word they remembered whether or not they recalled the words in the order presented or remembered every word in a given block. On average, this task began approximately 21.5

minutes after stressor onset and took approximately 11 minutes to complete. Participants saw different sentences at each session.

2.2.10 Emotional Memory Task: Recall Phase

Immediately following completion of the working memory task, participants were tested for their free recall of the photos presented during the encoding phase of the emotional memory task. On average, this portion of the emotional memory task began approximately 34 minutes after stressor onset and took approximately 4 minutes to complete.

2.2.11 Emotional Memory Task: Association Test Phase

Immediately following the recall portion of the emotional memory task, participants were tested for their memory of photo-location association of the photos presented during the encoding phase of the emotional memory task. Subjects viewed the same picture displayed on two different locations of the computer screen, simultaneously, and indicated the correct location of the picture by key press. Participants were only shown the same pictures they viewed during the encoding phase of that same session. On average, this portion of the emotional memory task began approximately 38 minutes after stressor onset and took approximately 2 minutes to complete.

2.2.12 Estradiol Condition and Analyses

Drug assignments for the ELITE trial have yet to be unblinded. Due to the delay in unblinding, we conducted all analyses on estradiol level, rather than condition. Estradiol influence on cortisol response, working memory, and emotional memory was assessed by comparing performance of women within the top and bottom quartiles of estradiol levels (n=10). Average estradiol level for women in the bottom quartile was 1.2935 pg/ml, and was 97.51125 pg/ml for women in the top quartile.

2.2.13 Statistics

Pearson's correlation analyses were conducted on questionnaires completed during sessions 1 and 2. Positive affect, negative affect, CES-D scores, pre and post water stress ratings, and pre and post water pain ratings were correlated with estradiol levels. These analyses were conducted on the entire sample of 42 women. All remaining analyses were conducted using the quartile-based Low Estradiol (LE) and High Estradiol (HE) groups.

Analyses of variance (ANOVA) were conducted for effects of estradiol level and stress on cortisol response, working memory performance, recall of emotional and neutral pictures, and picture-location associations. Significance was set at $p \leq 0.05$.

Analyses for working memory performance were conducted for the proportion of words recalled within each load, as well as the overall proportion of words recalled, collapsed across loads. Analyses for the emotional memory recall test and emotional memory picture-location association test also were conducted for the proportion of the pictures and locations remembered within each valence and collapsed across valences.

2.3 Results

2.3.1 Participants: Demographic Information for Low E2 versus High E2 Women

Low Estradiol (LE) and High Estradiol (HE) women did not differ in subjective ratings of their stress level at the start of session 1 or session 2, nor did they differ in their ratings for their stress levels on study days compared to their stress levels on "normal days". Nor did LE and HE women differ in their positive affect, negative affect, or depression scores at session 1 or session 2.

LE and HE women did differ in years of education ($t=-2.214$, $df=18$, $p=.040$), and subjective ratings of overall health at session 1 ($t=-2.75$, $df=18$, $p=.013$) and session 2 ($t=-3.349$,

df=18, p=.004). Overall subjective health ratings were based on a scale from 1 to 9, with 1 being very poor health and 9 being excellent health, that both groups averaged ratings over 7 suggests both groups of women felt subjectively healthy. While women in the LE and HE groups differed in their years of education, they did not differ in their performance on the WTAR (t=-.471, df=18, p=.643).

Lastly, of course, estradiol levels did differ between the low and high estradiol women (t=-2.936, df=18, p=.009). See table 2.2.

2.3.2 Correlation Analyses between Estradiol Levels and Questionnaire Responses

There was no relationship between estradiol levels and positive affect, negative affect, pre-water pain, post water pain, or post water stress during session 1. There was, however, a significant negative correlation between estradiol levels and pre-water stress ratings (r=-5.30, p=.016), such that women with higher estradiol levels reported feeling less stressed before placing their hand in water during session 1. No relationships between estradiol levels and any questionnaire were found during session 2.

2.3.3 Subjective Ratings of Stress and Pain Immediately Before and After Stress Exposure

Due to the negative correlation between estradiol and pre-water stress ratings, we conducted a 2 (stress: cold v warm water) x 2 (estradiol) within-by-between MANCOVA, controlling for session 1 stress condition, on pre-water stress ratings. This analysis revealed that LE women reported higher levels of subjective stress prior to placing their hands in water than did HE women (F=6.41, p=.021).

Despite the higher baseline levels of subjective stress immediately prior to water exposure in LE women, follow-up 2 (stress: cold v warm) x 2 (time: pre v post stress) x 2 (estradiol: low v high) within-by-between MANCOVA, controlling for session 1 condition,

found no three way interaction between stress, time, and estradiol (F=1.543, p=.231; see figure 2.1), indicating there was no difference in the magnitude of change in subjective stress ratings from pre stress to post stress between LE and HE women during either the stress or control sessions. Follow-up 2 (time: pre v post stress) x 2 (estradiol: low v high) analyses for the stress session alone, while still controlling for session 1 condition, also found no two way interaction (F=2.915, p=.106), indicating that there was no difference in the magnitude of change from pre stress to post stress between LE and HE women during the stress session. Likewise, no interaction was found when the analysis was conducted for the control session alone (F=.422, p=.524).

A 2 (stress: cold v warm) x 2 (time: pre v post stress) x 2 (estradiol: low v high) within-by-between ANOVA revealed no overall stress x time x estradiol interaction, suggesting there was no difference between LE and HE women in the change of their subjective *pain* ratings from immediately before placing their hand in water to the peak amount of pain felt while their hand was in the water during both the stress and control sessions (F=.351, p=.561; see figure 2.2). Follow-up 2 (time: pre v post stress) x 2 (estradiol: low v high) analyses for the stress session only revealed a main effect of time (F=51.676, p<.001), such that subjective pain ratings increased from pre stress to post stress (means: pre=4.95, post=54.15). However, there was no estradiol x time interaction (F=1.046, p=.32), suggesting that there was no difference in the change from pre to post stress between LE and HE women. Similar analyses conducted for the control session revealed a main effect of time (F=5.766, p=.027), with subjective pain ratings decreasing from pre stress to post stress (means: pre=4.5, post=0.4). However, there was no estradiol x time interaction (F=2.885, p=.107), suggesting that there was no difference in the change from pre to post stress between LE and HE women.

2.3.4 Cortisol Response for Low E2 versus High E2 Women During the Stress and Control Sessions

A 2 (stress: cold v warm) x 2 (estradiol) within-by-between ANOVA comparing baseline cortisol levels between LE and HE women in the stress and control sessions, revealed that baseline cortisol levels were comparable during the stress and control sessions (F=.721, p=.407). The analysis also failed to find a stress x estradiol interaction (F=1.176, p=.292), indicating LE and HE women had comparable baseline levels of cortisol at each session. In order to account for the higher pre-water stress levels reported by LE women during session 1, a follow-up ANCOVA looking at only session 1 baseline cortisol levels while controlling for pre-water stress levels was conducted. This comparison also revealed that LE and HE women had comparable baseline cortisol levels (F=.000, p=.989).

A 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) x 2 (estradiol: low v high) within-by-between ANOVA on cortisol levels revealed no main effect of stress (F=1.313, p=.267), but a main effect of time (F=4.668, p=.044), such that cortisol levels increased from baseline to 15 minutes post stress onset, and a stress x time interaction (F=8.559, p=.009), revealing that cortisol levels increased from baseline to 15 minutes post stress onset in the stress session, but decreased between the two time points during the control session; see figure 2.3. Follow-up analyses revealed that the increase from baseline to 15 minutes post stress onset was significant for the stress session (F=9.977, p=.005), but the decrease observed for the control session was not significant (F=.161, p=.693).

This analysis also uncovered a time x estradiol interaction (F=7.881, p=.012), such that when collapsed across sessions, LE women displayed increases in cortisol from baseline to 15 minutes post stress onset, while HE women displayed a decrease in cortisol; see figure 2.4.

Follow-up analyses conducted on cortisol levels during the stress session only revealed a main effect of time (F=9.977, p=.005) and a time x estradiol interaction (F=5.58, p=.03) revealing that LE women showed a much larger increase in cortisol levels from baseline to 15 minutes post stress onset than the HE women; see figure 2.5. Additional follow-up analyses found that the increase in cortisol observed in LE women was significant (F=9.047, p=.015), but the increase observed in HE women was not statistically significant (F=1.005, p=.342).

Further follow-up analyses controlling for pre-water stress levels during the stress session still found the same pattern of results. A 2 (time: baseline v 15 minutes post stress onset) x 2 (estradiol) within-by-between ANCOVA, using pre-water stress ratings as a covariate, uncovered a main effect of time (F=10.205, p=.005), such that cortisol levels increased from baseline to 15 minutes post stress onset. The analysis also uncovered a time x estradiol interaction (F=7.30, p=.015), suggesting that main effect of time was largely attributable to the larger cortisol increase exhibited by LE women (means: baseline=.148 μg/dl, 15 minutes post onset= .354 μg/dl) versus the lack of increase in HE women (means: baseline=.150 μg/dl, 15 minutes post onset= .150 μg/dl), despite the two groups having comparable baseline cortisol levels during the stress session (F=.001, p=.974, means: LE=.148 μg/dl, HE= .150 μg/dl).

2.3.5 Working Memory: Word Recall

A 2 (stress: cold v warm) x 5 (load: 2 sentences through 6 sentences) x 2 (estradiol: low v high) within-by-between ANOVA revealed a marginal main effect of stress (F=3.843, p=.066), trending towards worse working memory performance during the stress session than control session. A main effect of load was uncovered (F=29.870, p<.001), revealing that performance decreased as load increased. No estradiol x load or stress x load interactions were detected, suggesting LE and HE women showed the same pattern of performance decrement as loads

increased in difficulty, with the same pattern also observed across stress and control conditions. There was, however, a marginal stress x estradiol interaction (F=4.138, p=.057), where LE women showed a larger difference in performance across stress and control sessions (means: control=.598, stress=.511) while HE women performed almost the same in each session (means: control=.543, stress=.542). The difference in means between LE and HE women for control session performance did not differ statistically (t=.828, df=18, p=.418).

We next conducted a 2 (stress: cold v warm) x 2 (estradiol: low v high) ANOVA on overall working memory performance, collapsed across loads. This analysis again revealed a marginal effect of stress (F=3.815, p=.067) and marginal stress x estradiol interaction (F=3.815, p=.067). Trends also were for worse performance during the stress session, and greater impairment after stress in LE women than HE women. Follow-up analyses for the LE women alone revealed an effect of stress (F=5.099, p=.05), where they exhibited worse performance during the stress session than during the control session. HE women, however, showed no decrement in performance after stress compared to their control session (F=.000, p=1; see figure 2.6).

2.3.6 Emotional Memory: Free Recall of Emotional and Neutral Pictures

A 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) x 2 (estradiol: low v high) within-by-between ANOVA on picture recall revealed no main effect of stress (F=.614, p=.444), but did find a main effect of valence; see figure 2.7. This analysis also failed to find stress x valence, stress x estradiol, or valence x estradiol interactions.

We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) x 2 (estradiol: low v high) within-by-between ANOVA on picture recall revealed no main effect

of stress (F=.297 p=.592), but did find a main effect of valence (F=16.141, p=.001; means: negative=.304, neutral=.183). This analysis also failed to find stress x valence, stress x estradiol, or valence x estradiol interactions, although the stress x valence interaction was marginal (F=3.349, p=.084; see figure 2.8) with a smaller difference between the proportion of negative pictures and neutral picture remembered during the stress session than the difference observed in the control session. Further follow-up analyses revealed that the difference between recall of negative and neutral pictures during the stress session was not statistically significant (F=.673, p=.423), but the difference between recall of negative and neutral pictures during the control session was significant (F=9.176, p=.007).

Analyses looking at positive versus neutral pictures found no effects of stress or valence, or any interactions. However, the main effect of valence was marginal with F=4.315 and p=.052 (means: positive=.275, neutral=.2). Likewise, analyses looking at negative versus positive pictures found no effects of stress or valence, or any interactions; nor did any of the comparisons approach significance.

2.3.7 Emotional Memory: Memory for Picture Location

A 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) x 2 (estradiol: low v high) within-by-between ANOVA on memory for where on the computer screen pictures were presented during the encoding phase revealed no main effect of stress (F=.086, p=.773), valence (F=.208, p=.654) or stress x valence, stress x estradiol, or valence x estradiol interactions.

We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) x 2 (estradiol: low v high) within-by-between ANOVA on memory for where pictures were

presented on the computer screen during encoding revealed no main effect of stress (F=.497 p=.49), valence (F=2.718, p=.117), or stress x valence, stress x estradiol, or valence x estradiol interactions. Likewise analyses looking at memory for where pictures were presented on the computer screen during encoding between positive versus neutral pictures and positive versus negative pictures found no effects of stress or valence, or any interactions.

2.4 Discussion

Study 1 tested three main hypotheses regarding the effects of estradiol treatment on HPA reactivity to a stressor. Hypothesis 1 focused on the cortisol response to a stressor and stated that HE women would display a blunted cortisol response to an ice water stressor compared with LE women. The results of the study support this hypothesis. The notion that estradiol treatment would minimize cortisol release created the foundations for the second and third hypotheses, such that reductions in cortisol release should mitigate the effects of stress on other domains because less GC would be available to act on and affect other processes. Following this reasoning, the second hypothesis focused on the ability of estradiol treatment to change the effects of stress on working memory. We stated that HE women would show less stress-induced *interference* of working memory than LE women, and indeed our results support this hypothesis as well. The third hypothesis focused on how estradiol treatment might alter the effects of stress on emotional memory. We stated that HE women would show less stress-induced *enhancement* of emotional memory than LE women, however this hypothesis was not supported by the current study.

2.4.1 Differences In Overall Health Ratings And Education Between HE And LE Women

Low E2 and High E2 women differed in their subjective ratings of their overall health and education, with HE women reporting higher overall health ratings and having a higher

education. Overall health ratings were made based a scale of 1 to 9, with 1 being very poor health and 9 being excellent health. The average rating for both groups of women was above 7, suggesting that both groups of women felt relatively healthy. The slightly higher rating provided by HE women, however, is not unexpected. Menopausal women taking estradiol supplements are often relieved of the discomforts associated with menopause such as hot flashes (Nelson, 2004), vaginal dryness, other urogenital changes, and decreased libido (Bachmann & Leiblum, 2004). The HE women, presumably taking estradiol supplements through the ELITE trial, should have fewer menopausal symptoms than the LE women, and this may contribute to their slightly higher ratings of overall health.

HE women were found to have more years of education than the LE women, albeit the difference was relatively small since the average for both groups was over 16 years of education. In fact, of the 10 women in each group, 9 LE women had 16 or more years of education, versus all 10 of the HE women. Further, to check that the years of education did not alter vocabulary capabilities, we compared LE and HE women on their WTAR performance and found no difference. Given that WTAR performance and years of education were positively correlated ($r=.490$, $p=.001$) and with linear regression analysis revealing years of education to be a significant predictor of WTAR performance ($F=12.654$, $p=.001$) in our sample of women, finding that LE and HE women did not differ in their WTAR performance despite the difference in years of education suggests the LE and HE groups were comparable in their overall level of education.

2.4.2 Subjective Ratings of Pre-water Stress Levels Between LE and HE Women

With regard to the subjective ratings of pre and post water stress and pain levels, we did find a difference between LE and HE women on their subjective ratings of pre-water stress levels

during session 1. This finding suggests that LE women may have felt more anticipatory stress immediately prior to placing their hand in water than the HE women. Importantly, when first arriving in the laboratory women completed two scales for the amount of stress they were feeling that day on a scale from 1 to 9. The first scale asked how stressed they felt that day and was marked as 1 being very low, 5 being usual, and 9 being very high. They also completed a scale rating "how does your stress level today compare to your usual stress level?" with 1 being much lower, 5 being same as usual, and 9 being much higher. LE and HE women did not differ on either of these scales at the beginning of the session, suggesting the LE women had a larger subjective increase in stress levels from the beginning to the session to the moments immediately before placing their hand in water.

This change from having comparable stress levels at the beginning of the session to higher stress levels immediately before placing their hand in water for the first time does fall under our general hypothesis that estradiol treatment, or higher estradiol levels, are associated with a minimized stress response. If HE women are less likely to feel anticipatory stress in the moments before a stressful event, they may be offered psychological protection from the anticipation of stressor onset, which may contribute to reducing their stress response.

2.4.3 Baseline Cortisol Levels in LE and HE Women

Despite higher subjective ratings of stress prior to hand immersion during session 1, LE and HE women had comparable baseline cortisol levels during that session. Unlike session 1, subjective ratings of pre-water stress levels did not differ during session 2, and again neither did baseline cortisol levels.

Our hypothesis that HE women experience blunted cortisol responses to a stressor may partially address why this group had lower subjective stress ratings during session 1, but does not

address why LE women did not then have higher baseline cortisol levels during this session. A potential explanation for this may be a result of the time course of the cortisol response. Recall that cortisol levels peak between 15-45 minutes after stressor onset (Kern et al., 2008; Kirschbaum et al., 1999; Kirschbaum et al., 1992; Kudielka, Buske-Kirschbaum, et al., 2004; Kudielka et al., 1998; Kudielka & Kirschbaum, 2005; Kudielka et al., 1999; Kudielka, Schommer, et al., 2004), and that the baseline saliva sample was taken immediately *before* women rated their pre-water subjective stress levels. It may be the case that baseline cortisol levels could have differed if stress ratings were collected prior to the saliva sample and if there had been ample time between the two events. This, however, would not be an ideal protocol for assessing changes in subjective stress before and after stress onset.

2.4.4 Cortisol Response in LE and HE Women

HE women displayed a blunted cortisol response after ice water exposure compared with the LE women, suggesting that higher estradiol levels, presumably as a result of taking estradiol supplements, dampens the cortisol response to a stressor. This pattern was maintained when controlling for session 1 pre-water stress ratings and stress session pre-water stress ratings, suggesting this effect is robust.

Based on the literature reviewed here, two possibilities exist, one based on potential short-term effects of estradiol, and the other based on potential long-term effects of estradiol. The first, potential short-term, effect is that the HE women had higher levels of corticosteroid binding globulin (CBG), limiting the amount of unbound, biologically active cortisol available. Saliva samples assess the amount of unbound cortisol, and so it may be the case that HE and LE women released the same amount of cortisol but that the HE women were protected from much of the cortisol released as it bound to CBG. This mechanism would still exert a protective effect

on neural tissue and cognition as the proportion of the bound hormone would be unable to act on tissue.

The second, potential long-term, effect is that the HPA axis of HE women functions more optimally than the HPA axis of LE women. Women assigned to estradiol in the ELITE trial had been using estradiol for almost 5 years, on average, which may be enough time to protect the HPA axis from age-related degradation in the negative feedback loop of the system. Maintenance of efficient functioning of the axis would allow for better regulation of cortisol release in response to a stressor, lower peaks in cortisol levels after stress exposure, and faster shutdown of the HPA axis after stressor removal. The pattern of results for saliva sample 3 suggests that the HE women do not reach the same peak levels of cortisol as the LE women, at least within the timeframe of this study (i.e., within 38 minutes of stress onset). Although the difference between LE and HE groups 38 minutes post stress onset was nonsignificant ($t=.923$, $df=18$, $p=.368$), the means for the HE and LE groups do trend toward a pattern of HE women remaining much lower than LE women with regard to cortisol release post stress exposure (means: LE 38 minuets post onset=.387 μg/dl, HE 38 minutes post onset=.286 μg/dl).

A third possibility based on results stemming from this study, could be that HE women experience less anticipatory stress prior to potential stress exposure. Whereas LE women may experience higher anticipatory stress, resulting in an additive effect of anticipatory stress and actual stress exposure on cortisol response, HE women may only experience cortisol responses to a direct stressor. An alternative explanation along similar lines is that HE women are more impervious to the effects of feeling stress. Although there was no interaction between pre-water and post-water stress ratings between the LE and HE women, and no significant difference when post-water ratings were compared directly ($t=-1.299$, $df=18$, $p=.210$), HE women did have a

numerically higher post-water stress rating than LE women (means: LE=36.2, HE=53.5). Yet, despite reporting a numerically higher stress level after stressor removal, these women still showed markedly lower cortisol release than the LE women. This pattern may point to a level of resistance against stress in women with high estradiol levels, which may aid in maintaining proper function of the HPA axis and neural integrity of regions most affected by GC exposure.

2.4.5 Effects of Stress on Working Memory and Emotional Memory in LE and HE Women

Based on the cortisol results of this study it would be expected that HE women would show less decline in working memory performance from the control to stress session than the LE women, and this seems to be the case. Given that HE women exhibited less cortisol release, less GC should have been available to interfere with neural regions involved in working memory performance. Again, the decreased availability of cortisol could result from less cortisol release in response to the stressor, or from higher CBG levels minimizing the amount of cortisol that can act on tissue. Unfortunately, this study cannot address which mechanism is at play. In order to assess total cortisol levels (i.e., bound and unbound) one would need blood samples. Collecting blood samples for a stress study could have confounding effects in that the insertion of the needle for blood draw could be considered a stressor and increase cortisol levels in the absence of a stressor or have an additive effect of stress during the stress session.

Yet, regardless of the means by which cortisol levels were reduced, the effect remains that HE women seem to have some level of protection against stress when it comes to their working memory. Protection against stress in this domain of cognition might prove to be beneficial to postmenopausal women. Recall that perceived high pressures at work and home were associated with higher cortisol levels in middle-aged women (Eller et al., 2006; Evolahti et al., 2006). The perceived high pressure was a result of having multiple tasks and timelines to

adhere to in both cases, suggesting women experiencing this high pressure are using their working memory to address their many tasks. Showing that estradiol can minimize the negative effects of the stress on this cognitive domain could have important implications for how women cope with, respond to, and perform their daily tasks when feeling this increased pressure and associated increases in cortisol levels.

Less clear are the lack of effects in the emotional memory tests. Despite the evidence showing that stress can affect memory for emotional items (Buchanan & Lovallo, 2001; Payne et al., 2007; Smeets et al., 2006), this study failed to find any direct stress effects for any combination of valence and stress interactions in picture recall or memory for where pictures were presented on the computer screen.

With regard to picture recall, valence effects were found for comparisons of emotional and neutral pictures, and negative and neutral pictures, but no other effects were found. Meanwhile, no effects at all were uncovered for memory of where pictures were presented during encoding. While the failure to find stress effects in HE women does make sense given their minimal cortisol response to the ice water stressor, it is not clear why the LE women also failed to exhibit an effect of stress of emotional memory given the significant increase in cortisol elicited by the stressor. In fact, we hypothesized that LE women would show an enhanced effect of stress on emotional memory as a result of lower estradiol levels being associated with better emotional memory (Sakaki & Mather, 2012) coupled with exhibiting a higher cortisol response to the stressor. One possibility is that the effects of stress and low estradiol competed to eliminate any effects of estradiol, stress or stress-by-estradiol interaction on emotional memory. Emotional memory, although nonsignificant, was negatively correlated with salivary estradiol levels (see figure 2.9). However, although also nonsignificant, emotional memory was negatively

correlated with cortisol levels in the LE women (see figure 2.10). While the LE women should have exhibited better emotional memory due to the low estradiol levels, the dramatic increase in cortisol levels may have dampened this effect, essentially negating any effects, and contributing to the failure to see effects of stress in this group.

2.4.6 Conclusion

This study suggests that estradiol treatment after menopause may have protective effects against stress, with regard to cortisol release and working memory. Although this finding may have important implications for post-menopausal women, the mechanism remains unclear and demands further attention.

Whether these effects are a result of circulating estradiol levels that can be achieved with short-term estradiol treatment, or if they are a result of the cumulative five years that women were using estradiol through the ELITE trial, remains unclear. This is an important issue to parse out as it is still unclear if extended long-term estradiol treatment is feasible for post-menopausal women, or if following an intermittent on-off scheduling for estradiol treatment is safer. In the instance of the short-term mechanism of action for this protective effect, estradiol's ability to reduce the stress response would be based solely on circulating estradiol levels. This short-term mechanism would likely be attributable to upregulation of CBG levels, thereby minimizing the amount of bioavailable, unbound cortisol. It also is possible that the reintroduction of estradiol into the system would result in a renewed upregulation of GC receptors leading to more efficient and optimal HPA axis function. The long-term mechanism would involve the ability of long-term estradiol treatment to protect the neural regions involved in shutting down the HPA response. While this mechanism is suggested to be the most beneficial in terms of neural integrity, cognitive function, and delaying of neurodegenerative disorders of aging, it cannot be

implemented until estradiol treatments are found safe for extended long-term use. Once the mechanism of protection is understood, this line of research has the potential to further inform the medical community of the effects of estradiol treatment. With the potential application of this work to the general population, it is important to continue pursuit of the potential additional benefits of this estradiol-stress interaction, its limitations, and its risks.

CHAPTER 3

Study 2: Influence of estradiol fluctuations during the menstrual cycle on the stress response, and stress effects on working memory and emotional memory.

3.1 Introduction

As briefly discussed in Chapter 1, results are mixed as to whether, or how, stress or cognition are affected by the natural hormone fluctuations characteristic of the menstrual cycle. During the course of the 28-day cycle, women experience increases and decreases in a number of hormones other than estradiol, such as progesterone, follicle stimulating hormone, and luteinizing hormone. The varying levels of each hormone, and the different combination of levels of each hormone during different phases of the menstrual cycle, make it difficult to ascertain which hormones may be involved with any observed changes in behavior. This increased variability likely contributes to the varying results obtained for effects of the menstrual cycle on behavior.

For this study we were not interested in the effects of the menstrual cycle, per se, but rather to determine if the effects of estradiol treatment observed in study 1 also hold true for young-adult, spontaneously cycling, women. In order to limit our examination to the effects of estradiol we focused on the portion of the menstrual cycle where only estradiol fluctuates, while the other HPG hormones remain at relatively low and stable levels, the follicular phase of the menstrual cycle. The follicular phase of the menstrual cycle, or the first half of the cycle, begins on the first day of menstruation and lasts until just before ovulation. The follicular phase is characterized by low levels of progesterone, follicle stimulating hormone, and luteinizing hormone, but increasing estradiol levels. During the early follicular phase, or when women are menstruating, estradiol also is at its lowest levels. A few days later, or the days leading up to

ovulation, women experience a sharp increase in estradiol levels, until it reaches its highest levels of the monthly cycle.

In order to pick up on the largest differences between phases, studies often focus on the follicular phase, where only estradiol levels vary, versus the luteal phase, where both progesterone and estradiol are higher. Thus limiting our examination to the follicular phase where estradiol alone varies, may make it difficult to uncover robust differences, however, in order to examine the effects of estradiol fluctuations in young spontaneously cycling females we must limit the examination to only this phase. Our hypotheses for this second study were similar to study 1. Specifically, that higher estradiol levels would be associated with attenuated cortisol release in response to a stressor, that higher estradiol levels would be associated with less stress-induced *interference* in working memory performance, and higher estradiol levels would be associated with decreased stress-induced *enhancement* of emotional memory.

3.2 Methods

3.2.1 Participants

Twenty-seven young-adult, spontaneously-cycling, undergraduate females from USC (18-24 years) were recruited for this study. Each participant attended four sessions. Two sessions occurred during the low estradiol/low progesterone, Early Follicular phase (EF) and two sessions occurred during the high estradiol/low progesterone, Late Follicular (LF) phase. Whether participants were first seen in the early or late follicular phase was counterbalanced.

3.2.2 Inclusionary and Exclusionary Criteria

Young women participating in the study were free from the following conditions and medications: heart disease, peripheral vascular disease, diabetes, Reynaud's phenomenon, cryoglobulinemia, vasculitis, lupus, tingling or numbness in the hands and/or feet, or any other

serious chronic illness. Subjects also were nonsmokers, were not taking hormone contraceptives, beta-blocker medications, corticosteroid-based medications, or psychoactive drugs. In order to participate subjects further were not allowed to have used hormone birth control in the last 6 months or been pregnant in the last year. Subjects also were fluent in English, and had normal or corrected vision.

3.2.3 Sessions

Participants came in for 4 sessions, two sessions during the early follicular phase and two sessions in the late follicular phase, order of first phase seen was counterbalanced. Within each phase participants underwent one stress and one control session, order also counterbalanced. The EF phase was defined as days 1-5 of the menstrual cycle, with the first day of menses being day 1. The LF phase was defined as days 8-12 of the menstrual cycle, with the first day of menses being day 1. Women were screened for cycle regularity before participating. During this screening, women reported the expected start date of their next menstrual cycle and were not seen for their sessions until they reported the start of their menses to study personnel. Women first seen during the EF phase completed all 4 sessions within the same menstrual cycle, whereas women first seen during the LF phase completed their 4 sessions across two menstrual cycles. In order to reduce individual variability in stress hormone levels, all sessions were conducted in the afternoons between 1pm and 7pm, with no session starting later than 5pm.

Sessions lasted approximately 55 minutes, depending on factors such as time needed to produce sufficient saliva samples, time taken to read and sign informed consent, and time taken to complete questionnaires at the beginning the sessions.

3.2.4 Hormone Sampling

Three saliva samples were taken to assess cortisol, estradiol, and progesterone levels, and were collected before the stress manipulation, prior to starting the behavioral tasks (on average, 17 minutes after stressor onset), and after completion of the behavioral tasks (on average, 43 minutes after stressor onset). In order to minimize variations in hormone levels, participants were asked to refrain from exercise and food/drink (except water) within one hour, sleep within two hours, and caffeine and alcohol within three hours of their session start time. Participants were then asked to drink an 8oz bottle of water upon arrival to the lab in order to ensure proper hydration for saliva production and collection of a clean saliva sample. The first saliva sample was not taken until a minimum of 10 minutes had elapsed since the participant finished the 8oz water bottle.

Salivary samples are a reliable source for determining biologically available, unbound, levels of hormones (Tunn et al., 1992; Vining et al., 1983). Saliva sample 1 was used to assess baseline cortisol and sex hormone levels; to collect a large enough sample, participants passively drooled 1.25mL of saliva into a collection tube. The remaining 2 samples assessed cortisol levels after water exposure as well as sex hormones. Samples were labeled with a barcode containing no personal information and then stored at 0°C until all data collection was completed. Once all samples had been collected they were analyzed in house. Sex hormone levels have not yet been processed. Once the samples are analyzed, estradiol and progesterone levels from all three samples will be averaged to obtain the most accurate estradiol and progesterone levels for that particular session.

3.2.5 Stress Manipulation

To induce a stress response, participants completed the Cold Pressor Task (Hines Jr. & Brown, 1936; Lovallo, 1975), which has been shown to reliably induce cortisol secretion (Bullinger et al., 1984; Edelson & Robertson, 1986; Lighthall et al., 2009; Lighthall et al., 2011; Mather et al., 2010). In the CPT participants submerge their non-dominant hand, up to the wrist, in ice water (0-5°C) for one to three minutes. The control condition uses warm water (37-40°C).

3.2.6 Psychological Measures

Participants completed questionnaires for demographics, emotional state, mood, symptoms of PMS, and daily stress. Measures were completed upon arrival at the lab and prior to the CPT.

The measures used were: 1) Health and Demographics form, to determine sleep habits, stress, education level, and income; 2) the Daily Stress Inventory (Almeida et al., 2002) to assess the current level of stress, 3) the Positive and Negative Affective Scale (Watson et al., 1988) to assess emotional state; 4) the Center for Epidemiological Studies Depression Scale (Radloff, 1977) to determine mood; 5) the Premenstrual Tension Syndrome Visual Analogue Scale to determine symptoms of PMS (Steiner, Peer, Macdougall, & Haskett, 2011). Subjects also were screened for intellectual capabilities using the Wechsler Test of Adult Reading (Wechsler, 1981) as a measure of verbal intelligence. Lastly, immediately before and after the stress manipulation subjects completed a visual analog scale indicating the 1) the amount of pain and stress they felt at the moment before stress exposure and 2) the peak amount of pain and stress they felt *during* the stress manipulation.

3.2.7 Behavioral Tasks

Behavioral tasks began after collection of Saliva Sample 2, or approximately 18 minutes after stressor onset. The delay between stressor onset and commencement of behavioral tasks ensured participants were experiencing peak cortisol responses during the tasks. Other studies have shown that peak cortisol responses are observed between 15-45 minutes after stressor onset (Kern et al., 2008; Kirschbaum et al., 1999; Kirschbaum et al., 1992; Kudielka, Buske-Kirschbaum, et al., 2004; Kudielka et al., 1998; Kudielka & Kirschbaum, 2005; Kudielka et al., 1999; Kudielka, Schommer, et al., 2004). In the time between completing the questionnaires and collection of the second saliva sample, participants completed a word search as a filler task until 14 minutes had passed since stressor onset.

3.2.8 Emotional Memory Task: Encoding Phase

We used the same task used in Mather et al. (under review) to assess whether emotional items were remembered differently depending on estradiol levels and cortisol levels. Participants viewed pictures of positive, negative, and neutral valence shown in different locations on a computer screen during an encoding phase. After viewing the pictures participants completed a working memory task, followed by a free recall test for the pictures they viewed during the encoding phase, and then tested for their memory of picture-location associations.

Participants viewed pictures of negative, positive, or neutral valence on a computer screen. They viewed 24 pictures in total, 12 emotional and 12 neutral. Of the 12 emotional pictures, 6 were negative and 6 were positive. Of the 12 neutral pictures, 6 were neutral versions of negative photos, and 6 were neutral versions of positive photos. Each participant only saw either the negative, positive, or neutral version of each picture. Eight versions of the task were created and each participant viewed four versions. Of the four versions each participant viewed,

none of the negative and neutral or positive and neutral photo pairs overlapped. Photos were presented on different locations of the screen. Each photo was presented for 2000ms. Between each individual photograph, subjects were shown a yellow or green dot and indicated the color of the dot by key press on a keyboard. The dot task helped ensure participants were paying attention to the screen throughout the duration of the task. Participants were tested for their recall of the photos as well as for photo-location associations after completing the working memory task. On average, this portion of the task began 18 minutes after stressor onset and took approximately 2 minutes to complete. Participants viewed different pictures at each session. The versions of the task viewed across sessions were counterbalanced.

3.2.9 Working Memory Task

The sentence span task was used to assess working memory. In this task, sentences were shown one at a time on the computer screen and participants were asked to make their best judgment as to whether the sentence "makes sense" or was "nonsense" **and** to remember the last word of every sentence presented within a given block. "Makes sense" and "Nonsense" judgments were recorded by key press on a computer keyboard, while word recall was recorded on a paper scoring sheet by the experimenter.

Sentences were collected from various sources and have been used in other reading or sentence span tasks (Copeland & Radvansky, 2001; Daneman & Carpenter, 1980; N. P. Friedman & Miyake, 2004). Nonsense sentences were created as done in Turner and Engle (1989), "[Semantically and Syntactically] 'Incorrect' sentences were made nonsense by reversing the order of the last four…preterminal words e.g. 'The grades for our finals will be posted outside the classroom door' to 'The grades for our finals will be classroom the outside posted door'". Sentences were presented on the center of the screen with the last word in all

capital letters (e.g. The boy said HELLO), for a duration of 4 seconds. At the end of the 4-second sentence presentation, the screen changed and displayed “Makes Sense” and “Nonsense”, on the left and right side of screen, respectively. Subjects made a key press indicating whether they thought the just-viewed sentence made sense or did not. This was followed by a 500ms inter-trial interval, and then presentation of the next sentence. Participants completed 13 blocks in total. Blocks 1 and 2 were practice blocks and consisted of a 1-sentence load and 2-sentence load, respectively. At the end of each load the subject was prompted to tell the experimenter the last word of the 1 or 2 sentences they just viewed. The main portion of the task included 4 blocks at the 2-sentence load, 3 blocks of the 3-sentence load, and 2 blocks each of the 4-, 5-, and 6-sentence loads. At the end of each block participants were prompted to tell the experimenter the last word of the 2, 3, 4, 5, or 6 sentences they just viewed, in the order they were presented in.

Analyses were conducted using a lenient scoring criterion. The lenient scoring criterion involved giving women 1 point for each word they remembered whether or not they recalled the words in the order presented or remembered every word in a given block. On average, this task began approximately 23 minutes after stressor onset and took approximately 11 minutes to complete. Participants saw different sentences at each session.

3.2.10 Emotional Memory Task: Recall Phase

At least thirty-five minutes following stress onset, participants were tested for their free recall of the photos presented during the encoding phase of the emotional memory task. On average, this portion of the emotional memory task began 36 minutes after stressor onset and took approximately 3 minutes to complete.

3.2.11 Emotional Memory Task: Association Test Phase

Immediately following the recall portion of the emotional memory task, participants were tested for their memory of where photos were presented during the encoding phase of the emotional memory task. Subjects viewed the same picture displayed on two different locations of the computer screen, simultaneously, and indicated the correct location of the picture by key press. Participants were only shown the same pictures they viewed during the encoding phase of that same session. On average, this portion of the emotional memory task began approximately 40 minutes after stressor onset and took approximately 1.5 minutes to complete.

3.2.12 Statistics

Pearson's correlation analyses were conducted on questionnaire responses and cortisol levels. Specifically, positive affect, negative affect, CES-D scores, PMS symptoms, pre and post water stress ratings, and pre and post water pain ratings were tested for correlations with baseline cortisol levels. T-tests were also conducted on these measures to examine potential differences between the two phases. Since all measures, except the CES-D and WTAR, were completed before the participant placed their hand in water or knew what water condition they were receiving, T-tests were conducted on the first session of each phase. CES-D and WTAR scores were compared across the first session of each phase, as they were considered stable enough to not be affected by stress exposure.

Analyses of variance (ANOVA) were conducted for effects of menstrual cycle phase and stress on cortisol response, working memory performance, recall of emotional and neutral pictures, and picture-location associations. Significance was set at $p \leq 0.05$.

Analyses for working memory performance were conducted for the proportion of words recalled within each load and the overall proportion of words recalled (collapsed across loads).

Analyses for the emotional memory recall test and emotional memory picture-location association test also were conducted for the proportion of the pictures and locations remembered within each valence and collapsed across valences.

3.3 Results

3.3.1 Participants: Demographic Information

Young women were between the ages of 18 and 24 (average age: 20.77 years) and had between 12 and 18 years of education (average years of education: 14.79). Ethnic and racial breakdown was 77.8% non-Hispanic and 22.2% Hispanic, 55.6% Asian, 18.5% Caucasian, 7.4% biracial, 14.8% other, and 3.7% declined to state. Participants were primarily undergraduate or graduate students at the University of Southern California, or just recently graduated with degrees in Occupational Therapy (7.4%).

3.3.2 Correlation Analyses between Cortisol Levels and Questionnaire Responses

We first conducted Pearson's correlation analyses looking at relationships between baseline cortisol levels during the first EF and LF phase sessions and questionnaire responses during the first EF and LF phase sessions. The only relationship found during the first session of the EF phase was a positive relationship between baseline cortisol levels and the PMS symptom of experiencing a greater inability to sleep ($r=.435$, $p=.023$). The only relationship found during the first session of the LF phase was a positive relationship between baseline cortisol levels and scores on the CES-D ($r=.405$, $p=.036$).

We next conducted Pearson's correlation analyses looking at relationships between baseline cortisol levels during the stress session of the EF and LF phases and questionnaire responses during the stress session of the EF and LF phase. During the stress session of the EF phase there was no relationship between baseline cortisol levels and positive affect, negative

affect, depression scores, pre and post stress onset pain ratings, or pre and post stress onset stress ratings. Neither was there a relationship between baseline cortisol levels and the following symptoms of PMS: 1) depressed mood, 2) overeating or food cravings, 3) changes in sleep patterns related to sleeping more, 4) feeling overwhelmed or out of control, 5) experiencing more frequent emotional mood swings, 6) decreased interest in activities, 7) feeling lethargic, easily fatigued, or having a lack of energy, or 8) breast tenderness, bloating, or water retention. However, baseline cortisol levels were positively correlated with the following symptoms of PMS: 1) feeling tense, restless, or anxious ($r=.400$, $p=.039$), 2) feeling more irritable or hostile ($r=.434$, $p=.024$), 3) difficulty concentrating ($r=.404$, $p=.037$), and 4) changes in sleep patterns related to being unable to sleep ($r=.411$, $p=.033$). There were no relationships between baseline cortisol and any measures during the stress session of the LF phase.

We next conducted Pearson's correlation analyses looking at relationships between baseline cortisol levels during the control sessions of the EF and LF phases and questionnaire responses during the control sessions of the EF and LF phase sessions. The pattern was similar to that seen for the stress session. During the control session of the EF phase there was no relationship between baseline cortisol levels and positive affect, negative affect, depression scores, or pre- and post-stress onset pain ratings. Neither was there a relationship between baseline cortisol levels and the following symptoms of PMS: 1) depressed mood, 2) overeating or food cravings, 3) changes in sleep patterns related to sleeping more, 4) feeling overwhelmed or out of control, 5) experiencing more frequent emotional mood swings, 6) decreased interest in activities, 7) feeling lethargic, easily fatigued, or having a lack of energy, 8) feeling tense, restless, or anxious, 9) difficulty concentrating, or 10) breast tenderness, bloating, or water retention. However, baseline cortisol levels were positively correlated with the following

symptoms of PMS: 1) feeling more irritable or hostile (r=.437, p=.023), 2) changes in sleep patterns related to being unable to sleep (r=.401, p=.038), as well as pre stress onset stress ratings (r=.435, p=.023) and post stress onset stress ratings (r=.463, p=.015). There were no relationships between baseline cortisol and any measures during the control session of the LF phase.

3.3.3 T-tests between EF and LF phases on Questionnaire Responses

Comparisons between the first session of the EF phase and first session of the LF phase revealed no differences between phases with exception of 2 PMS symptoms: 1) feeling lethargic, easily fatigued, and/or having a lack of energy (t=3.045, df=26, p=.005) and 2) breast tenderness, bloating, and/or water retention (t=3.257, df=26, p=.003). There was marginal difference in feeling overwhelmed or out of control (t=1.936, df=26, p=.064). In all three cases, ratings were higher during the EF phase than the LF phase.

We next compared responses on measures during the stress and control sessions with the EF or LF phases. During stress and control sessions of the EF phase, women only differed on their post stress onset pain ratings (t=14.496, df=26, p<.001) and post stress onset stress ratings (t=10.156, df=26, p<.001). Likewise, during the stress and control sessions of the LF phase, women only differed on their post stress onset pain ratings (t=15.058, df=26, p<.001) and post stress onset stress ratings (t=10.637, df=26, p<.001). See table 3.2.

3.3.4 Subjective Ratings of Stress and Pain Immediately Before and After Stress Exposure

We conducted a 2 (stress: cold v warm) x 2 (time: pre v post stress) x 2 (phase: EF v LF) within subjects ANOVA on pre and post stress onset subjective stress ratings. The analysis revealed no main effect of phase, but did reveal a main effect of stress (F=79.597, p<.001), with higher subjective stress ratings during the stress sessions than the control sessions, and a main

effect of time (F=17.280, p<.001), with lower pre stress onset stress ratings than post-stress onset stress ratings. There were no phase x stress, phase x time, or three-way phase x stress x time interactions. There was, however, a stress x time interaction (F=121.972, p<.001; see figure 3.1), with subjective stress ratings increasing during the stress sessions, and decreasing during the control sessions. Follow up analyses revealed that the increase in stress ratings during the stress session was significant in both phases (EF: t=-7.005, df=26, p<.001; LF: t=-6.555, df=26, p<.001), and the decrease in stress ratings during the control session was significant in both phases (EF: t=5.122, df=26, p<.001; LF: t=4.058, df=26, p<.001).

We conducted a similar 2 (stress: cold v warm) x 2 (time: pre v post stress) x 2 (phase: EF v LF) within subjects ANOVA on pre and post stress onset subjective *pain* ratings. Unlike the subjective stress ratings, this analysis did reveal a main effect of phase (F=5.284, p=.030), with women reporting higher pain during the EF phase than during the LF phase, as well as a main effect of stress (F=224.057, p<.001), with higher subjective pain ratings during the stress sessions than the control sessions, and a main effect of time (F=361.497, p<.001), with lower pre stress onset pain ratings than post stress onset pain ratings. However, like the stress ratings there were no phase x stress, phase x time, or three-way phase x stress x time interactions. There was, however, a stress x time interaction (F=298.766, p<.001; see figure 3.2), with subjective pain ratings increasing during the stress session, and slightly decreasing during the control session. Follow up analyses revealed that the increase in pain ratings during the stress session was significant in both phases (EF: t=-16.027, df=26, p<.001; LF: t=-15.378, df=26, p<.001), while the decrease in pain ratings during the control session was nonsignificant in both phases (EF: t=.851, df=26, p=.402; LF: t=.086, df=26, p=.932).

3.3.5 Cortisol Response during the EF and LF phases

A 2 (stress: cold v warm) x 2 (phase: EF v LF) within-subject ANOVA comparing baseline cortisol levels between the EF and LF phases during the stress and control sessions, revealed that baseline cortisol levels were comparable during the stress and control sessions ($F=.535$, $p=.471$). The analysis also failed to find a stress x phase interaction ($F=.423$, $p=.521$), indicating women had comparable baseline levels of cortisol at each session during both the EF and LF phases.

A 2 (stress: cold v warm) x 3 (time: baseline v 15 minutes post stress onset v 40 minutes post stress onset) x 2 (phase: EF v LF) within subject ANOVA on cortisol levels revealed only a marginal effect of stress ($F=3.326$, $p=.08$; see figure 3.3), but a main effect of time ($F=5.757$, $p=.006$; see figure 3.4), such that cortisol levels increased from baseline to 15 minutes post stress onset then deceased again from 15 minutes post stress onset to 40 minutes post stress onset. Further, the significant stress x time interaction ($F=10.04$, $p<.001$; see figure 3.5), revealed that the pattern of cortisol changes differed between stress and control sessions, with cortisol levels increasing from baseline to 15 minutes post stress onset in the stress session, then decreasing from 15 minutes post stress onset to 40 minutes post onset, but slightly decreasing during each time point in the control sessions. However, this analysis failed to find a main effect of phase, or phase x stress, phase x time, or phase x stress x time interactions.

We then conducted a follow-up 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) x 2 (phase: EF v LF) within subject ANOVA on cortisol levels. This reduced design revealed no main effect of stress ($F=2.916$, $p=.1$), but a main effect of time ($F=8.912$, $p=.006$), such that cortisol levels increased from baseline to 15 minutes post stress onset. Further, the significant stress x time interaction ($F=20.91$, $p<.001$), revealed that the pattern of cortisol

changes differed between stress and control sessions, with cortisol levels increasing from baseline to 15 minutes post stress onset in the stress session, and slightly decreasing from baseline to 15 minutes post stress onset in the control sessions. However, this analysis also failed to find a main effect of phase, or phase x stress, phase x time, or phase x stress x time interactions.

Lastly, we conducted further analyses on only the stress or control session, and only the EF or LF phase. A 2 (phase: EF v LF) x 2 (time: baseline v 15 minutes post stress onset) analysis for the stress session only revealed no effect of phase or a phase x time interaction, but did reveal an effect of time ($F=17.528$, $p<.001$). No effects were found for the same analysis during the control sessions. We then conducted a 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) analysis for the EF phase only. This analysis revealed no main effect of stress (see figure 3.6), but did reveal a main of time ($F=4.556$, $p=.042$) and a stress x time interaction ($F=4.431$, $p=.045$; see figure 3.7), with cortisol increasing from baseline to 15 minutes post stress onset during the stress session, but slightly decreasing across the two time points during the control session. Follow-up analyses on cortisol levels at baseline and 15 minutes post stress onset, however, failed to reveal differences (baseline: $t=-.637$, $df=26$, $p=.530$; 15 minutes post onset: $t=1.145$, $df=26$, $p=.263$).

We next conducted a 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) analysis for the LF phase only. Unlike the EF comparisons, this analysis did reveal a main effect of stress ($F=7.274$,$p=.012$; see figure 3.8), as well as a main effect of time ($F=6.878$, $p=.014$), and a stress x time interaction ($F=11.763$, $p=.002$; see figure 3.9), with cortisol increasing from baseline to 15 minutes post stress onset during the stress session, but slightly decreasing across the two time points during the control session. Follow-up analyses on cortisol

levels at baseline and 15 minutes post stress onset, did reveal differences at 15 minutes post stress onset, but not at baseline (baseline: t=-.432, df=26, p=.670; 15 minutes post onset: t=3.827, df=26, p=.001).

3.3.6 Working Memory: Word Recall

A 2 (phase: EF v LF) x 2 (stress: cold v warm) x 5 (load: 2 sentences through 6 sentences) within subject ANOVA revealed only a main effect of load (F=53.226, p<.001), but no main effect phase or stress, nor any interactions. Neither were any effects of phase or stress found when a 2 (phase: EF v LF) x 2 (stress: cold v warm) within subject ANOVA was conducted on overall word recall (i.e., collapsed across loads).

3.3.7 Emotional Memory: Free Recall of Emotional and Neutral Pictures

Within subject ANOVAs were conducted on phase, stress, and valence. Valence comparisons were conducted for negative images versus neutral images, positive images versus neutral images, negative images versus positive images, and emotional (negative and positive) images versus neutral images.

A 2 (phase: EF v LF) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) within subject ANOVA on picture recall revealed main effects of phase (F=4.683, p=.040; see figure 3.10), with women recalling more pictures during the LF phase than the EF phase, and valence (F=25.950, p<.001), with more emotional pictures recalled than neutral. However, the main effect of stress was only marginal (F=3.141, p=.088) with a trend toward higher recall during the stress session than control session. This analysis also failed to find any significant interactions.

We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (phase: EF v LF) x 2 (stress: cold v warm) x 2 (valence: negative

pictures v neutral pictures) within subject ANOVA on picture recall revealed no main effect of stress (F=1.856, p=.185), but did find main effects of phase (F=6.166, p=.020; see figure 3.11) and valence (F=37.357, p<.001; negative > neutral). This analysis also failed to find stress x valence, stress x phase, valence x phase, or overall phase x stress x valence interactions.

Analyses looking at positive versus neutral pictures found no effects of phase, stress or valence, nor phase x stress, stress x valence, or phase x stress x valence interactions. However, the phase x valence interaction was significant (F=4.381 and p=.046; see figure 3.12), with women showing better recall of positive pictures than neutral pictures during the EF phase, but better recall of neutral pictures than positive pictures during the LF phase. Contrasts for negative versus positive pictures revealed no effects of phase or stress, but did reveal a main effect of valence (F=27.283, p<.001), with negative pictures recalled more than positive pictures. The only interaction found to be significant was the phase x valence interaction (F=8.074, p=.009; see figure 3.13), with the difference between the proportion of negative pictures recalled versus positive pictures being larger during the LF phase than the difference between recall of negative and positive pictures during the EF phase.

3.3.8 Emotional Memory: Memory for Picture Location

ANOVAs were conducted on phase, stress, and valence. Valence comparisons were conducted for negative images versus neutral images, positive images versus neutral images, negative images versus positive images, and emotional (negative and positive) images versus neutral images.

A 2 (phase: EF v LF) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) within subject ANOVA on memory for picture location revealed no main effects of phase, stress, or valence. Nor did we find any phase x stress, phase x valence, or phase x stress x

valence interactions. The stress x valence interaction, however, was significant (F=4.791, p=.038; see figure 3.14), with better memory for where neutral pictures were presented than where emotional pictures were presented during the stress session, but better memory for where emotional pictures were presented than for where neutral pictures were presented during the control session.

We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (phase: EF v LF) x 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) within subject ANOVA on memory for where pictures were presented during encoding revealed a main effect of phase (F=4.303, p=.048; see figure 3.15), with better memory for location of presentation during the LF phase than during the EF phase. There was, however, no main effect of stress or valence. This analysis also failed to find phase x stress, phase x valence, or phase x stress x valence interactions. However, the analysis did reveal a marginally significant stress x valence interaction (F=4.166, p=.052; see figure 3.16) with better memory for where neutral pictures were presented than where negative pictures were presented during the stress session, but better memory for where negative pictures were presented than where neutral pictures were presented during the control sessions.

Analyses looking at positive versus neutral pictures found no main effects of phase, stress, or valence, nor any phase x stress, phase x valence, stress x valence, or phase x stress x valence interactions. Contrasts for negative versus positive pictures also failed to find any main effects or interactions.

3.3.9 Cortisol Response between Responders and Nonresponders during the EF and LF phases

Because we failed to observe effects of stress on working memory we ran analyses on "responders" versus "nonresponders", as well as responders only and nonresponders only for

cortisol levels, working memory, and emotional memory. Responders (n=19 in both phases) were defined as women who experienced any increase in cortisol from baseline to 15 minutes post stress onset during the stress session. Nonresponders (n=8 in both phases) were defined as women who showed no change, or a decrease, in cortisol levels from baseline to 15 minutes post stress onset during the stress session. Because the responders and nonresponders did not remain the same across phases analyses were run separately for the EF and LF phases.

A 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) x 2 (response: responders v nonresponders) within-by-between subject ANOVA on cortisol levels during the EF phase only revealed no main effects of response category, stress, or time. Nor did it reveal stress x response or stress x time interactions. The response x time interaction, however, was significant ($F=13.38, p=.001$), showing that when collapsed across stress conditions responders experienced an increase between the two time points, while nonresponders experienced a decrease. The analysis also revealed a marginal overall response x stress x time interaction ($F=3.941$, $p=.058$, see figure 3.17), suggesting the responders and nonresponders exhibited a different pattern of cortisol responses during stress and control sessions.

A similar 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) x 2 (response: responders v nonresponders) within-by-between subject ANOVA on cortisol levels during the LF phase only revealed main effects of response ($F=4.333$, $p=.048$), with responders exhibiting higher cortisol levels than nonresponders, and stress ($F=6.147$, $p=.02$) with higher cortisol levels during the stress session than control session, but no main effect of time. Unlike the EF phase, only the response x stress interaction was found to be nonsignificant, suggesting that both responders and nonresponders exhibited similar patterns of cortisol levels between stress and control sessions. The response x time interaction ($F=9.259$, $p=.005$) revealed that

when collapsed across stress conditions responders experienced an increase between the two time points, while nonresponders experienced a decrease.

The stress x time interaction ($F=5.287$, $p=.03$) revealed that cortisol levels increased from baseline to 15 minutes post stress onset during the stress session, and very slightly decreased during the control session. The analysis also revealed an overall response x stress x time interaction ($F=18.301$, $p<.001$; figure 3.18), suggesting the responders and nonresponders exhibited a different pattern of cortisol responses between time points during stress and control sessions.

We then looked at responders and nonresponders during the stress session only within each phase. For the EF phase, a 2 (response: responders v nonresponders) x 2 (time: baseline v 15 minutes post stress onset) between-by-within subject ANOVA revealed a main effect of response ($F=21.936$, $p<.001$), with responders exhibiting higher cortisol levels than nonresponders, but only a marginal effect of time ($F=3.716$, $p=.065$), with a trend toward increasing from baseline to 15 minutes post stress onset. The response x time interaction, however, was significant ($F=21.936$, $p<.001$; see figure 3.19), with responders exhibiting an increase between the two time points and nonresponders exhibiting a decrease. The same analysis for the LF phase revealed a main effect of response ($F=17.774$, $p<.001$), with responders exhibiting higher cortisol levels than nonresponders, and a main effect of time ($F=4.769$, $p=.039$), with cortisol increasing from baseline to 15 minutes post stress onset. The response x time interaction also was significant ($F=17.774$, $p<.001$; see figure 3.20), with responders exhibiting an increase between the two time points and nonresponders exhibiting a decrease. These same analyses conducted for the control sessions found no main effects of response, time, or any response x time interactions in either the EF or LF phases.

We next looked at responders during the stress and control sessions within each phase. For responders in the EF phase, a 2 (stress: cold v warm) x 2 (time: baseline v 15 minutes post stress onset) within subject ANOVA revealed no main effect of stress (F=.133, p=.719), with cortisol levels being only slightly higher during the stress session than during the control session, but there was a main effect of time (F=15.819, p=.001), with cortisol increasing from baseline to 15 minutes post stress onset. The stress x time interaction also was significant (F=6.552, p=.02), with cortisol significantly increasing between the two time points during the stress session (F=29.502, p<.001) and slightly but nonsignificantly decreasing during the control session (F=.03, p=.865; see figure 3.21). The analysis for the LF phase found a marginal effect of stress (F=3.567, p=.075), with a trend toward higher cortisol levels during the stress session than the control session, but there was a main effect of time (F=12.942, p=.002), with cortisol increasing from baseline to 15 minutes post stress onset. The stress x time interaction also was significant (F=33.122, p<.001), with cortisol significantly increasing between the two time points during the stress session (F=27.905, p<.001) and numerically but nonsignificantly decreasing during the control session (F=.352, p=.56; see figure 3.22).

3.3.10 Working Memory: Word Recall in Responders versus Nonresponders during the EF and LF phases

Because working memory analyses collapsed across responders and nonresponders failed to reveal any effects, we next split women into groups of responders (n=19 in both phases) and nonresponders (n=8 in both phases) and conducted between-by-within analyses on working memory performance during the EF and LF phases separately. We first ran a 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 5 (load: 2 sentences through 6 sentences) between-by-within subject ANOVA for the EF phase. This analysis failed to find

main effects of response or stress, but did find a main effect of load ($F=21.878$, $p<.001$) with the proportion of words correctly recalled decreasing as loads increased in difficulty. Further, interactions of response x load and stress x load were found to be nonsignificant. However, the response x stress interaction was marginal ($F=3.923$, $p=.059$; see figure 3.23), with responders exhibiting worse performance during the stress session than control session and nonresponders showing better performance during the stress session than control session. Similarly, the overall response x stress x load interaction was marginal ($F=2.142$, $p=.081$), suggesting the different patterns of performance between the stress and control sessions in responders differed from the different patterns of performance between the stress and control sessions in nonresponders. This same analysis for stress and control sessions during the LF phase, failed to find any significant or marginal main effects or interactions, with the exception of load ($F=22.489$, $p<.001$).

We next ran the same contrast but only looking at responders in the EF and LF phase. Here, a 2 (stress: cold v warm) x 5 (load: 2 sentences through 6 sentences) within subject ANOVA completed on responders during the EF phase revealed a marginal main effect of stress ($F=7.19$, $p=.07$; see figure 3.24), with worse performance during the stress session than the control session, and an effect of load ($F=19.117$, $p<.001$). The stress x load interaction, however, was nonsignificant. A similar contrast looking at responders in the LF phase revealed a different pattern of effects. While there was still a main effect of load ($F=22.235$, $p<.001$), there was a robust nonsignificant effect of stress ($F=.002$, $p=.962$; see figure 3.25) and no stress x load interaction, suggesting that despite showing a larger, albeit nonsignificant, cortisol response to the stressor during the LF phase, women failed to experience stress-induced interference of working memory performance. These same analyses examining patterns in nonresponders revealed only effects of load (EF: $F=7.056$, $p<.001$; LF: $F=7.05$, $p<.001$). Failure to find effects

of stress or stress x load interactions are not surprising given these women did not exhibit a cortisol response to the stressor.

3.3.11 Emotional Memory: Free Recall of Emotional and Neutral Pictures in Responders versus Nonresponders during the EF and LF phases

Although we did observe effects in the overall analyses collapsed across responders and nonresponders, we conducted within-by-between analyses examining picture recall in responders versus nonresponders to remain consistent with the cortisol and working memory analyses. Mixed design ANOVAs were conducted on phase, stress, and valence between responders and nonresponders during the EF and LF phases separately. Valence comparisons were conducted for negative images versus neutral images, positive images versus neutral images, negative images versus positive images, and emotional (negative and positive) images versus neutral images.

A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) between-by-within subject ANOVA on picture recall during the EF phase only revealed a main effect of valence ($F=11.031$, $p=.003$), with better recall of emotional pictures than of neutral pictures. We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) within-by-between subject ANOVA on picture recall also failed to find any main effects or interactions, with the exception of valence ($F=13.017$, $p=.001$), with better recall of negative pictures than neutral pictures. Analyses looking at positive versus neutral pictures, and negative versus positive pictures, in responders versus nonresponders found no effects of phase, stress, valence, or any interactions.

A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) within-by-between subject ANOVA on picture recall during the LF phase only revealed only a main effect of valence ($F=7.442$, $p=.011$), with better recall of emotional pictures than neutral pictures, and no other effects or interactions. We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) between-by-within subject ANOVA on picture recall also failed to find any main effects or interactions, with the exception of valence ($F=18.215$, $p<.001$), with better recall of negative pictures than neutral pictures. Analyses looking at positive versus neutral pictures in responders versus nonresponders found no effects of phase, stress, valence, or any interactions. However, comparisons between negative and positive pictures found a main effect of valence ($F=21.359$, $p<.001$), with better recall of negative pictures than positive pictures, but no other main effects or interactions.

3.3.12 Emotional Memory: Memory for Picture Location in Responders versus Nonresponders during the EF and LF phases

Similar to the cortisol and working memory analyses, we then conducted within-by-between analyses examining memory for location of picture presentation in responders versus nonresponders. Mixed ANOVAs were conducted on phase, stress, and valence between responders and nonresponders in the EF and LF phases separately. Valence comparisons were conducted for negative images versus neutral images, positive images versus neutral images, negative images versus positive images, and emotional (negative and positive) versus neutral images.

A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) between-by-within subject ANOVA on memory for picture location during the EF phase revealed no main effects or interactions. We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) between-by-within subject ANOVA on memory for picture location also failed to find any main effects or interactions. Analyses looking at positive versus neutral pictures and negative versus positive pictures also failed to reveal any main effects or interactions.

A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: emotional pictures v neutral pictures) between-by-within subject ANOVA on memory for picture location during the LF phase revealed no main effects or interactions. We next conducted follow-up analyses for each valence of picture presented: negative, positive, and neutral. A 2 (response: responders v nonresponders) x 2 (stress: cold v warm) x 2 (valence: negative pictures v neutral pictures) between-by-within subject ANOVA on memory for picture location also failed to find any main effects or interactions. However, analyses looking at positive versus neutral pictures in responders versus nonresponders found a response x stress interaction ($F=5.909$, $p=.023$; see figure 3.26), with responders showing better location memory during the control session than the stress session and nonresponders showing better location memory during the stress session than the control session. No other main effects or any interactions were found. Comparisons between negative pictures and positive pictures revealed no main effects or interactions for memory of picture locations.

3.4 Discussion

Study 2 aimed to determine if estradiol fluctuations during the menstrual cycle mimic effects observed in post-menopausal women in study 1. Of interest was whether changes in estradiol levels affected the stress response and/or working memory and emotional memory performance during stress in the same manner observed in postmenopausal women taking estradiol or placebo in study 1. We hypothesized that the fluctuations of estradiol during the first half of the menstrual cycle would result in the same pattern of effects observed in study 1. Our first hypothesis stated that women would display a blunted cortisol response to an ice water stressor during the high estradiol LF phase compared to the response exhibited during the low estradiol EF phase. The results of this study do not strongly support this hypothesis. The notion that higher estradiol levels would minimize cortisol release created the foundations for the second and third hypotheses, such that reductions in cortisol release should mitigate the effects of stress on other domains because less GC would be available to act on and affect other processes. Following this reasoning, our second hypothesis stated that women would show less stress-induced *interference* of working memory during the high estradiol LF phase than the low estradiol EF phase. Given that the cortisol response results did not support an attenuated cortisol response to an ice water stressor it was unclear whether or not hypothesis 2 would be supported. However, our results do support this hypothesis, with responders during the LF phase failing to show a decrease in performance during the stress session and EF responders exhibiting worse performance during the stress session. The third hypothesis focused on how estradiol treatment might enhance the effects of stress on emotional memory. We predicted women would show less stress-induced *enhancement* of emotional memory during the high estradiol LF phase than during the low estradiol EF phase, however while the emotional memory results did reveal an effect of

phase, there were no phase-by- stress interactions, even when looking at responders versus nonresponders – thus not supporting our third hypothesis.

3.4.1 Differences in Subjective Ratings of Pain

Pre and post stress onset stress ratings did not differ between the 2 phases. However, there was a main effect of phase when looking at pre and post stress onset *pain* ratings. The difference in the overall pain ratings was in the direction of greater pain during the EF phase than the LF phase. Given the within subject nature of our experimental design, this difference seems to be a result of the phase, rather than a confounding factor like cohort effects. The LF phase has been associated with higher pain thresholds relative to the EF phase in previous studies (Hellström & Anderberg, 2003; Riley III, E. Robinson, Wise, & Price, 1999), this may be related to the higher ratings of PMS symptoms during the EF phase in this study. The general level of discomfort experienced during menstruation may make women more sensitive to other forms somatic pain, as would be experienced by holding one's hand in ice water for an extended period of time.

3.4.2 Cortisol Response to a Stressful Event during the EF and LF phases

Our stress manipulation failed to result in a main effect of stress, suggesting no difference in cortisol levels between the stress and control sessions, although we did observe a stress-by-time interaction with increases during the stress session and decreases during the control session. Yet, follow up analyses on just the EF and LF phases alone revealed a main effect of stress for the LF phase, with higher cortisol levels during the stress session than the control session, but only an effect of time and a stress-by-time interaction during the EF phase. Failure to find consistent stress effects with the cold pressor is not unheard of, nor the first time to occur in our lab (Clewett, Schoeke, & Mather, 2013). However, given that the majority of studies conducted

in our lab to employ the cold pressor task result in reliable increases in cortisol levels (Lighthall et al., 2009; Lighthall et al., 2011; Mather et al., 2010), we decided to examine differences in responders and nonresponders.

These analyses did reveal different patterns between phases, but not in line with our hypotheses. We hypothesized that cortisol responses would be larger during the EF phase than the LF phase, however, when analyses focused on responders only there was a robust nonsignificant effect of stress on overall cortisol levels during the EF phase, but a marginal effect of stress during the LF phase. This pattern suggests that cortisol levels increased more during the LF phase than during the EF phase. While our original overall analyses failed to find a significant difference between cortisol levels at baseline and at 15m post stress onset, a paired samples t-test comparing the 13 women classified as responders during both phases did show a significant difference between the change score from baseline to 15m post stress onset ($t=-2.889$, $df=12$, $p=.014$; means: change for EF=.0635 μg/dl, change for LF=.1492 μg/dl).

Finding that cortisol levels increased more during the LF phase is surprising given the evidence that women show less HPA activation in response to a stressor than men, presumably partially due to female sex hormones (Davis & Emory, 1995; Kirschbaum et al., 1999; Kirschbaum et al., 1992; Kudielka, Buske-Kirschbaum, et al., 2004; Kudielka et al., 1998). This pattern, however, is in direct opposition to the animal literature which shows that females show greater HPA activation to a stressor than males, in both rodents (Handa, Burgess, Kerr, & O'Keefe, 1994; Viau & Meaney, 1991) and non-human primates (Roy, Reid, & Van Vugt, 1999). This effect in the animal literature may be a result of estradiol's ability to upregulate CRH expression (Lalmansingh & Uht, 2008), which should cause a greater release ACTH and glucocorticoids.

This mechanism may have contributed to the higher cortisol release observed during the LF phase. Unfortunately, we cannot investigate this until the saliva samples collected for this study have been analyzed for estradiol levels. In this case, estradiol and cortisol should be positively correlated, as the higher estradiol levels would increase CRH expression leading to more CRH available for release when faced with a stressor, followed by increased downstream HPA activity and greater cortisol release.

Another possibility is that our LF women were already experiencing an increase in progesterone, making their phase profile look more similar to the luteal phase. In this instance, we would expect a greater cortisol response, as women often exhibit greater cortisol responses to a stressor during the luteal phase (Andreano et al., 2008; Kirschbaum et al., 1999). Again, however, we cannot investigate this further until the saliva samples are analyzed for progesterone.

3.4.3 Relationship between Stress and Working Memory during the EF and LF phases

When analyzed irrespective of whether or not women responded to the ice water stressor, we found no effects of stress or phase on working memory performance. However, when running analyses on responders during the EF and LF phases we found different patterns of effects. In these analyses it was found that EF responders performed worse during the stress session than during the control session, but that LF responders performed comparably across sessions. This finding supported our hypothesis that higher estradiol levels would be associated with less stress-induced interference of working memory. This finding is interesting, and a bit unexpected, since women experienced greater cortisol responses to the ice water stressor during the LF phase. Finding that women experienced greater cortisol responses during the LF phase suggests that they should also have experienced greater interference with working memory performance.

That women experienced less stress induced interference during the LF phase when they were experiencing greater cortisol responses suggests that something about the LF phase is protective against these higher cortisol levels. One possibility concerns another effect of estradiol – increasing GC receptor expression in the brain. Thus, it is possible that although higher estradiol levels during the LF phase may increase CRH expression, the higher estradiol levels also should increase glucocorticoid receptor expression in the brain (Ferrini & De Nicola, 1991; Ferrini et al., 1995). The increase in GC receptors should lead to more efficient GC detection and swifter shut down of the stress response despite releasing more cortisol initially. Thus, while the brain regions involved in working memory may be initially exposed to more cortisol, the potential for more rapid reduction of cortisol release due to more efficient detection of higher levels may limit the amount of time cortisol is available to interfere with working memory processes.

Another interesting result our analyses uncovered was a response-by-stress interaction for working memory performance during the EF phase, but not the LF phase. The EF phase interaction indicated that women classified as nonresponders performed better under stress than during the control session, versus responders who performed better during the control session than during stress. One possible explanation refers back to the inverted-U shaped effect of stress on performance first reported by Yerkes and Dodson (1908). Perhaps during the EF phase, the cortisol response displayed by nonresponders was enough arousal to place participants in the range of optimal performance, versus responders, whose cortisol response was too large, causing too much arousal and thereby interfering with performance.

Although there was no response-by-stress interaction during the LF phase, there was a trend towards better performance under stress for nonresponders, and no change in performance

in responders. Interestingly, the effect of response was also marginal, with *nonresponders* trending toward *better* performance than responders in both the stress and control sessions. In this instance, the Yerkes-Dodson principle may still apply. In this scenario, the amount of arousal experienced by the nonresponders during the LF phase may have been near the optimal level to help facilitate performance, similar to that suggested for the EF nonresponders, despite the failure to increase cortisol levels. On the other hand, the increase in cortisol levels in the responders was enough to prevent enhancement of performance, but not enough to hinder performance as observed during the EF phase. This may be another way in which the LF phase protects working memory under stress; that the change in the hormonal profile leads to greater tolerance of stress despite showing larger physiological responses, thereby protecting women from the maladaptive effects of stress exposure on this form of cognition.

3.4.4 Relationship between Stress and Emotional Memory during the EF and LF phases

Analyses examining memory for where pictures were presented during encoding did not reveal many effects, with exception of a main effect of phase when comparing memory for negative versus neutral pictures, and a stress-by-valence interaction for emotional versus neutral pictures. The failure to find more robust effects of stress on this test of emotional memory may be due to the relative ease of the task. While on average the encoding phase of this task occurred 21 minutes prior to the location memory test, this interval may be too short to pick up effects of stress prior to encoding in our sample of women. Recall that each woman only viewed 24 pictures during each session, and that the location memory test consisted of showing only the pictures they viewed during that session both in the correct location and an incorrect location simultaneously. It may be the case that the added cue of viewing the pictures again, with one in

the correct location, provided enough of a cue to aid in recalling location regardless of whether stress was applied prior to encoding or not.

With regard to the main effect of phase, women exhibited better memory for picture presentation location during the LF phase than the EF phase. This effect may reflect overall better memory during the LF phase relative to the EF phase, although other studies report mixed results regarding whether various forms of memory are worse during the EF phase (Phillips & Sherwin, 1992), better during the EF phase (Maki, Rich, & Shayna Rosenbaum, 2002), or whether no difference exists between menstrual cycle phases (Kuhlmann & Wolf, 2005). The stress-by-valence interaction suggested memory for the location of emotional pictures was better than memory for the location of neutral pictures during the control session, and vice versa during the stress session. This is contradictory to typical reports of stress enhancing memory for emotional stimuli (Buchanan & Lovallo, 2001; Payne et al., 2007; Smeets et al., 2006). Because of the failure to find more robust effects of stress, and the contradictory stress-by-valence interaction, we conducted follow-up analyses looking at responders and nonresponders to see whether including nonresponders in the overall analysis muddied the results.

Unfortunately, analyses comparing nonresponders and responders during the EF phase failed to find any effects at all. During the LF phase, the only effect found was a response-by-stress interaction in the positive versus neutral picture comparison, with responders showing better location memory during the control session than the stress session and nonresponders showing better location memory during the stress session than the control session. The sparse significant results in the location memory analyses, despite looking at responders and nonresponders alone, again may be due to the participants finding this particular memory test relatively easy and thus performing well on the test regardless of whether or not they

experienced stress. Further, it may be the case that a stronger stressor would have affected emotional memory. Recall that as tasks become easier greater stress is required to enhance performance (Dodson, 1915), thus it is possible that the ice water stressor did not induce a sufficient amount of stress to affect the relatively easy picture-location test.

Analysis of the free recall test did reveal more effects, perhaps because the greater difficulty of this test was sensitive to the manipulations employed. First, comparing memory for emotional versus neutral pictures did uncover effects of phase and valence, and even a marginal effect of stress, but no interactions. Women recalled more total pictures during the LF phase than during the EF phase and emotionally valenced pictures were better recalled than neutral pictures regardless of phase. Because the emotional versus neutral pictures comparison includes all 24 pictures viewed during encoding, the failure to find a phase-by-valence interaction may indicate that women experience better overall memory during the LF phase than they do during the EF phase, irrespective of content of the pictures. The effect of valence followed the expected pattern of better recall of emotional pictures than neutral pictures, yet the marginal effect of stress trended toward better recall during the stress session than the control session. This pattern is not necessarily what would be expected. Although stress should enhance memory for emotional items, it should not necessarily cause an overall increase in recall of all items. This trend may again be a related to arousal induced enhancement of overall performance as discussed for the stress and working memory interactions. Perhaps the amount of stress experienced was optimal for performance rather than detrimental, leading to slightly better recall rates after stress exposure.

Patterns differed for the more specific valence comparisons. When looking at recall of negative and neutral pictures we only observed effects of phase and valence, with greater recall

during the LF phase than during the EF phase, which may have contributed to the effect observed in the overall analysis. This differed from the analyses comparing recall of positive and neutral pictures. This analysis revealed no main effects, but did uncover a phase-by-valence interaction with women showing better recall of positive pictures than neutral pictures during the EF phase, but showing better recall of neutral pictures than positive pictures during the LF phase. Interestingly, although not significant in every comparison, women numerically recalled more pictures during the LF phase then the EF phase. However, the valence most recalled differed across analyses, with more negative than neutral, more neutral than positive, and more negative than positive pictures recalled during the LF phase. Thus, while the LF phase may be related to better memory, the valence may in the end matter, despite the failure to find significant effects in all comparisons or a phase-by-valence interaction in the overall emotional versus neutral picture comparison. These same analyses comparing responders versus nonresponders did not change the pattern of results.

One possible explanation for the better recall of negative pictures during the LF phase may be related to reports of better recognition of negative facial expressions outside of the high progesterone luteal phase (Derntl, Kryspin-Exner, Fernbach, Moser, & Habel, 2008). The greater recognition of the emotionally valenced items may have made them more salient and therefore more likely to be recalled during the LF phase. However, this assumes that estradiol alone can modulate the ability to recognize and remember emotionally valenced stimuli, which this study cannot attest to. Another possibility is that our LF women were already experiencing increases in progesterone making them exhibit memory performance more commonly reported within the luteal phase – which, counterintuitive to the above-reported increased recognition of negative facial expressions outside of the luteal phase – is characterized as having better memory for

emotional stimuli, particularly negative stimuli (Ertman, Andreano, & Cahill, 2011; Sakaki & Mather, 2012). Again, we are unable to examine this possibility until we have processed the saliva samples for sex hormones levels.

3.4.5 Conclusion

This study suggests that the late follicular phase of the menstrual cycle may exert some form of protective effects against the effects of stress on working memory and may be related to better delayed recall over relatively short intervals regardless of stress exposure. Although, firm conclusions on patterns of emotional memory throughout the menstrual cycle phase currently cannot be made because sex hormone levels are still unknown.

It is unclear how the LF phase may be exerting its effect against stress given women displayed higher cortisol responses to the ice water stressor during this phase. Likewise, the dichotomy of phase and stress interacting for the working memory task, but not the emotional memory task, despite higher cortisol levels, suggests that the way phase protects against stress effects on working memory is different from the way phase and stress each affect emotional memory. This is not entirely surprising since different brain areas govern these two forms of cognition. Further, since estradiol is not reducing the cortisol levels in the phase that experiences some buffering from stress, this rules out one potential mechanism of protection – that higher estradiol levels reduce HPA response to a stressor, but provides possible alternative mechanisms. First, the estradiol levels of our women during their LF phase may alter the speed with which HPA activity is shut down after initiation of hormone release due to GC receptor upregulation. Second, given that 1) different brain regions govern working memory and emotional memory, and 2) it does not appear that higher estradiol levels are able to attenuate initial cortisol release, the actual hormone profile of our LF women (versus the anticipated hormone profile based on

time since the first day of menses) may alter the way cortisol acts on those specific brain regions. Third, rather than changing the action of cortisol directly in those regions, it may be that higher estradiol levels lead to higher binding of estradiol in those same regions and that this higher rate of estradiol action in the brain somehow counteracts cortisol effects. These last alternative explanations could also explain why some forms of cognition experience protection from stress while others seem to be unaffected.

CHAPTER 4

Estradiol differentially alters the effects of stress in post-menopausal and young spontaneously cycling women.

4.1 Introduction

This work set out to investigate the potential protective effects of estradiol against the stress response and effects of stress on working memory and emotional memory in post-menopausal and young spontaneously cycling women. We tested 3 general hypotheses in each study. First, we proposed that higher estradiol levels, either as a result of estradiol supplementation or menstrual cycle phase, would be associated with reduced cortisol response to a stressful event. Second, that higher estradiol levels would be associated with less stress-induced interference of working memory, and third, that higher estradiol levels would be associated with less stress-induced enhancement of emotional memory. In each study we uncovered support for some but not all of our a priori hypotheses.

Our first study, examining the effects of estradiol treatment in post-menopausal women, did reveal that the highest estradiol levels observed in our subsample of ELITE patients was related to significantly blunted cortisol responses to the ice water stressor – supporting our first hypothesis in post-menopausal women. We also found that the women with the highest estradiol levels, who displayed the blunted cortisol response, also were protected from the negative effects of stress on working memory. So much so, that these women failed to show any difference in their performance between stress and control sessions, in contrast with the low estradiol women who displayed significant increases in cortisol, and significantly worse working memory during the stress session – supporting our second hypothesis in post-menopausal women. However,

estradiol treatment did not appear to influence emotional memory in this study, nor did stress – thus not supporting our third hypothesis in post-menopausal women.

Our second study, examining the effects of low and high estradiol during different menstrual cycle phases, failed to support all three hypotheses. In this study, although not significant, cortisol responses to the ice water stressor were greater during the high estradiol LF phase than during the low estradiol EF phase – thus not concretely negating our first hypothesis, but at minimum suggesting the higher estradiol phase might be associated with greater cortisol release in response to a stressor. Surprisingly, despite trending toward greater cortisol release during the LF phase, women experienced protection from stress-induced interference in working memory performance during the high estradiol LF phase – supporting our second hypothesis in young spontaneously cycling women. Lastly, while menstrual cycle phase did seem to influence emotional memory, stress did not do so reliably, and there were no phase-by-stress interactions – thus these findings did not support our third hypothesis.

We had proposed that estradiol would have the same effect in both post-menopausal and younger women. And, in particular, that the variable effects obtained across menstrual cycle studies was a result of looking at phases with such different hormone profiles, rather than isolating specific hormones, which is admittedly difficult. However, after the exciting results of the post-menopause study, we set out to isolate the effects of estradiol as best we could in a sample of young naturally cycling women, which is why we focused on the low-estradiol/low-progesterone, early follicular phase and the high-estradiol/low-progesterone, late follicular phase. Thus, we were surprised that we did not obtain similar effects across the board. Yet, the systems governing these effects in pre- and post-menopausal women likely differ given the physiological differences between the two groups.

4.2 Differences in cortisol response to an ice water stressor in young spontaneously cycling and post-menopausal women

The pattern of cortisol responses between low and high estradiol groups differed in the two studies. In study 1, we observed a estradiol-by-stress interaction where post-menopausal women with the top quarter of estradiol levels (HE) displayed nonsignificant increases in cortisol in response to the ice water stressor, while the post-menopausal women with the bottom quarter of estradiol levels (LE) displayed significant increases in cortisol levels in response to the ice water stressor. In study 2, we did not detect a phase-by-stress interaction. However, the numerical patterns trended toward greater increases in cortisol levels in response to the ice water stressor during the high estradiol phase of the menstrual cycle (LF), while exhibiting smaller increases in cortisol in response to the ice water stressor during the low estradiol phase of the menstrual cycle (EF).

The mechanisms by which estradiol is working may differ in the two populations of women, perhaps as a result of relative HPG axis function. For the postmenopausal women in study 1, the discontinuation of estradiol and progesterone release from the ovaries results in dysregulation of the entire HPG system because of the removal of negative feedback. The absence of ovarian hormones leads to loss of inhibition of GnRH release from the hypothalamus, and increased synthesis and release of LH and FSH (Atwood et al., 2005). Reintroduction of estradiol should reactivate some of the negative feedback lost through menopause, but may not completely reestablish optimal HPG regulation. Although the axis may be partially reestablished, estradiol treatment may not mimic the cyclicity of activation and inhibition observed in young naturally cycling women. This difference in HPG axis activity might change the way the HPG

axis influences HPA activity and thereby change the effects of estradiol levels on the stress response.

One way communication between the axes may differ could be the effects of estradiol on CRH upregulation. The rapid and large increase observed during the LF phase of the menstrual cycle may lead to upregulation of CRH. The greater expression of this releasing hormone would be expected to cause greater release of ACTH and cortisol, causing greater stress reactivity during this phase. In post-menopausal women, estradiol treatment may not elevate E2 levels enough to induce the upregulation of CRH, thus preventing this heightened stress response. The failure to induce increased CRH expression, coupled with the possible increase of CBG limiting the amount of free cortisol available to act on tissue, and the possible increase of GC receptors leading to more efficient and swift shutdown of the HPA axis, may explain why post-menopausal women taking estradiol experience smaller cortisol responses to a stressor than young women in the high estradiol phase of the menstrual cycle.

An additional contributor to these possible differences in HPA-HPG communication is the role of progesterone. Although women in the clinical trial were also given progesterone, nurses anecdotally reported that many women did not comply with the treatment that would be associated with the progesterone (i.e. use of the vaginal cream). On the other hand, the women in study 2 may have already been experiencing rises in progesterone. If this were the case, then the women in study 1 would only be experiencing alterations in estradiol, allowing us to observe only changes in estradiol, whereas the younger women would be experiencing alterations in both estradiol and progesterone, accounting for the numerically larger increase in cortisol during the LF phase and potentially for the different patterns observed across the two studies.

4.3 How estradiol is preventing stress from interfering with working memory in young spontaneously cycling and post-menopausal women

Interestingly, despite displaying different cortisol response profiles, both the HE women from study 1 and women during the LF phase of study 2 experienced protection from stress-induced interference of working memory. That both groups of women experienced protection, despite exhibiting different cortisol response profiles, suggests different mechanisms are responsible for the protection observed in the pre- and post-menopausal women. In the post-menopausal women from study 1, protection from stress-induced interference in working memory could be due to estradiol reducing HPA reactivity to a stressor, thereby reducing cortisol release. Reduced cortisol release means less cortisol would be available to disrupt the brain regions involved in working memory, thus leading to resilience against stress. A possible alternative to this is that estradiol treatment upregulated CBG. In this case, the same amount of cortisol could be released by LE and HE women, but the higher CBG levels in HE women would result in less biologically active cortisol available to act on tissue. Recall that saliva samples only measure free, biologically active, levels of cortisol, so our data cannot speak to which of these mechanisms might contribute to the decreased cortisol levels or protection against stress-induced interference in working memory.

For the young naturally cycling women in study 2, protection from stress-induced interference in working memory is likely due to a different mechanism. One possibility is the potential estradiol-induced upregulation of GC receptors. The greater levels of GC receptors in the brain would lead to more sensitive detection of increases in cortisol levels, leading to more efficient and swift reduction of CRH release and HPA reactivity, despite exhibiting larger cortisol increases initially. Alternatively, the larger cortisol response to the ice water stressor

coupled with the protection of working memory processes could involve an inverted-U shaped function. Resilience against stress in this phase may result in a shift of how much stress one can experience while still remaining in the optimal range for performance, before reaching the tipping point that sends one into a detrimental amount of arousal that impairs performance. This increased resilience could also be a result of the increased GC receptors. In the instance of the inverted U curve of arousal and performance, basal levels of cortisol bind to the type 1, mineralocorticoid receptor, and as cortisol levels increase the hormone binds to the type 2, glucocorticoid receptor. Binding to the type 2 receptor may contribute to stress related dysfunctions since these are the receptors filled during stress and have been associated with decreased performance (Seckl & Olsson, 1995). However, since estradiol has been shown to upregulate both type 1 and type 2 receptors (Ferrini & De Nicola, 1991), women should have more type 1 receptors available to fill during the LF phase before reaching levels requiring activation of the type 2 receptor. This type of mechanism would shift the range of GC receptor occupation related to optimal performance versus detrimental performance.

4.4 Differences in the effects of stress on emotional memory in young spontaneously cycling and post-menopausal women

Patterns of emotional memory also differed between the two studies. Post-menopausal women from study 1 showed no effects of stress or estradiol treatment on emotional memory. Meanwhile, although there were no strong effects of stress on emotional memory in the young women from study 2, effects of phase and phase-by-valence interactions were detected. We found that women correctly recalled a greater number of pictures during the LF phase than during the EF phase and that women recalled more positive pictures than neutral pictures during

the EF phase, but more neutral pictures during the LF phase, and more negative pictures than positive pictures during the LF phase.

For the HE women in study 1, the failure to find effects of stress make sense given their minimal cortisol response to the ice water stressor. However, the significant increase in cortisol observed in the LE women suggests strong effects of stress should have been observed in the emotional memory task. However, while the data did not reveal effects of stress, it is possible that we are seeing effects of the inverted-U relationship between stress and performance. The large stress response in the LE women might have induced too much arousal, leading to robust type 2 GC receptor occupation and activation, leading to detrimental performance. This high-arousal induced reduction in performance would then look similar to the lower performance observed during the control session resulting from not enough arousal, making performance in both sessions look similar.

The failure to observe strong effects of stress in the young women from study 2 may also be attributed to the inverted U function, just as with the LE women from study 1. However, the manner in which the mechanism influences performance would be different. The trend was opposite to what was expected, with recall slightly better during the stress session. In this instance, the amount of stress experienced would have been just enough to enhance overall performance. As for the effects of phase and valence, the better memory performance during the LF phase seemed to be at least partially attributable to greater memory of negative stimuli. This trend toward better memory of negative stimuli may be a result of better recognition of negative stimuli outside of the high progesterone luteal phase (Derntl et al., 2008); the better recognition of the negative stimuli may have made it more salient and thus easier to encode and remember. However, while recognition of negative stimuli is associated with lower progesterone phases of

the menstrual cycle, it is the high progesterone phases that are associated with better memory for emotional stimuli, particularly negative stimuli (Ertman et al., 2011; Sakaki & Mather, 2012). Thus, like the cortisol results, it may be that our LF women were already experiencing increases in progesterone leading to enhanced memory of negative stimuli. Again, unfortunately we cannot speak to this possibility until saliva samples have been analyzed for sex hormones.

4.5 Closing remarks

The results of these studies do suggest that hormone profiles influence the stress response, and can change the way stress affects working memory processing, although the mechanisms by which this is occurring appears to differ between pre- and post-menopausal women. Further work is required in both age groups to better determine how estradiol is exerting its effect in women before and after menopause. Nonetheless, the studies do confirm that sex hormones play an important role in our physiological responses to stressful stimuli and events, as well as to cognition. Further, this work speaks to the importance of continuing this line to research to better understand how these systems interact to sometime protect cognition. Although this work is limited to only adding to the understanding of how changes during the menstrual cycle affect our responses to stress and cognitive processing, the work with post-menopausal women can further inform the medical community on potential effects of estradiol after menopause.

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Appendix A: Tables

Table 2.1: Average timeline for Study 1 sessions

Time (min)	**Time (min) Since Stressor Onset**	**Task**
10	-25	Informed consent/ HIPAA Waiver/Drink water
10	-10	Questionnaires/Rest
5	-5	Baseline saliva sample/Baseline pain and stress rating
3	0-3	Stress manipulation
1	3-4	Post stress manipulation pain and stress rating
10	4-14	Questionnaires and Word Search
3	14-17	Saliva sample 2
2	18-20	Emotional Memory Task, Encoding Phase
11	21-32	Sentence Span Task
4	34-38	Emotional Memory Task, Recall Phase
2	38-40	Emotional Memory Task, Association Test Phase
3	41-44	Saliva sample 3
5	49-54	Debriefing
Total time: 69 (60-80 to account for individual timing differences)		

Table 2.2: Sex hormone levels, demographics, emotional state, and mood

	LE	HE	df	t	p
Estradiol levels	**1.29 pg/ml**	**97.51 pg/ml**	**18**	**-2.936**	**.009**
Progesterone levels	80.12 pg/ml	50.72 pg/ml	18	.443	.663
Age at menopause	50.13	51.38	17	-.677	.507
Age at randomization	59.59	62.03	18	-.665	.514
Years on study drugs	4.94	4.88	18	.376	.711
Age at time of participation	64.88	66.91	18	-.644	.527
Years of education	**16.2**	**17.6**	**18**	**-2.214**	**.040**
WTAR	44.80	45.80	18	-.471	.643
S1– Overall health rating	**7.65**	**8.70**	**18**	**-2.750**	**.013**
S1 – Stress levels on day of session	2.9	1.9	18	1.467	.160
S1 – Stress on day of session compared to normal non-study days	5.0	5.2	18	-2.169	.791
S1 – Positive affect	34.50	30.30	18	1.143	.268
S1 – Negative affect	11.20	10.50	18	1.505	.094
S1 – Pre stress, pain	9.9	1.0	18	1.619	.123
S1 – Pre stress, stress	**9.0**	**2.5**	**18**	**2.652**	**.016**
S1 – CES-D	5.2	2.0	18	1.303	.193
S2 – Overall health rating	**7.9**	**8.8**	**18**	**-3.349**	**.004**
S2 – Stress levels on day of session	2.2	1.5	18	1.481	.156
S2 – Stress on day of session compared to normal non-study days	5.0	4.6	18	.937	.361

S2 – Positive affect	36.4	32.1	18	1.233	.234
S2 – Negative affect	10.8	10.3	18	1.197	.247
S2 – Pre stress, pain	6.8	1.2	18	1.496	.152
S2 – Pre stress, stress	11.0	5.1	18	1.079	.295
S2 – CES-D	6.1	4.0	18	.736	.471

*S1=Session 1, S2=Session 2; Significant values are bolded; the df=17 for age at menopause was due to a missing value from the ELITE data set.

Table 3.1: Average timeline for Study 2 sessions

Time (min)	**Time (min) Since Stressor Onset**	**Task**
10	-10	Informed consent/Drink water/Questionnaire
2	-2	Baseline saliva sample/Baseline pain and stress rating
3	0-3	Stress manipulation
1	3-4	Post stress manipulation pain and stress rating
10	4-14	Word Search
3	14-17	Saliva sample 2
2	18-20	Emotional Memory Task, Encoding Phase
11	21-32	Sentence Span Task
3	35-38	Emotional Memory Task, Recall Phase
1.5	38-40	Emotional Memory Task, Association Test Phase
3	41-44	Saliva sample 3
5	49-54	Debriefing
Total time: 54.5 minutes (41-62 minutes to account for individual timing differences)		

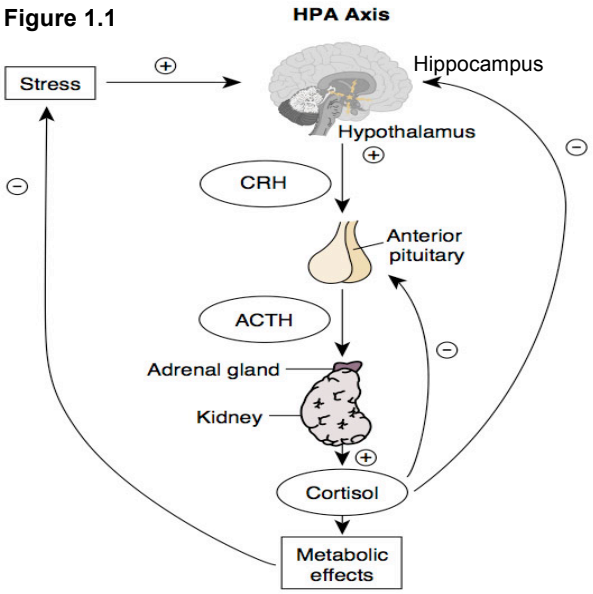
Table 3.2: Emotional state, mood, and PMS symptoms during first session of each phase

	EF	**LF**	**df**	**t**	**p**
Positive Affect	24.26	25.52	26	-.829	.415
Negative Affect	13.48	13.52	26	-.063	.950
CES-D	18.0	17.52	26	.346	.732
Depressed mood – PMTS-VAS	20.07	22.22	26	-.538	.595
Tense, restless, anxious – PMTS-VAS	29.74	32.19	26	-.807	.427
Emotional, mood swings – PMTS-VAS	26.37	27.52	26	-.254	.802
Irritable, hostile – PMTS-VAS	17.74	16.44	26	.382	.706
Decreased interest in activities – PMTS-VAS	24.11	17.37	26	1.557	.132
Difficulty concentrating – PMTS-VAS	28.59	22.22	26	1.416	.169
Lethargy, easy fatigability, lack of energy – PMTS-VAS	**40.11**	**23.89**	**26**	**3.045**	**.005**
Overeating, food cravings – PMTS-VAS	25.56	22.19	26	.637	.530
Change in sleep patterns: unable to sleep – PMTS-VAS	16.93	17.59	26	-.144	.886
Change in sleep pattern: sleeping more – PMTS-VAS	26.15	20.70	26	.830	.414
Feeling overwhelmed or out of control – PMTS-VAS	**34.63**	**26.15**	**26**	**1.936**	**.064**
Breast tenderness, bloating, water retention – PMTS-VAS	**33.74**	**13.74**	**26**	**3.257**	**.003**
Pre Stress, Stress	26.37	23.11	26	.756	.459
Pre Stress, Pain	9.74	5.44	26	1.670	.107

*Significant values are bolded

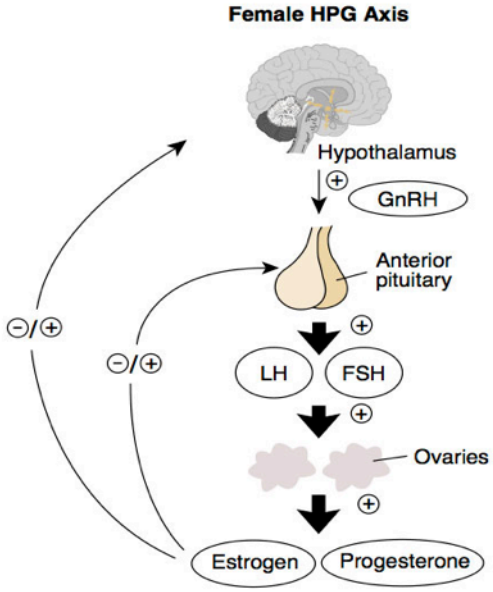
Appendix B: Figures

Figure 1.1



University of New South Wales Embryology: http://php.med.unsw.edu.au/embryology/index.php?title=File:HPA_axis.jpg

Figure 1.2



University of South Wales Embryology: http://php.med.unsw.edu.au/embryology/index.php?title=File:HPG_female_axis.jpg

Figure 2.1: Subjective ratings of stress before and after water exposure

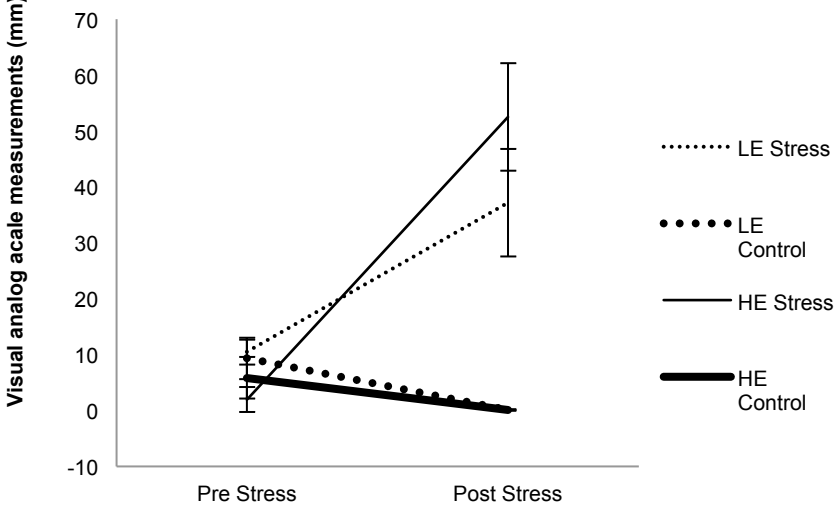


Figure 2.2: Subjective ratings of pain before and after water exposure

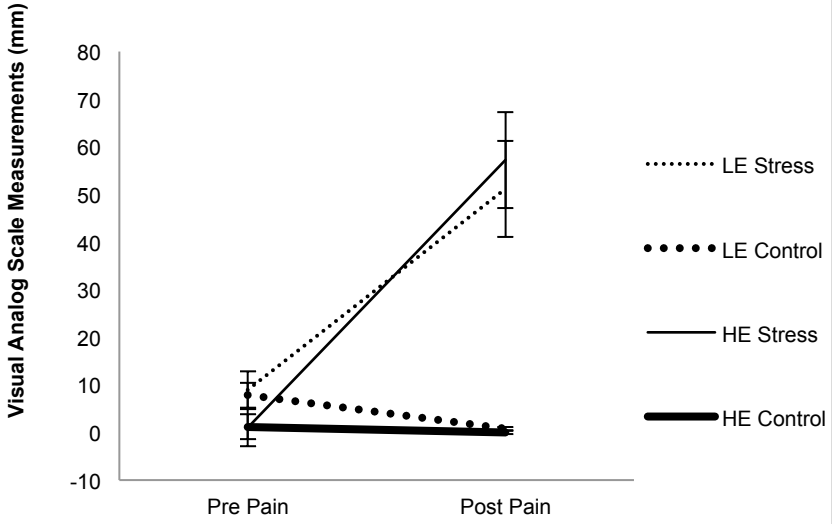
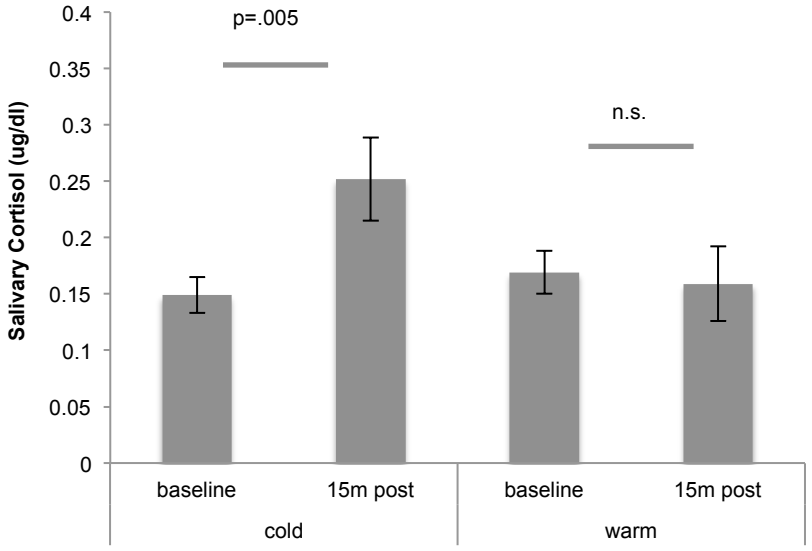


Figure 2.3: Cortisol during the stress and control sessions collapsed across HE and LE women



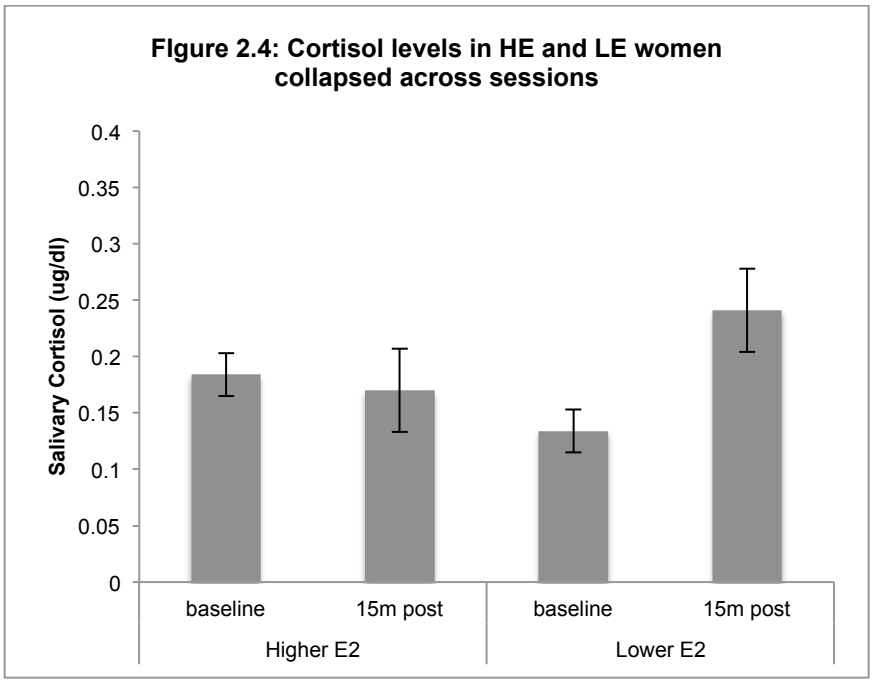


FIgure 2.4: Cortisol levels in HE and LE women collapsed across sessions
Salivary Cortisol (ug/dl)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
baseline
15m post
baseline
15m post
Higher E2
Lower E2

Figure 2.5: Cortisol levels in HE and LE women during the stress session only

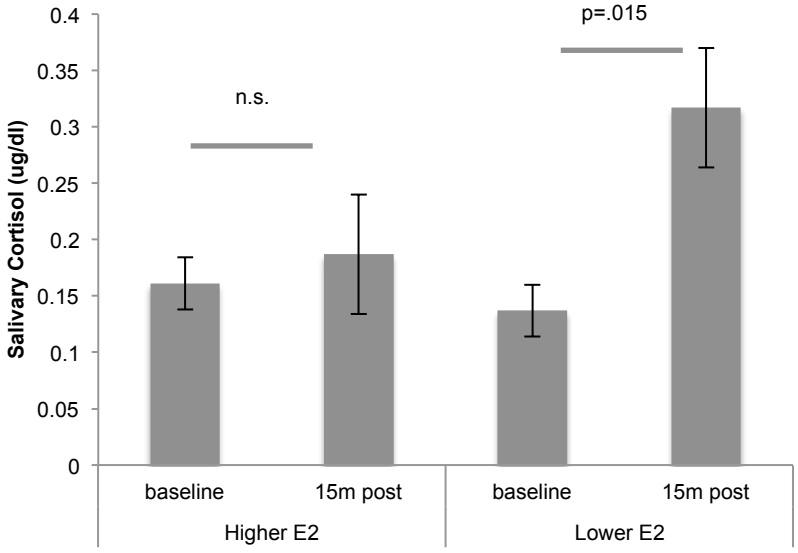


Figure 2.6: Working memory performance in HE and LE women

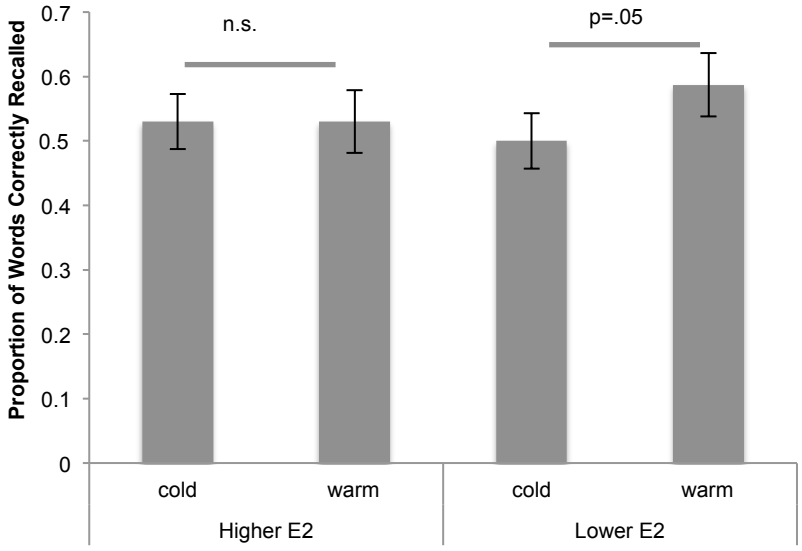


Figure 2.7: Picture recall collapsed across session and estradiol: Emotional versus neutral pictures

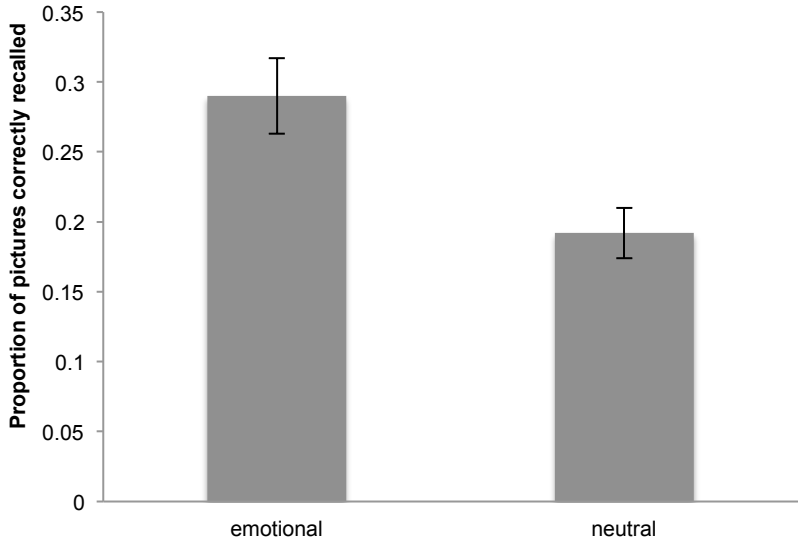


Figure 2.8: Emotional memory picture recall during the stress and control sessions

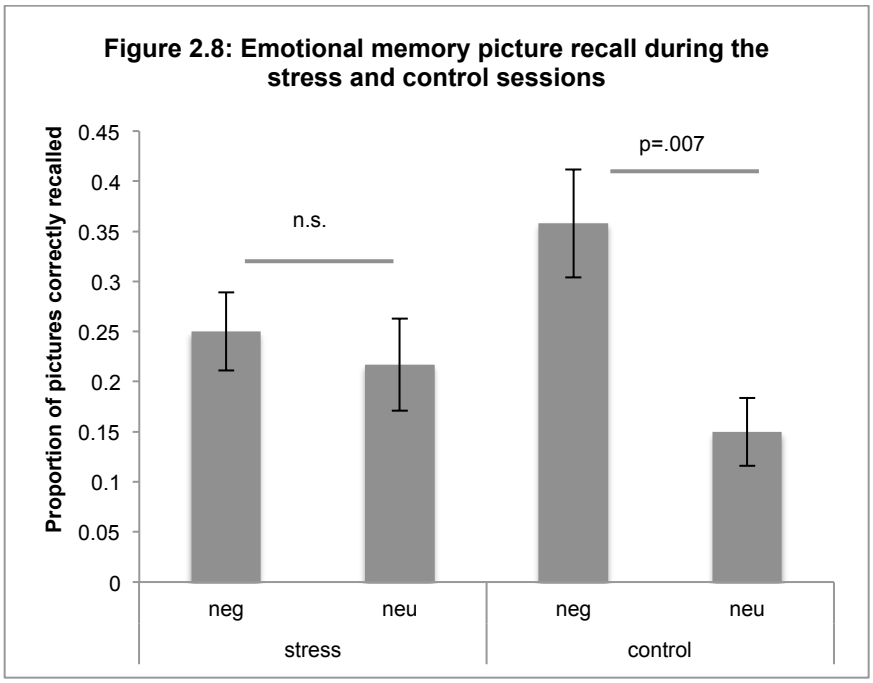


Figure 2.9: Recall of emotional pictures by all women during the stress session

Figure 2.10: Recall of emotional pictures by LE women in the stress session

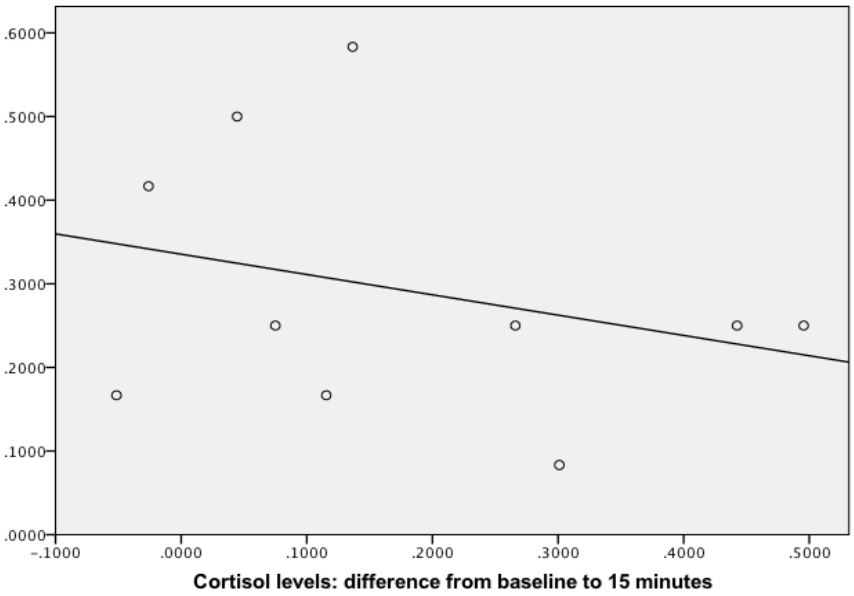


Figure 3.1: Subjective ratings of stress before and after water exposure

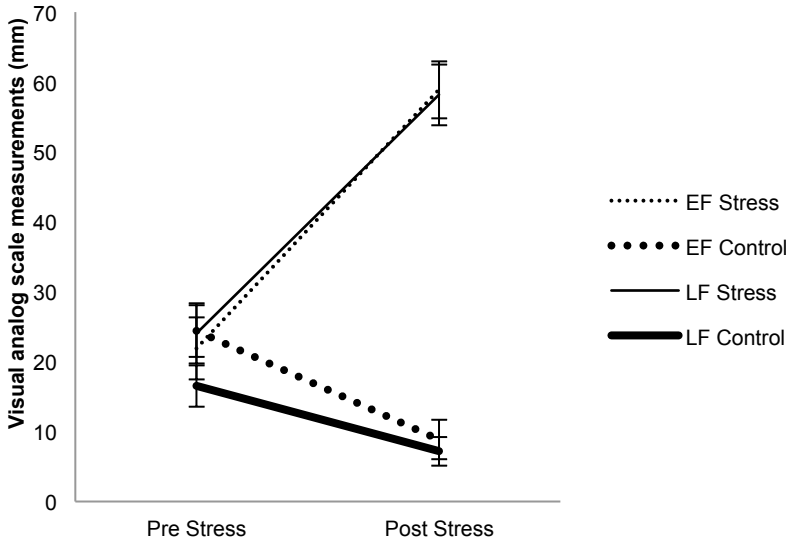


Figure 3.2: Subjective ratings of pain before and after water exposure

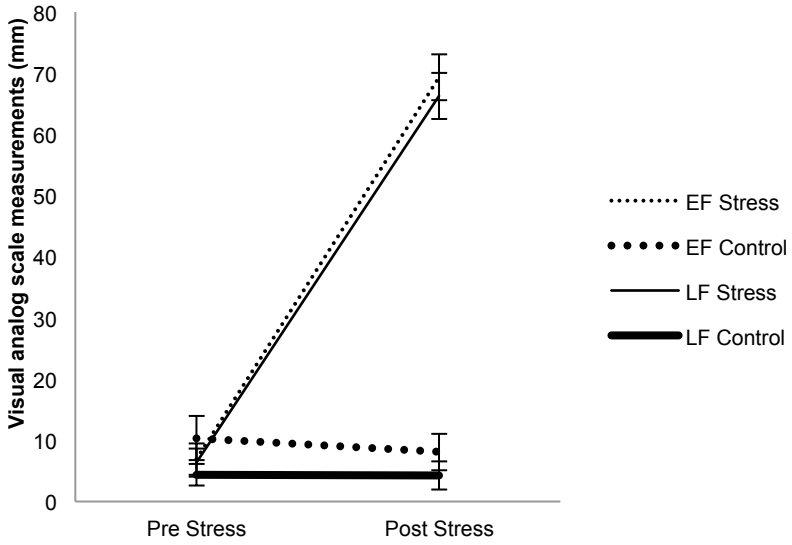


Figure 3.3: Cortisol levels collapsed across phase and all 3 time points

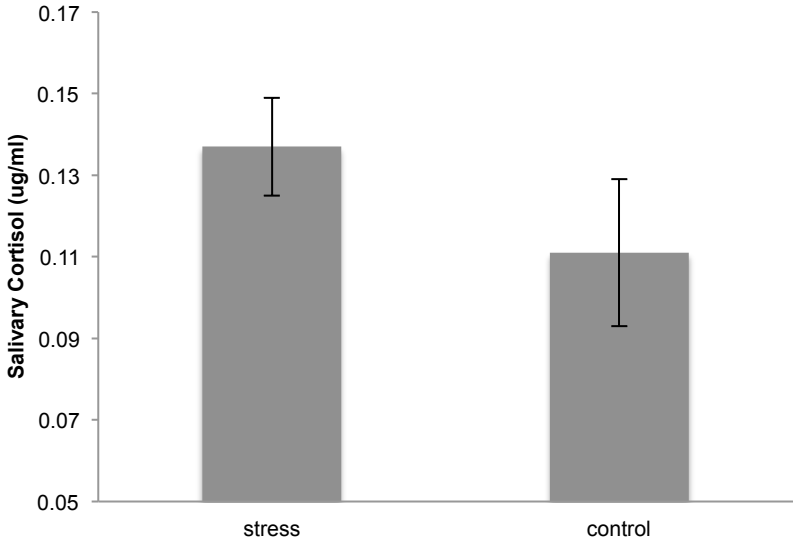


Figure 3.4 Cortisol levels collapsed across session and phase

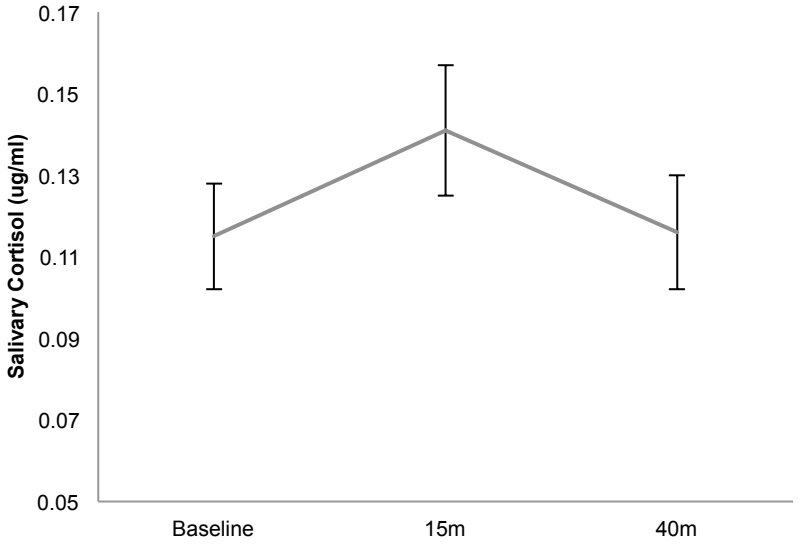


Figure 3.5: Cortisol levels collapsed across phase

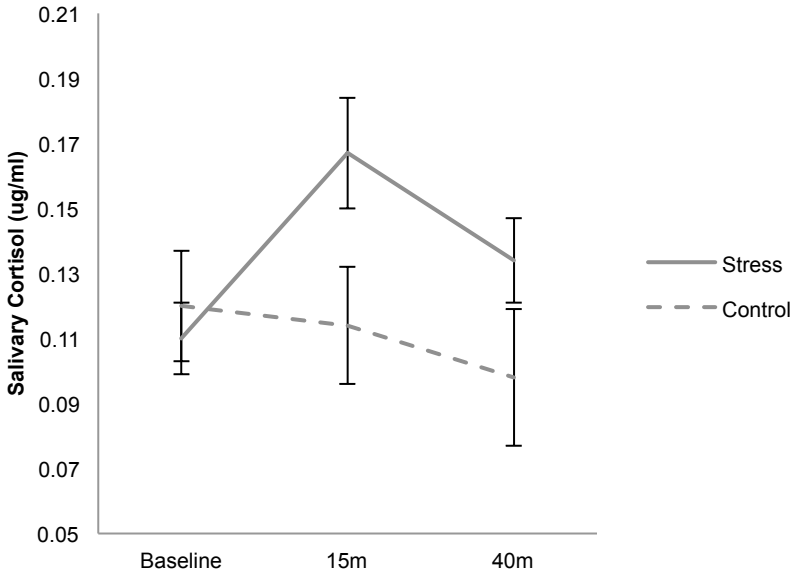


Figure 3.6: Cortisol levels during the EF phase: Collapsed across time points

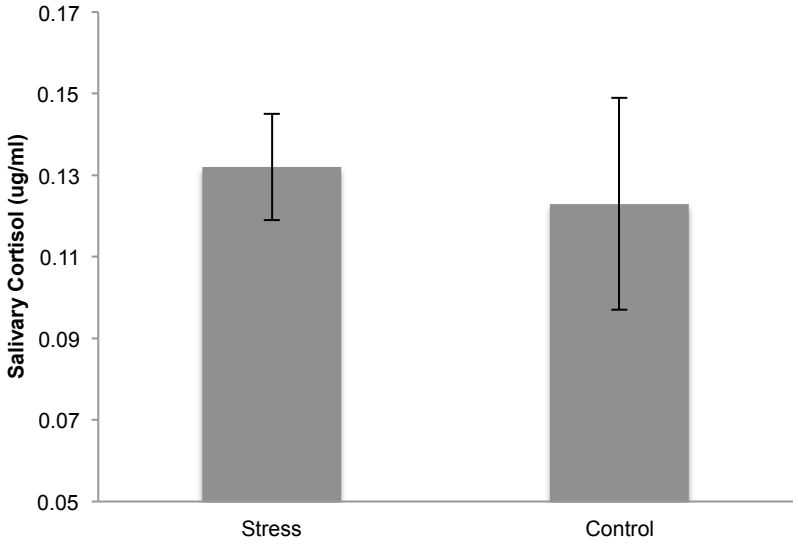


Figure 3.7: Cortisol levels during the EF phase: Baseline versus 15m post onset

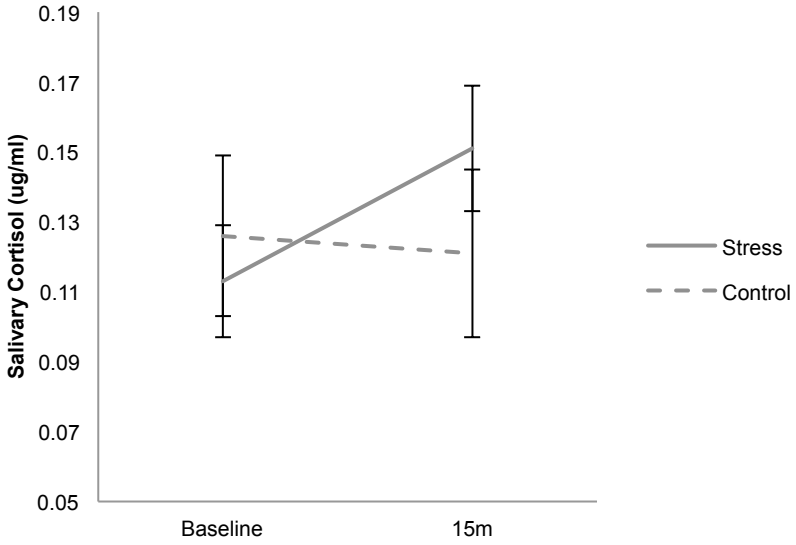


Figure 3.8: Cortisol levels during the LF phase: Collapsed across time points

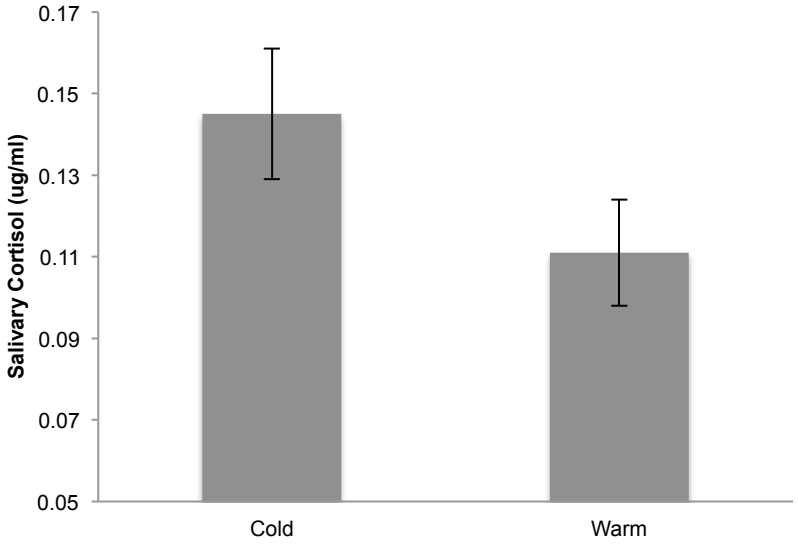


Figure 3.9: Cortisol levels during the LF phase: Baseline versus 15m post onset

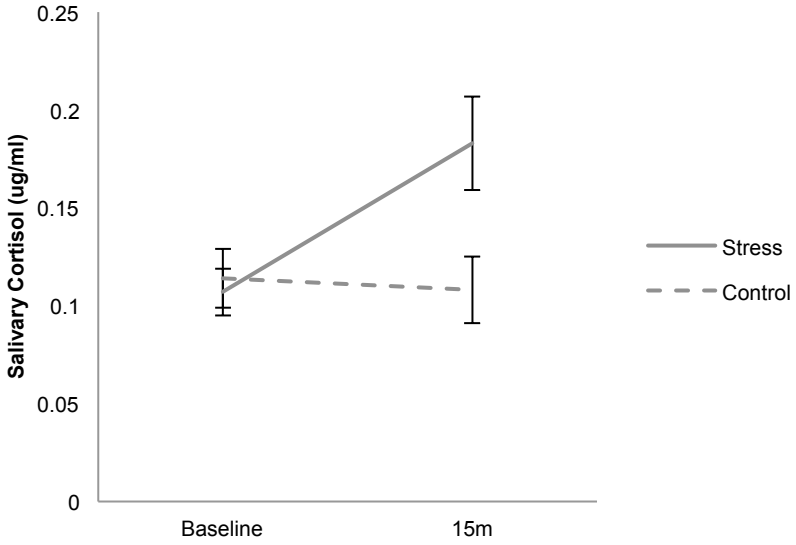


Figure 3.10: Picture recall across stress and valence: Emotional versus neutral pictures

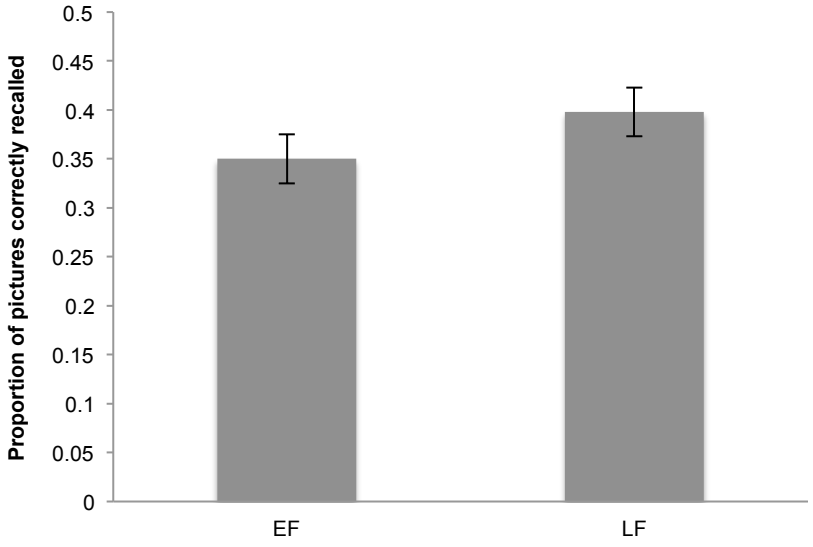
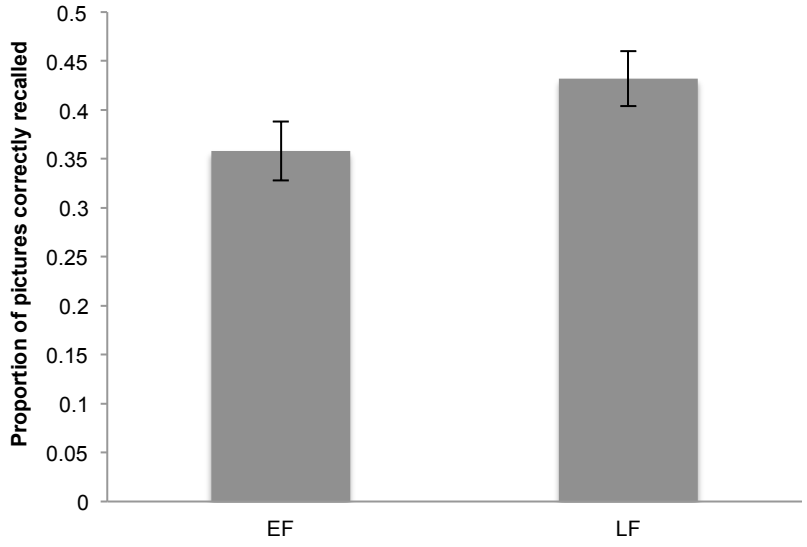


Figure 3.11: Picture recall across stress and valence: Negative versus neutral pictures



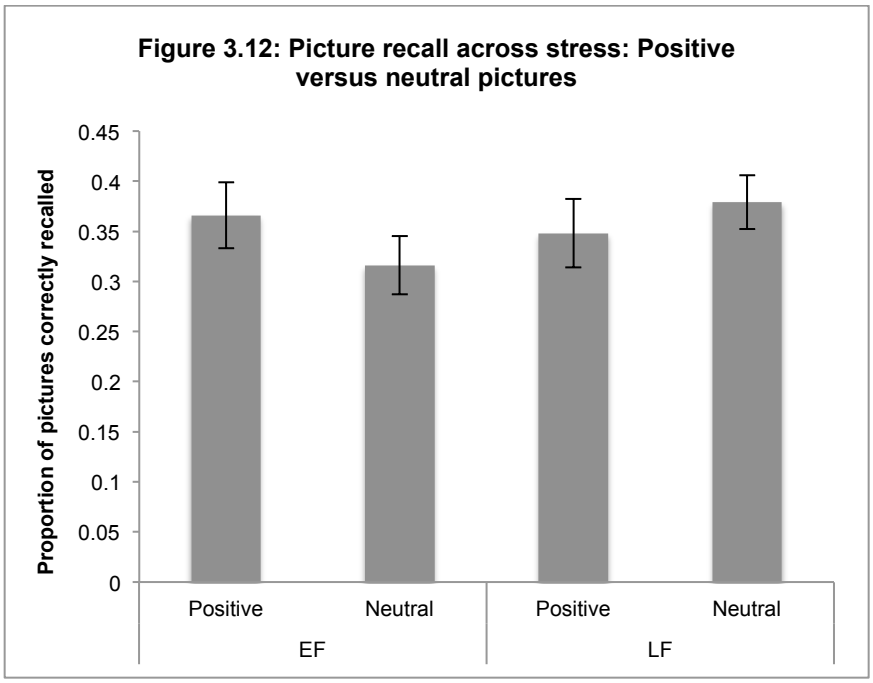


Figure 3.12: Picture recall across stress: Positive versus neutral pictures
Proportion of pictures correctly recalled
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Positive
Neutral
Positive
Neutral
EF
LF

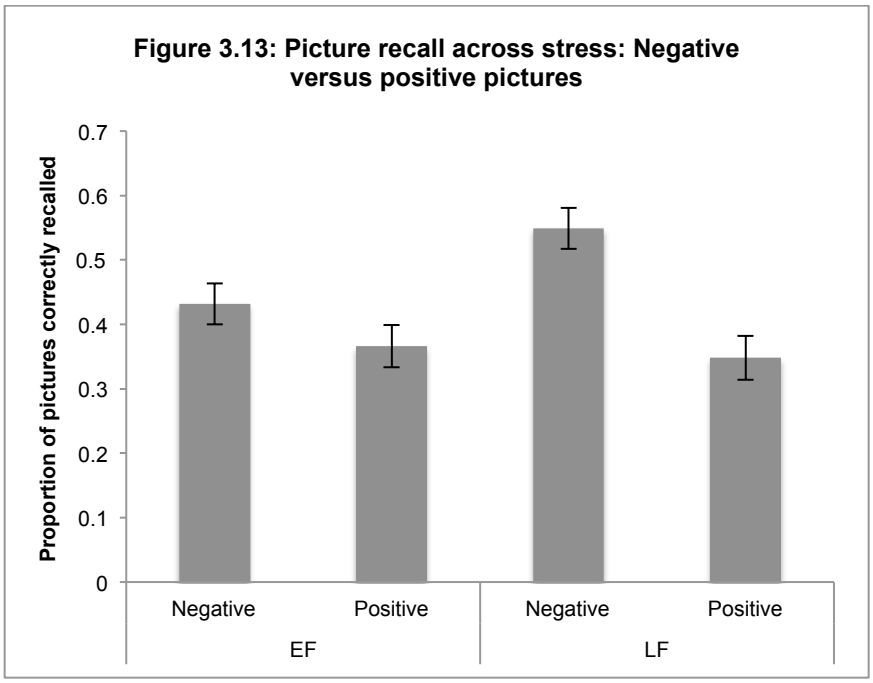


Figure 3.13: Picture recall across stress: Negative versus positive pictures
Proportion of pictures correctly recalled
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Negative
Positive
Negative
Positive
EF
LF

Figure 3.14: Picture location memory across phase: Emotional versus neutral pictures

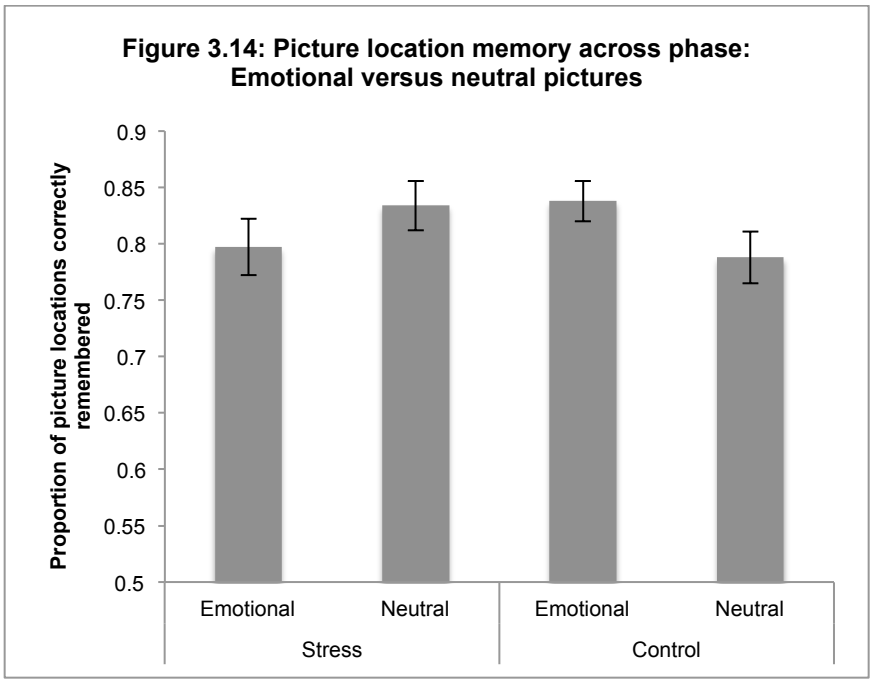


Figure 3.15: Picture location memory across stress and valence: Negative versus neutral pictures

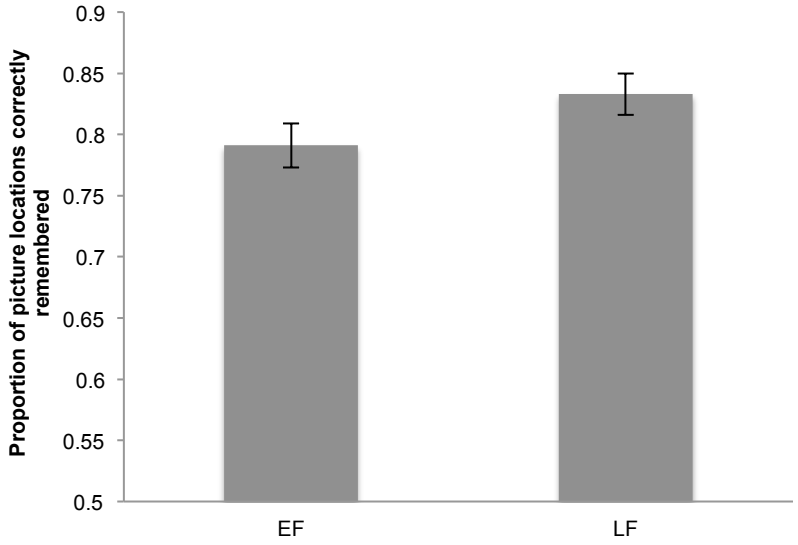


Figure 3.16: Picture location memory across phase: Negative versus neutral pictures

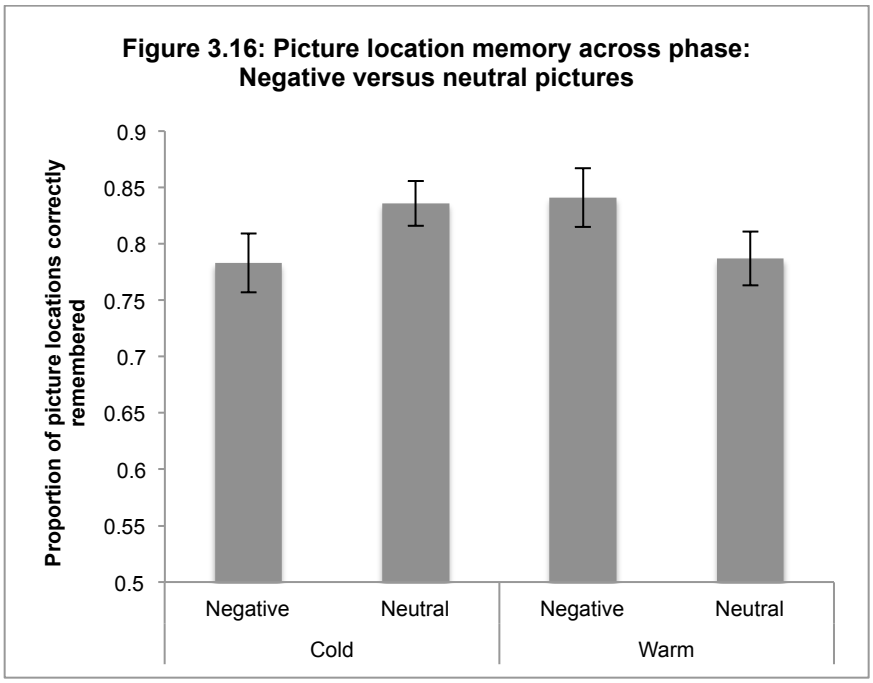


Figure 3.17: Cortisol levels between Responders and Nonresponders during the EF phase: Stress versus control sessions

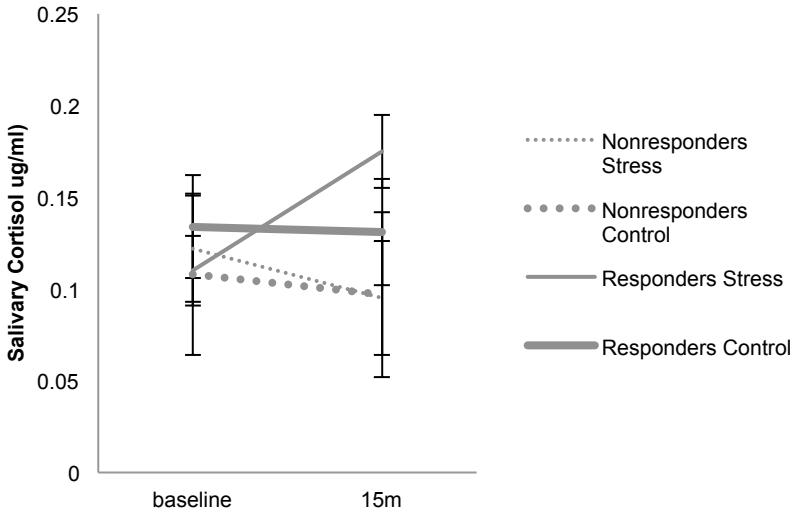


Figure 3.18: Cortisol levels between Responders and Nonresponders during the LF phase: Stress versus control sessions

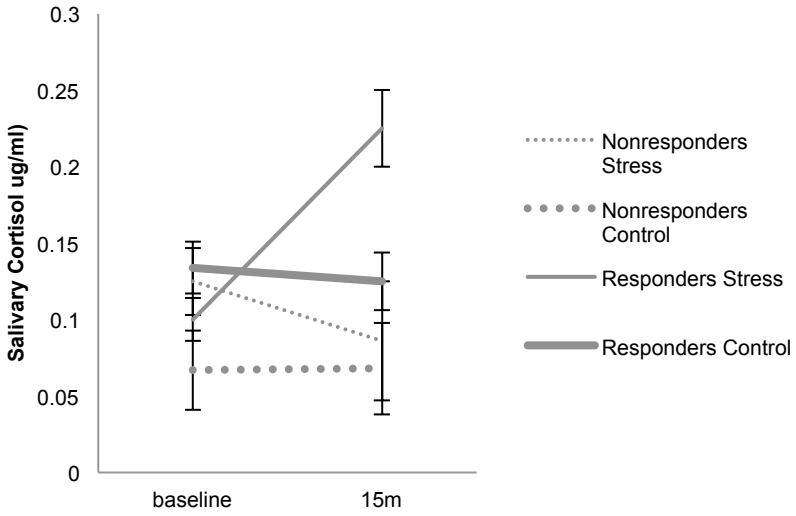


Figure 3.19: Cortisol levels in Responders and Nonresponders during the stress session in the EF phase

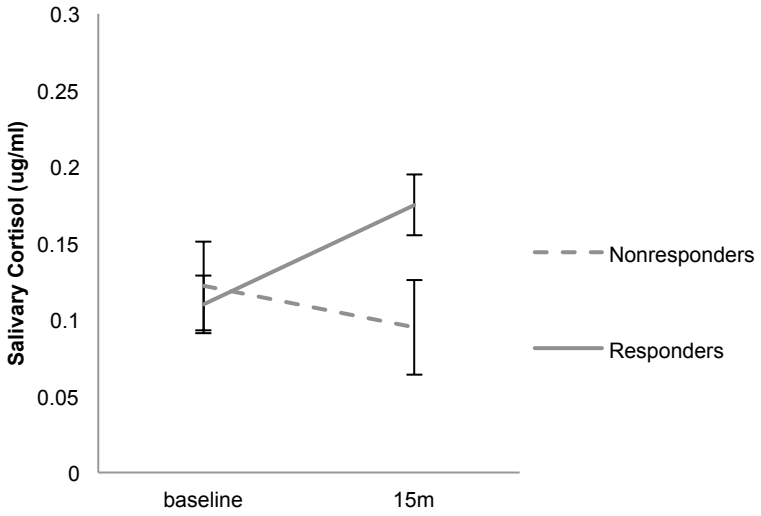


Figure 3.20: Cortisol levels in Responders and Nonresponders during the stress session in the LF phase

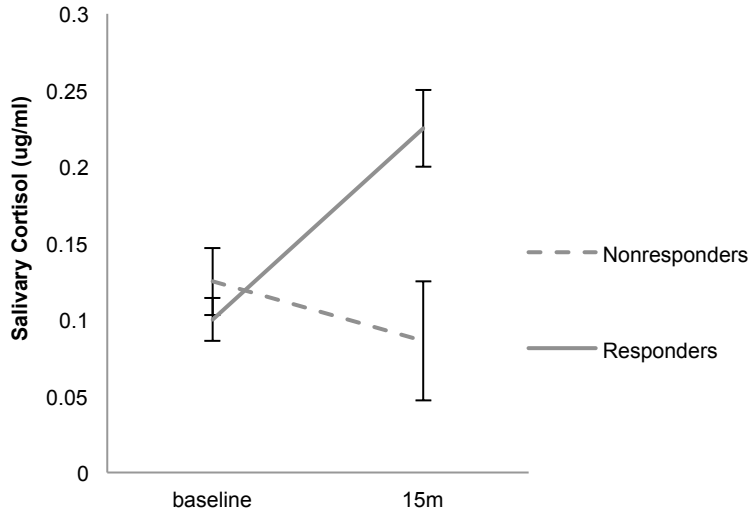


Figure 3.21: Cortisol levels in Responders in the EF phase: Stress versus control sessions

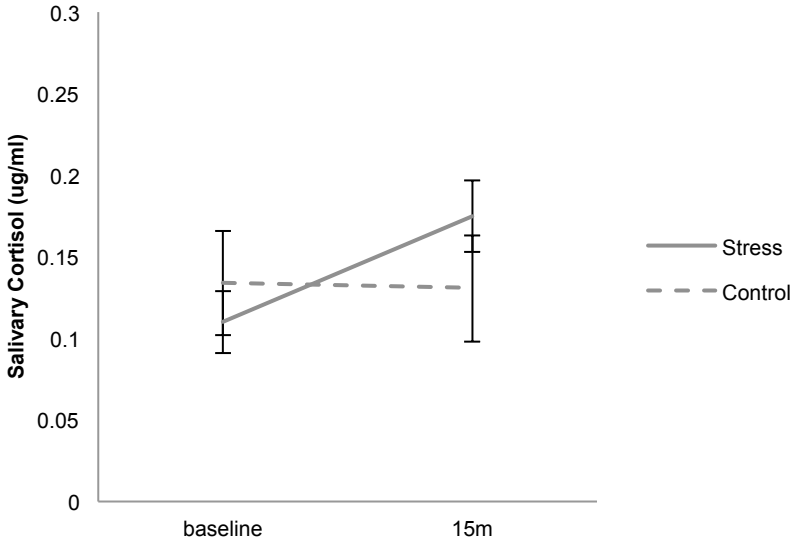


Figure 3.22: Cortisol levels in Responders in the LF phase: Stress versus control sessions

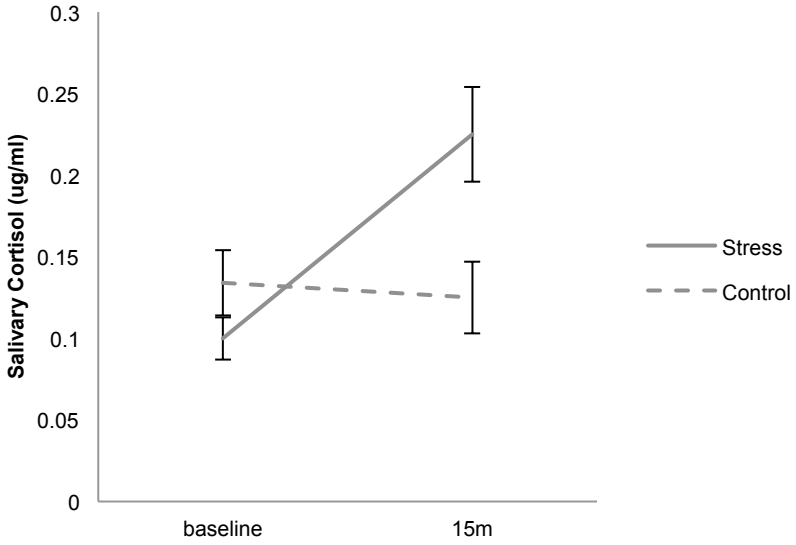


Figure 3.23: Working memory performance in the EF phase: Stress versus control session

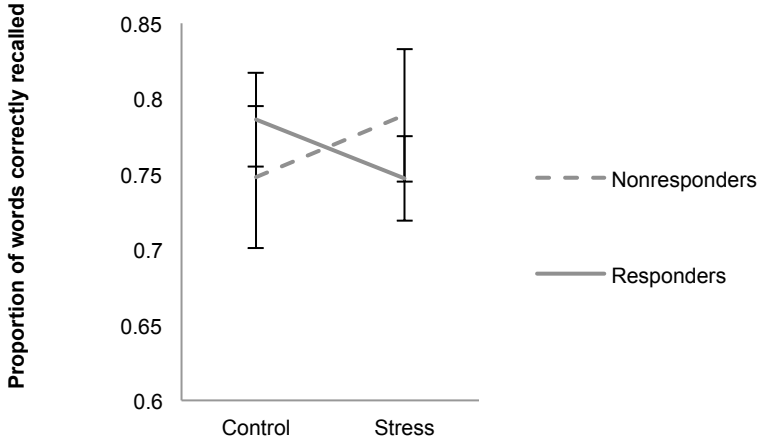
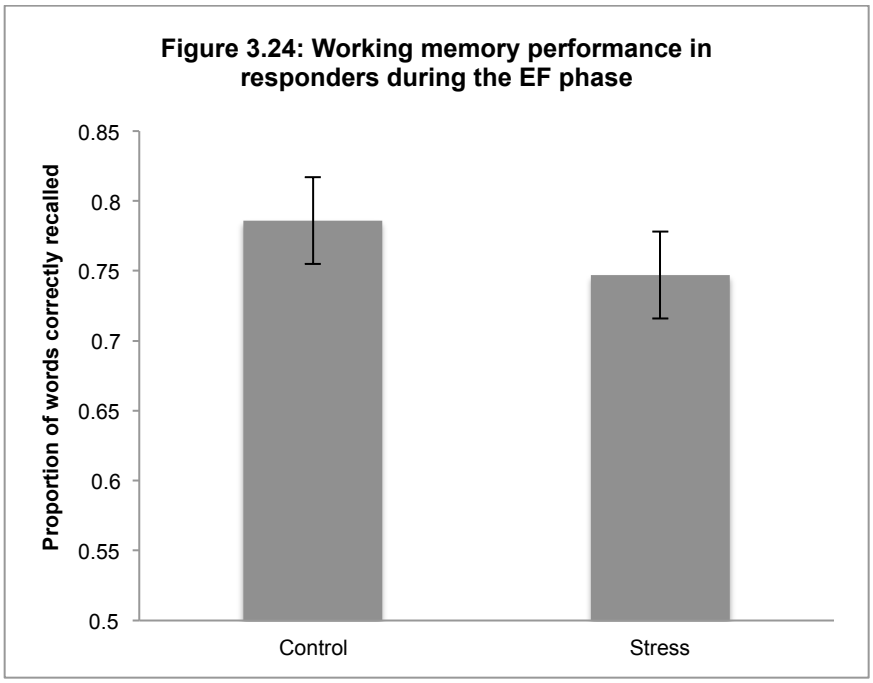


Figure 3.24: Working memory performance in responders during the EF phase



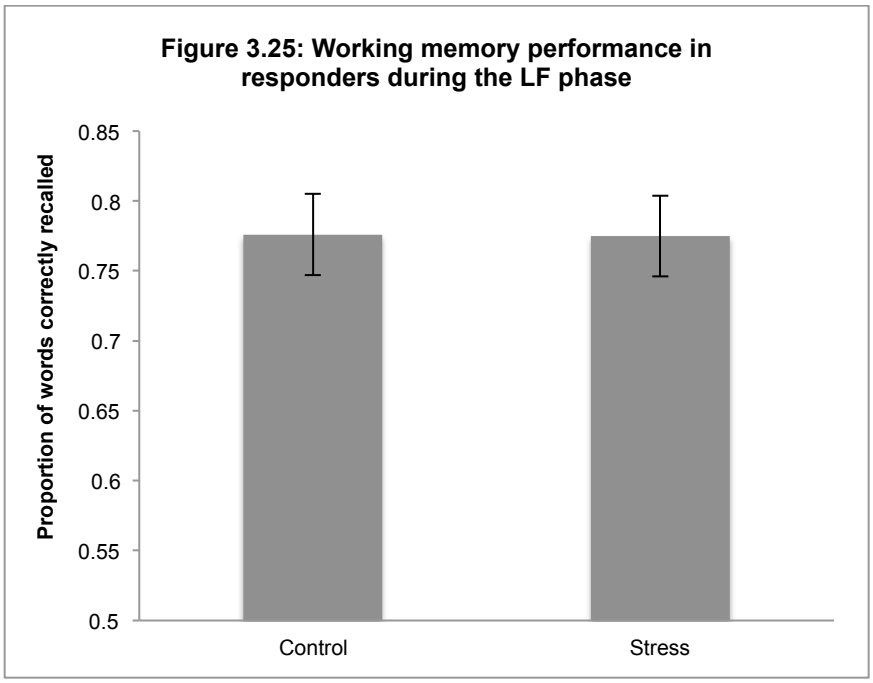


Figure 3.25: Working memory performance in responders during the LF phase
Proportion of words correctly recalled
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
Control
Stress

Figure 3.26: Picture location memory in the LF phase: Positive versus neutral pictures