11-15-2006

Differential Endogenous Estrogen Exposure Influences Prefrontal Cortex Response to Acute Stress

Katya Rubinow

Differential Endogenous Estrogen Exposure Influences Prefrontal Cortex Response to Acute Stress

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

Katya B. Rubinow

2006

Differential Endogenous Estrogen Exposure Influences Prefrontal Cortex Response to Acute Stress

Rubinow KB, Shansky RM, Arnsten AF. Department of Neurobiology, Yale University, School of Medicine, New Haven, CT.

Abstract

The present study was conducted to determine the effect of differential endogenous estrogen exposure in rats on stress-induced changes in spatial working memory. Subjects comprised male (n=8) and female (n=10) Sprague-Dawley rats, which were trained to complete a T maze, delayed alternation task. Performance was scored as a percentage of trials during which the correct maze arm was selected. Subjects' scores were recorded after 1 and 2 hours of restraint stress, as well as after 1 hour of unimpeded movement in a cage placed in the testing room. Restraint stress was effected through physical confinement within plastic, cylindrical tubing. Female subjects underwent each of the testing conditions twice, during periods of high and low endogenous estrogen exposure, as ascertained by microscopic examination of vaginal epithelial cells for estrous cycle stage determination. Females in proestrus (elevated endogenous estrogen exposure) subjected to 1 hour of restraint performed significantly worse than their baseline scores ($p=0.0017$) or females in estrus (low endogenous estrogen exposure) after 1 hour of restraint ($p=0.00014$). After 1 hour of restraint, females in proestrus also committed an increased rate of perseverative errors compared to females in estrus, although this increase did not achieve statistical significance ($p=0.06$). No appreciable differences existed among subject groups in baseline performance or subsequent to 2 hours of restraint stress. Resultant data indicate impaired working memory among female rats under conditions of stress in the context of elevated endogenous estrogen exposure. This study, then, suggests a potential synergistic effect of stress and estrogen in compromising prefrontal cortex function and, therefore, may lend insight into the observed sex-related disparity in the incidence of major depressive disorder and other anxiety-related mood disorders.

Acknowledgements

Most importantly, I would like to thank Dr. Amy Arnsten for so graciously welcoming me into her lab and allowing me to participate not only in her research but also in a wonderful complement of journal meetings and mentoring sessions. Dr. Arnsten has been invariably generous with her time and teaching, during both the research and writing phases of this project. It has been an enormous privilege to learn from her, and I am inordinately grateful for her willingness to act as my advisor.

In addition, I owe a substantial debt of gratitude to Dr. Rebecca Shansky. Despite being in the midst of completing her PhD, Becca always made time to discuss her research with me, to teach, and to share good music. Becca enabled my research experience to be both enriching and a sheer joy; she is an outstanding mentor who has also proven a great friend.

I would also like to thank my father, whose willingness to serve as my external reviewer afforded me both the opportunity to benefit from one of the pioneers in neuroendocrinology and an excuse to spend additional time with one of my favorite people in the world.

The completion of this paper would not have been possible without the support of the Department of Neurobiology and a research grant from the Office of Student Research.

Table of Contents

Introduction

Mood disorders have a profound impact on both personal and collective outcome, with Major Depressive Disorder (MDD) the most severe in terms of prevalence (15%) and social cost ($40 billion per year) associated with disease morbidity (1). The incidence of MDD among women is twice that among men, a differential that transcends distinctions of race, ethnicity, and socioeconomic class and further, that is evident in multinational epidemiologic data (2). However, despite the fact that depression is twice as common among women as men, few experimental data exist regarding the pathophysiology of depression specifically in women, including the potential importance of estrogen in contributing to this phenomenon. Although this pronounced sex-related differential could be partially attributable to sociocultural influences, the ubiquity of this striking discrepancy suggests some component of biological predisposition. A potential insight into this sex differential derives from the occurrence of specifically reproductive-related mood disorders among women, including premenstrual syndrome and menopausal depression. Such disorders demand consideration of the role of ovarian hormones in creating a biological context that predisposes some women to mood disturbances. Research to date has dispelled the possibility of a simple, causal relationship between level of hormone exposure and the development of MDD or reproductive-related mood disorders (3). Rather, extensive data collectively suggest the intricate interplay of genetic, biological, and environmental factors in the generation of a particular neurochemical milieu that predisposes some women to develop dysregulated mood states.

Environmental context undoubtedly contributes to the pathogenesis of depression, particularly as it produces sources of uncontrollable stress. As sociocultural forces broadly may exert certain pressures uniquely upon women, they may partially account for the sex-related variance in MDD incidence. Both epidemiological and genetic research indicate considerable interrelatedness among stress, anxiety, and depression. Epidemiologic data have demonstrated a strong association between stressful life events and the development of depression (4). Moreover, the interaction between anxiety and depression is reflected by the high incidence of co-morbidity of depressive and anxiety-related mood disorders (5, 6). Further, animal models employed to simulate depression involve "learned helplessness," a state that develops in animal subjects after repeated exposure to uncontrollable stress. The cognitive and behavioral impairment observed among animals under stressful conditions, therefore, resembles that associated with clinical depression, as well.

Genetics and Sex-Related Variance in Depression

Dispelling a purely environmental basis for this sex-related variance, however, emergent evidence offers support for a prominent role of genetics in the development of MDD. Linkage studies, for example, have suggested the potential involvement of cAMP Response Element Binding 1 protein (CREB1) in the pathophysiology of MDD (7). CREB1 is a transcription factor and represents a downstream target of an intracellular signaling cascade mediated proximally by protein kinase A (PKA). This cascade is coupled to many neurotransmitter receptors, and interaction has been reported between CREB1 and nuclear estrogen receptors. The association between CREB1 and MDD,

moreover, was observed exclusively among female probands, further supporting the notion that susceptibility to mood disorders has a biological basis. CREB-based research may yield further insights into the genetic predisposition toward MDD and the attendant underlying pathophysiology of the disease. However, the precise mechanism whereby differences in CREB phosphorylation might translate into mood disturbances remains unelucidated.

Similar to these CREB-associated findings, differences in estrogen receptor-α (ER-α) genotype were associated with the development of MDD, but also solely in women (8). Moreover, at the level of transcription, discrepant amounts of ER-α mRNA production have been observed in mental illness. Decreased ER-α mRNA levels were found in the amygdala of patients with MDD compared to controls, and women with MDD had diminished ER-α mRNA production in the dorsolateral prefrontal cortex (PFC) and throughout the hippocampus, with mRNA levels significantly below those of men with depression (9, 10).

These preclinical data, then, strongly suggest that vulnerability to mood disorders is, to an extent, biologically encoded, and further implicate ovarian hormones in the evolution of this vulnerability. To explore the degree to which ovarian hormones are implicated in mood disorders, the relationship between ovarian hormones and cognition requires examination. The influence of estrogen in particular has been established throughout the brain, with roles in neuroprotective, learning, and memory functions, among others. Critically, however, estrogen's role in the brain must be examined contextually, with a high degree of specificity for brain region and the associated facets of cognition.

Subcortical Effects of Estrogen

Ovarian hormones appear to effect structural changes in subcortical areas of the brain. Choi et al (2003) showed that both estrogen and progesterone administration affect the neuroanatomy of the CA1 region of the hippocampus, modifying dendritic spine number and synaptogenesis (11). Granholm et al (2003) examined the effects of estrogen treatment in a mouse model of Down Syndrome. Whereas non-treated mice exhibited significant loss of hippocampal dendritic spines and cholinergic neurons, estrogen-treated mice exhibited marked sparing of these losses (12). Furthermore, although estrogen did not affect amyloid-associated protein (APP) levels in the hippocampus, data from the same study demonstrated increased full-length striatal APP after estrogen treatment. This posited neuroprotective role of estrogen finds additional support in the work of Gulinello et al (2005), who found that estrogen administration decreased neuronal injury in the hippocampus subsequent to global ischemic injury (13). This study moreover found an associated preservation of visual and spatial memory, providing a functional corollary to the anatomic observations.

Ovarian hormones further have been implicated extensively in subcortical biochemistry and cognition. In a study conducted by Luine et al (2003), estrogen administration was associated with improvement in visual and place memory, tasks specific to hippocampal function (14). Rat data also demonstrate that estrogen augments neuronal excitability in the hippocampus and increases acetylcholine release during performance of a hippocampus-mediated task (15, 16). Increased NMDA receptor activation in these regions also has been observed subsequent to estrogen administration

(17). Estrogen further appears to interact with cholinergic neurons in the basal forebrain, with similar, enhancing effects on learning (18, 19). Of note, estrogen-induced enhancement of hippocampus-mediated cognition both augments acetylcholine release and appears acetylcholine-dependent, specifically through the acetylcholine M2 receptor (20). This observation attests to the complexity of estrogen's effects in the brain, as estrogen both creates and is dependent upon a particular neurochemical milieu to produce functional, cognitive outcomes.

In addition to its interaction with cholinergic systems, estrogen interfaces with serotonergic systems, as well. In a study by Rocha et al (2005), estrogen was shown to increase serotonin production in the dorsal raphe nucleus (DRN) of mice, and attendant antidepressant effects were observed (21). This increase in serotonin was ER-β dependent, as the antidepressant properties of estrogen were absent in ER-β knockout mice. Estrogen further mediates serotonin release through its modulatory role at the 5-HT1A receptor, which is both pre- and post-synaptic and therefore integral to serotonin signaling and negative feedback (22).

These data collectively support a significant role for estrogen in the brain, but the estrogen-mediated effects they suggest cannot be assumed for the prefrontal cortex (PFC). Research to date suggests that whereas estrogen enhances hippocampus-mediated learning, it plays a profoundly different role in the PFC. In fact, emergent data indicate that estrogen facilitates shifts in cognitive strategy toward hippocampus-mediated tasks and away from striatal or prefrontal cognition (23, 24). Such data demand examination of the relationship specifically between estrogen and the PCF, and they refute the broad

application of a cognition-enhancing role for estrogen throughout the brain; facets of cognition of highly region-specific, and so, too, appear the effects of estrogen.

The Prefrontal Cortex

The PFC warrants particular attention as a region of critical importance in the pathogenesis of MDD. Current models of MDD posit the centrality of the PFC in disease pathophysiology, a position that derives from the PFC's unique capacity to simultaneously manipulate information acquired from sources disparate in origin and time (25). The PFC maintains the availability of information, permits the assimilation of new information, and enables directed, focused attention that facilitates the translation of accessible information into goal-directed behavior. In aggregate, these functions constitute working memory, a defining function of the PFC, whereas animals with PFC lesions exhibit perseveration, impairment in sustained attention, and absence of behavioral inhibition. The interpretive, executive role of the PFC may also be understood with regard to the regulation of mood and emotional state, as the PFC represents the integrative and interpretive locus of limbic and sensory input, thereby determining overarching experience and commensurate mood state. This model finds support in an imaging study conducted by Levesque et al (2003), who recorded activation of different areas of the PFC in the experience of sadness and the active suppression thereof (26). Such data suggest that the PFC may be responsible for interpreting and re-interpreting experience, to the extent that an initial emotional response may be overridden in subsequent evaluation. Derailment of normal PFC function consequently would impede this volitional, state-changing capacity, and yield consequent mood dysregulation.

The primacy of the PFC in MDD pathogenesis is supported by abundant evidence of PFC dysfunction associated with major depression. Depressive states are commonly characterized by poor concentration, impaired motivational process, diminished verbal fluency, and perseverative thoughts, all areas of cognition regulated by the PFC. Lesions of the PFC similarly produce these deficits, suggesting perhaps that the pathophysiology of MDD involves some neurochemical "injury" to the PFC. Patients suffering from MDD moreover appear to exhibit cortical hypometabolism and diminished PFC volume (27, 28). Whereas mood presents an elusive prefrontal product in animal studies, working memory provides a surrogate for evaluating PFC function. A conceptual assumption must then be adopted, that is, that impairment in PFC-mediated learning corresponds to impairment in prefrontal mood regulation, and accordingly, that derailed working memory predicts a state of vulnerability to the generation of a depressive phenotype.

A critical relationship, then, emerges among the PFC, MDD, and stress, as stress is associated with both PFC impairment and depression. In animal studies, stress has been shown to cause diminished working memory function. This functional diminution has been attributed to stress-related changes in PFC neurochemistry, including increased release of norepinephrine (NE) and dopamine (DA); impairment in working memory appears a product of the consequent increased stimulation particularly of the NE α-1 and DA D1 receptors, with consequent signaling of IP_3/PKC and cAMP/PKA pathways, respectively (29). NE in particular is implicated in working memory impairment, as pre-treatment with an α-2 receptor agonist has proven protective for PFC function (30). Therefore, stress response has proven an important area of study for gaining insight into

the pathophysiology of depression and for evaluating differential susceptibility to the development of MDD. The question then arises as to the role of stress in mood disorders specifically in women, and the potential modulatory effect of ovarian hormones on the stress response, particularly as they interface in the PFC.

The PFC, Stress, and Estrogen

Substantial data exist supporting an influential role of estrogen on the primary stress response. Sex-based disparities in corticosterone release have been observed in a number of animal studies, in response to both acute and chronic stress (31). Furthermore, in humans, a subset of corticotrophin-releasing hormone (CRH) neurons in the hypothalamus are ER-α positive. Interestingly, a study by Bao (2005) et al found a significantly increased number of ER- α positive CRH neurons in patients with MDD (32).

Beyond this apparent interaction at the level of the hypothalamic-pituitary-adrenal axis, estrogen and stress interface again in the PFC. The disproportionate incidence of MDD among women and the centrality of the PFC in depression together demand attention to the influence of ovarian hormones on PFC activity. Beyond their activity in subcortical regions, ovarian hormones increasingly have been implicated in the mediation of direct effects in the PFC in women. Berman et al (1997), for example, demonstrated that progesterone and estrogen both were independently capable of changing regional cerebral blood flow to the PFC during performance of a PFC-specific task (33). Estrogen also appears to play a significant, modulatory role in the stress response specific to the PFC. Rodent and primate research demonstrates that ovarian hormones are powerful

regulators of cortical catecholamines, the neurotransmitters prominent in the stress response (34, 35, 36). States of acute stress are characterized by catecholamine up-regulation, including increased stimulation of the NE α-1 receptor and diminished activation of the functionally protective α-2 receptor (37). Whereas low to moderate levels of norepinephrine and dopamine are associated with optimal PFC function, impairment in PFC-mediated cognition develops with elevated levels of these catecholamines. Uncontrollable stress therefore constitutes a primary means by which PFC function may be compromised, and estrogen is again specifically implicated in this neurochemical picture, as it acts as a transcription factor for the cortical α-1 receptor (38) and appears to cause relatively decreased stimulation of the α-2 receptor (39). The literature consequently suggests a synergism between stress and estrogen, a collaborative favoring of subcortical cognition at the expense of prefrontal, executive functions. Confluent exposure to uncontrollable stress and estrogen must therefore be examined as a potential contributor to the genesis of MDD, insofar as it creates a neurochemical context that predisposes toward PFC derailment. PFC impairment indeed may have adaptive utility as a response to acute stress, enabling more reflexive, primitive reactions to emerge in response to a perceived environmental threat (40). However, chronicity of uncontrollable stress, particularly in the context of endogenous estrogen exposure, could produce a maladaptive state with prolonged PFC incapacitation and consequent mood dysregulation.

The Present Study

In aggregate, the elaborated data collectively underscore the importance of better delineating the relationship among stress, the PFC, and ovarian hormones, and of understanding this relationship with regard to its centrality in the sex-specific development of MDD. This relationship was explored in a recent study by Shansky et al (2004) which demonstrated the detrimental effects of estrogen exposure on performance of a PFC-mediated task after administration of the pharmacologic stressor FG7142, a benzodiazepine inverse agonist (41). Whereas female rats in states of low or absent (post-ovariectomy) estrogen exposure exhibited no performance impairment after FG7142 administration, female rats showed significantly compromised performance when exposed to high levels of estrogen. The methodological question remains, however, whether FG7142 as a pharmacologic stressor represents a valid approximation of physiologic stress. To address this question, the present study selectively examined the effects of estrogen on PFC function in rats after exposure to restraint stress. Restraint stress has been demonstrated in rats to increase plasma corticosterone as well as catecholamines, serotonin, and acetylcholine in the PFC of rats (42, 43, 44). Furthermore, Nakane et al (1994) showed that norepinephrine remains elevated for 20 minutes in the PFC after subjects' release from one hour of immobilization (45). Thus, restraint stress has been well-established as a reasonable model for simulating the effects of natural, uncontrollable stress. Resultant data from the present study corroborate the hypothesis that estrogen indeed plays a modulatory role in PFC function subsequent to restraint stress exposure. This study, then, provides further evidence that ovarian hormones represent a critical, regulatory link between stress and PFC-mediated

cognition. As mediators of the stress response, ovarian hormones thereby may confer vulnerability to mood disturbance and contribute substantially to the sex-related variance in depression.

Hypothesis and Specific Aims of the Study

Hypothesis

The basis of the present study is the posited role of estrogen in establishing a hormonal context of increased vulnerability to stress as demonstrated by PFC function, quantified by performance on a spatial delayed alternation task. Diminished ability to tolerate stress has been established as a predisposing factor for the development of depression, and greater stress tolerance appears protective. The null hypothesis is as follows: Estrogen does not play a modulating role in female rats' response to stress as measured by performance on a working memory task.

Specific Aims of the Study

The aims of the proposed study are as follows:

1.) To measure the response of male and female rats to restraint stress as a function of performance on a spatial delayed alternation task.

2.) To demonstrate whether a sex-specific response to stress appear to exist in rats. Specifically, the target of exploration is the whether male and female rats exhibit differential working memory performance subsequent to restraint, regardless of estrous cycle phase.

2.) To discern the presence or absence of a correlation between endogenous estrogen level and stress response in female rats, that is, to determine if the proestrus phase of the

estrous cycle (highest endogenous estrogen exposure) confers vulnerability to stress and/or the estrus phase (lowest endogenous estrogen exposure) confers protective effects from stress. Thus, the objective is to examine the potential interaction of estrogen exposure and acute stress in altering PFC function.

4.) To determine the appropriateness of use of the pharmacological agent FG7142 to simulate environmental stressors through comparison of their respective effects on PFC activity. As various experimental forms of acute stress are available, this study may help evaluate whether pharmacologic stress approximates the effects of physical stress on working memory.

5.) To gather preliminary data for the eventual hypothesizing of a sex-specific pathophysiology of depression in females, including the potential role of estrogen therein.

Methods and Materials

The overall study design comprised three stages, those of habituation, training, and testing. Habituation was essential for allowing study subjects to acclimate to the T maze, accepting food rewards directly from an experimentor, and human handling. Corresponding to these goals of acclimation, habituation was subdivided into three phases. Once subjects were accustomed to moving throughout the maze, accepting rewards, and manual transfer, they underwent training on a delayed alternation working memory task. Training was deemed complete when a subject scored 60-80% on consecutive testing days. Finally, during the testing stage, testing conditions were manipulated; subjects performed the T maze task alternatively after 1 hour of restraint stress, 2 hours of restraint stress, or 1 hour of unrestrained movement within a cage placed in the testing room. The last of these manipulations constituted control conditions.

Subjects

The subject population comprised male (n=8) and female (n=10) Sprague-Dawley rats (Camm, Wayne, NJ). Subjects were housed singly and exposed to a 12-hour light/dark cycle, with all testing conducted during the light phase. Subjects' diet consisted of Purina rat chow (15 g/day/rat), which was provided immediately after cognitive testing. Water was available ad libitum. Food rewards were administered during testing, and the choice of highly palatable miniature chocolate chips obviated the need for dietary restriction. Over the course of the 10 month study period, male subjects'

weights increased from a baseline mean of 240 g to 390 g. Female subjects' weights increased from a baseline mean of 240 g to 300 g.

Habituation

Subjects performed a delayed alternation task in a T maze (90 cm x 65 cm), which also served as the site of habituation and training. Complete subject habituation was considered achieved when an animal ate proffered chocolate chips directly from an experimentor's hand. Habituation occurred in three phases, with completion of each stage requiring the subject to consume 10 chocolate chips within 6 minutes of entering the T maze.

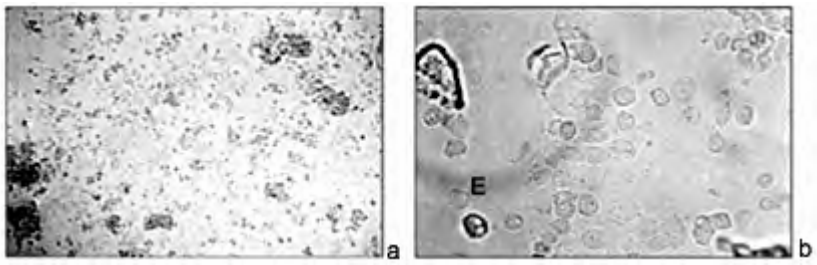
Upon initial introduction into the maze, each animal was permitted to explore freely. The first stage was deemed complete when the subject consumed 10 chips placed at the end of both arms of the maze; arm choice was unimportant. In the second stage, animals were again allowed to choose either arm of the maze but acclimated to accepting chips directly from an experimentor's hand upon reaching the end of an arm. The third phase of habituation introduced subjects to the experimentor's manual return of the animal to the maze starting gate after arrival at the end of a maze arm. Habituation to handling was imperative, as handling could otherwise constitute an inadvertent source of stress under intended unstressed conditions.

Estrous Phase Monitoring

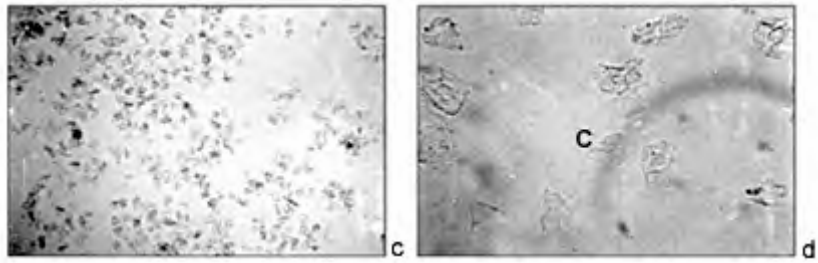
The rat estrous cycle comprises 4 stages, and each stage is normally 24 hours in duration. The proestrus phase is that of highest estrogen exposure, whereas the estrus

phase represents a nadir of endogenous estrogen exposure. Female subjects were vaginally lavaged with a cotton swab immediately subsequent to testing to determine estrous cycle phase. Lavage specimens were placed on microscope slides and stained with Cresyl violet. Specimens were then viewed by light microscopy to determine estrous cycle stage. Proestrus is characterized on histology by nucleated vaginal epithelial cells of irregular shape, often organized in clusters. Epithelial cells consistent with estrus may also be of irregular shape but are non-nucleated and generally organized singly. Estrous cycle phase determination was employed to select candidates for testing the following day, based on the expected cycle phase. Subjects were lavaged again after testing to ensure cycle phase had been predicted accurately. This measure was particularly important, as stress can contribute to estrous cycle irregularity.

Figure 1. Histologic Determination of Estrous Cycle Phase



PROESTRUS



ESTRUS

Figure 1. Photograph of vaginal epithelial cells characteristic of proestrus and estrus. Borrowed from Marcondes FK, et al (Braz J Biol vol 62 no.4a Sao Carlos Nov 2002).

Cognitive testing

A delayed alternation task was employed to examine spatial working memory. In addition to working memory, completion of the selected task demands behavioral inhibition and sustained attention. Performance is therefore interpreted as a measure of PFC function, an interpretation supported by data demonstrating impaired task performance in animals with compromised PFC function. This impairment has been

observed in animals with mechanical PFC lesions as well as those exposed to the pharmacologic stressor FG7142, an agent known to affect the PFC (46).

A period of training on the delayed alternation task followed completion of all 3 phases of habituation. Subjects initially were placed in the maze's gated start area and permitted free movement throughout the maze upon the gate's opening. Once the animal selected one arm of the maze, it received a food reward regardless of the side chosen. The animal then was returned manually to the start area behind the closed gate. On all subsequent trials, the subject was rewarded only if it selected the arm opposite from that which it had selected on the previous trial. If an animal committed an arm selection error, it was returned to the start area without reward. A total of 10 trials, not including the subject's initial arm selection, constituted a single testing session. Both test completion time and percentage of correctly performed trials were recorded. Animals were tested once daily, 5 days a week, and testing was conducted at the same time each day.

Scoring

Performance data were recorded as the percentage of trials during which the appropriate maze arm was selected. Therefore, a score of 50% corresponds roughly to chance, and scores below 50% indicate perseveration and impaired performance. An animal was deemed appropriate for restraint after achieving scores between 60-80% on 2 consecutive days of testing. On the third day, all male subjects were considered candidates for restraint. Female subjects were restrained only if vaginal lavage suggested they would be in either estrus or proestrus, the 2 cycle stages of interest, on the day of

restraint. Female subjects thus potentially could be tested under 6 different testing conditions. However, due to a variety of circumstances, not all data points were collected for each animal.

Inter-trial delay

Between trials, subjects experienced a brief delay. Initially, the inter-trial delay was minimized, of duration adequate only for the previously selected arm to be cleansed with alcohol (~ 2 sec). Cleansing was performed to eliminate the potential influence of olfactory cues on subsequent arm choice. If a subject scored 80% or higher on consecutive days, the inter-trial delay was increased by 5 seconds. The delay was continually raised by 5 second increments as needed to maintain average scores no higher than 80%.

Restraint

Prior to testing, subjects were confined to a plastic restraint tube variably for 1 hour or 2 hours. Control conditions consisted of the subject remaining unrestrained in its home cage in the testing room for 1 hour prior to testing. The order in which subjects underwent the various testing conditions (3 total for males, 6 for females) was randomized. Furthermore, testing sessions done subsequent to restraint were separated temporally by at least one week for any individual subject in order to minimize the effect of habituation to restraint on performance outcomes.

Statistical Analysis

Mean performance was determined for each of the three subject groups (males, females in proestrus, females in estrus) for each of the three testing conditions (control, 1 hour of restraint, 2 hours of restraint). T test analysis was employed to determine statistical differences within each subject group across any 2 testing conditions. T test analysis was similarly used to determine differences between 2 subject groups under the same testing condition. Repeated measures ANOVA was utilized to identify significant variance in performance across subject groups and within subject groups under alternative testing conditions. Mean number of perseverative errors and mean task completion times were also calculated for all subject groups across testing conditions. Pearson R values were calculated to determine any association between perseveration and time required to complete the task. In addition, ANOVA was employed to detect differences in task completion time across subject groups and testing conditions. If significant variance was found, tests of effects were performed to identify the specific source(s) of variance. Finally, ordinal values were assigned to each testing condition, and T test analysis was utilized to evaluate any incidental patterns in testing order.

My responsibilities

My responsibilities in the present study involved cognitive testing of female subjects. Subjects had completed maze habituation immediately prior to the onset of my participation and were at variable stages of learning the task; some subjects were immediately ready for testing, whereas others required a longer learning phase. On a daily basis, I conducted delayed alternation task testing with each of the 10 female rats. I

administered rewards and recorded each subject's respective performance with regard to overall score and task completion time.

Subsequent to task completion, I provided each animal with a daily ration of rat chow. When all subjects completed testing, I performed vaginal lavage on all animals for estrous cycle stage determination. I prepared all specimens and examined them by light microscopy. Subjects expected to be in estrus or proestrus the following day were identified as candidates for formal testing, either under control conditions or following restraint stress. After a subject underwent formal testing, I again lavaged the animal in order to confirm an accurate prediction of estrous cycle stage. In the event of a discrepancy between predicted and actual phase, performance data were discarded.

I entered performance data daily in spreadsheets and maintained a log of estrous cycle stage for each subject. After all performance data were collected, I participated in the analysis of acquired data from all subjects, both male and female.

I did not contribute to the processes of protocol development or subject habituation, nor was I involved in the training or testing of male subjects. After my involvement in data collection ceased, another study participant performed additional cognitive testing of the female subjects to augment the data set.

Results

PFC function was measured as a function of performance on the T-maze task, a delayed alternation task. After initially selecting one arm of the maze, the subject was required to choose the opposite arm on the subsequent trial and to continually choose alternating arms for a total of 10 trials, excluding initial arm choice. Performance was quantified as a percent of correctly performed trials, those for which the subject alternated its choice of maze arm.

Performance

Baseline Performance

In the absence of restraint stress, T-maze performance was comparable across subject groups. The mean scores achieved respectively by males, females in estrus, and females in proestrus were 75% (n=8, SD+/-5.3, SEM+/-1.9), 73% (n=9, SD+/-7.1, SEM+/-2.4), and 70% (n=6, SD+/-8.9, SEM+/-3.7). As analyzed by T test, no appreciable differences in performance existed between males and females in estrus ($p=0.6$), males and females in proestrus ($p=0.2$), nor between females under high and low estrogen exposure ($p=0.4$).

Performance following 1 hour of restraint stress

Subsequent to 1 hour of restraint stress, performance among both males and females in estrus showed no significant change from that achieved in the control trials ($p=0.6$ for males, $p=0.9$ for females in estrus). Among males, the mean score after restraint was 71% (n=8, SD+/-19.6, SEM+/-6.9), with the considerable standard deviation largely attributable to 2 outlying scores of 40% and 100%. The mean score among females in

estrus was 74% (n=9, SD+/-11.3, SEM+/-3.8), which actually represented an improvement over baseline performance. Females in proestrus, however, exhibited a significant decline in performance outcome as compared to control conditions ($p=0.0017$), with a mean score of 50% (n=10, SD+/-10.5, SEM+/-3.3) (Fig. 1). Furthermore, after 1 hour of restraint, females in proestrus performed significantly worse than females in estrus ($p=0.00014$). ANOVA with repeated measures demonstrated a significant between subjects effect ($F [2,14] = 6.14$; $p<0.01$) and within subjects effect of restraint ($F [2,14] = 7.31$; $p<0.003$). Analysis revealed a significant interaction between estrogen status and performance only after 1 hour of restraint ($F [2,14] = 14.0$; $p<0.0005$), whereas no appreciable effect of estrogen status on performance was observed under control conditions or subsequent to 2 hours of restraint ($p>0.1$).

Performance following 2 hours of restraint stress

After 2 hours of restraint, performance suffered among both the males and the females in estrus. As compared to performance under control conditions, the decline for males reached statistical significance ($p=0.01$) with a mean score of 58% (n=5, SD+/-14.8, SEM+/-6.6). The mean score for females in estrus was identical to that of the males at 58% (n=4, SD+/-18.9, SEM+/-9.5). This, too, constituted a significant impairment compared to baseline performance ($p=0.04$). Among females in proestrus, the mean score was 60% (n=6, SD+/-12.6, SEM+/-5.2); although scores among females in proestrus remained depressed compared to baseline performance, they did not differ significantly from those achieved after only 1 hour of restraint ($p=0.11$), and were strikingly similar to those among males or females in estrus after prolonged restraint.

Figure 1. Task performance following restraint stress.

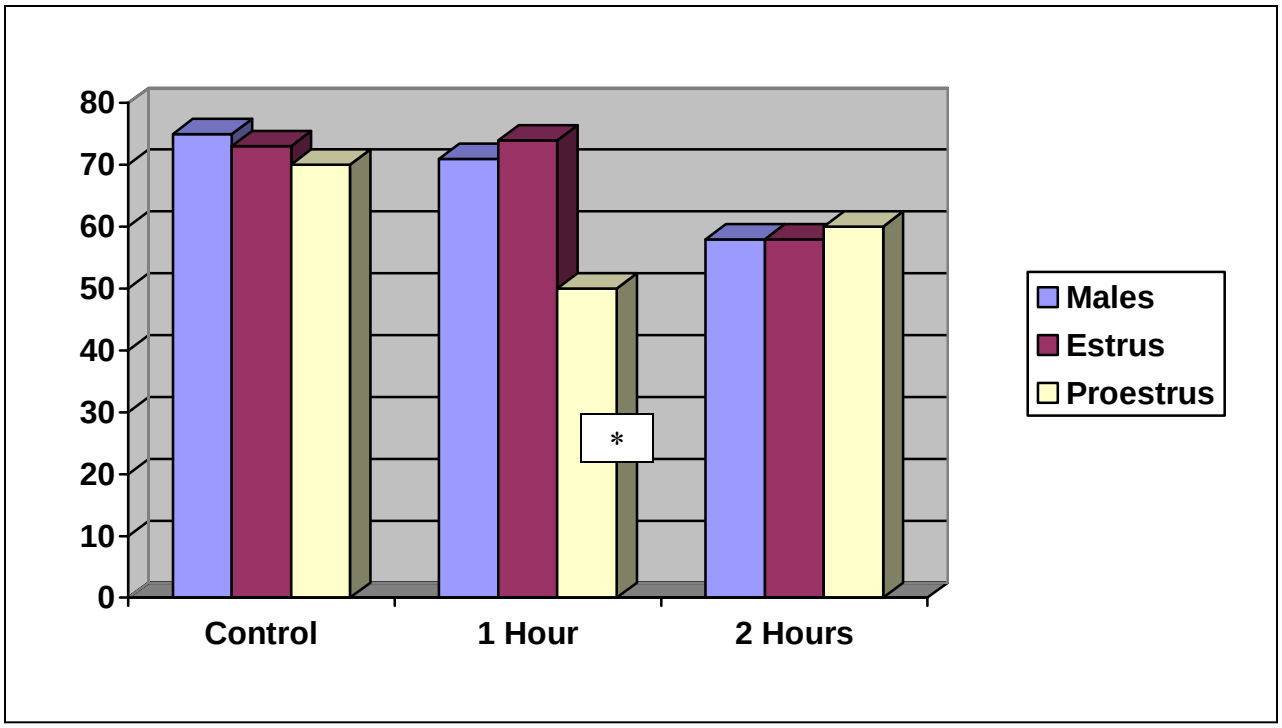


Figure 1. Performance data for all three subject groups across various study conditions. Y-axis values represent mean percentage of correctly performed trials. No significant differences in performance were evident under control conditions, nor after 2 hours of restraint stress. However, after 1 hour of restraint, females in proestrus demonstrated significant impairment in working memory, whereas males and females in estrus were not similarly impaired.

* - denotes significance

Perseveration

Perseveration was quantified as the maximum number of consecutive trials in a single testing period during which a subject selected the same arm of the maze. The mean number of perseverative errors committed by males in the absence of restraint stress was 2.3 (n=8, SD+/-0.46, SEM+/-0.2), and errors were committed at a comparable rate by females in both estrus and proestrus, with means of 2.6 (n=8, SD+/-0.74, SEM+/-0.3) and 2.4 (n=5, SD=/-0.6, SEM+/-0.3), respectively (Fig. 2).

After 1 hour of restraint, males and females in estrus achieved the same mean error rate of 2.5 errors (n=8, SD=/-0.8, SEM+/-0.3 for both subject groups) for each testing session. Females in proestrus, however, committed an average of 4.1 errors (n=9, SD+/-1.3, SEM+/-0.4). As evaluated by T test, the greater perseveration rate evident among females in proestrus after 1 hour of restraint did not achieve statistical significance. However, a trend toward increased perseverative error was observed among females in proestrus compared to females in estrus when both groups were tested subsequent to 1 hour of restraint stress (p=0.06).

After prolonged restraint, this disparity in perseverative error rate among subject groups disappeared. Males committed a mean error number of 3.2 (n=5, SD+/-0.84, SEM+/-0.4) after 2 hours of restraint, and the average perseverative error rate was 3.3 for both females in estrus (n=4, SD+/-1.3, SEM+/-0.7) and females in proestrus (n=3, SD+/-2.3, SEM+/-1.6).

Figure 2. Perseverative Error Rate

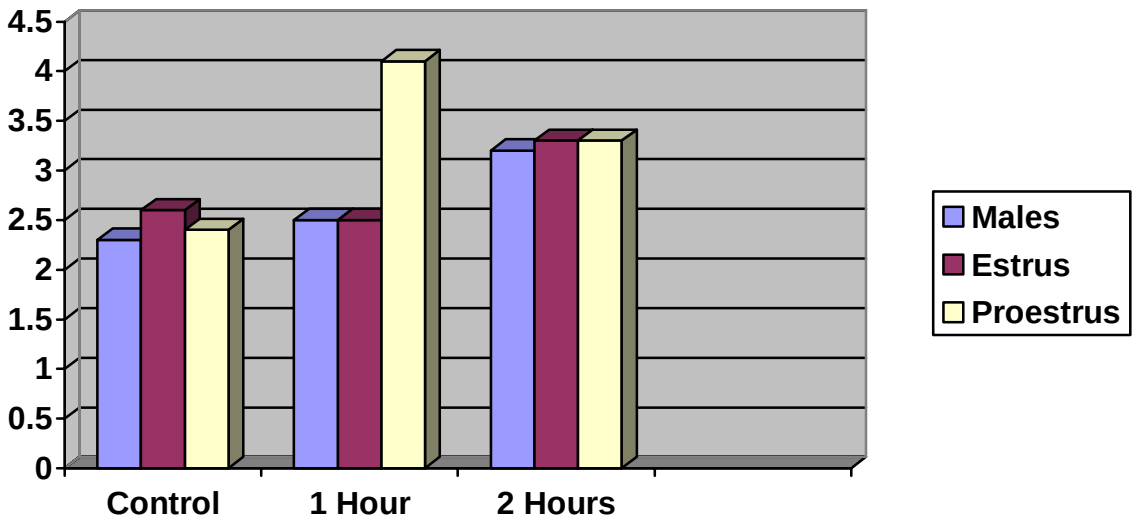


Figure 2. Mean number of perseverative errors committed across subject groups under different testing conditions. Y-axis represents mean number of errors for each testing session. Although no disparities in error rate achieved statistical significance, a trend toward increased perseveration among females in proestrus exposed to 1 hour of restraint stress was observed.

Task Completion Time

Task completion time was defined as the time elapsed between a subject's release into the T-maze and its final selection of maze arm upon completion of 10 alternating trials. Inter-trial delays were subtracted from time-to-finish values for those rats with inter-trial delays of 5 seconds or longer. T test analysis revealed an association between time-to-finish and testing condition exclusively among males, who completed the T maze task significantly faster when unrestrained than after 1 hour of restraint ($p=0.02$). No comparable association characterized task completion time among unrestrained and restrained females in estrus ($p=0.09$) or proestrus ($p=0.66$). Repeated-measures ANOVA revealed no between subjects effect of estrogen status on task completion time. However, a significant within subjects effect of restraint stress was observed ($F [2,13] = 6.8$; $p<0.005$). This result appears consistent with a freezing response subsequent to restraint stress. A small but significant interaction was identified between estrogen status and restraint ($F [4,26] = 3.14$; $p=0.03$), which tests of effects revealed to be a product of differences in task completion time under control conditions, with males completing the task faster than females ($F [2,13] = 3.19$; $p=0.075$). No significant differences in task completion time were observed across subject groups subsequent to either 1 or 2 hours of restraint ($p=0.63$, $p=0.48$).

Finally, Pearson R values were calculated to assess a possible association between perseveration and time-to-finish. This analysis was performed to evaluate the possibility of increased error rate representing an artifact of longer test completion times. If females in proestrus simply performed the task more slowly, they would assume a greater working memory burden to achieve comparable scores; each previous choice of maze

arm would have to be stored accessibly for a longer duration. However, no such association between task completion time and error rate existed for female subjects under any testing condition (R values ranged between -0.28 and 0.34). To the converse, females in proestrus had shorter task completion times after both 1 and 2 hours of restraint stress than their estrus-phase counterparts. Among males, a trend toward a positive correlation between task completion time and error rate was observed (R value = 0.67; $p>0.05$ for all other R values) (Table 1).

Table 1. Task Completion Time and Error Rate

Subject Group	Mean Time (min)	Mean Errors	Pearson R Value
Control/Estrus	4.3	2.6	0.19
Control/Proestrus	4.7	2.4	0.23
Control/Males	1.8**	2.3	0.44
1 Hour/Estrus	5.8	2.5	0.34
1 Hour/Proestrus	5.3	4.1*	0.15
1 Hour/Males	4.5	2.5	-0.14
2 Hour/Estrus	4.8	3.3	-0.10
2 Hour/Proestrus	3.8	3.3	-0.28
2 Hour/Males	5.0	3.2	0.67*

Table 1. Task completion times and mean errors committed by subjects across testing conditions. Male subjects completed the task significantly faster under unstressed conditions than following restraint stress. Among female subjects, no significant differences were evident in time-to-finish or perseverative error rate, although a trend toward increased error commission was observed among females in proestrus subject to 1 hour of restraint stress. No significant correlations existed between task completion time and perseverative error rate.

* - denotes trend

** - denotes significance

Testing order

The order in which rats were exposed to various testing conditions was assigned randomly for individual subjects. Subjects potentially could habituate to restraint stress, so a consistently applied sequence of testing conditions might inflate performance under conditions tested later as compared to those tested initially, rather than reflect an estrogen-mediated effect. To ensure that no pattern incidentally emerged among female subjects, ordinal values (1-6) were designated for each testing condition (control, 1 hour of restraint, 2 hours of restraint in estrus or proestrus) according to the order in which a given subject had been tested. T test analysis of ordinals revealed no significant association between the timing of exposure to 1 hour of restraint stress and estrous cycle stage at the time of exposure ($p=0.12$).

Male subjects were assigned ordinal values of 1-3 corresponding to testing conditions. No significant pattern of testing order was observed.

Discussion

The results from this study underscore the importance of context in estrogen-mediated effects on cognitive function. Whereas no significant differences in task performance existed among subject groups at baseline, female subjects in proestrus exhibited marked impairment in working memory after 1 hour of restraint stress. This observed decline in score corresponded to an increased rate of perseverative errors, and it further did not appear to be an artifact of differences in task completion time or testing order. However, in the absence of restraint stress, female subjects in proestrus performed comparably both to females in estrus and to males, demonstrating that estrogen exposure alone was not sufficient to impair PFC function.

Of note, no discrepant performance was observed between females in estrus and males subsequent to restraint stress. This comparability of performance is essential for establishing that no baseline, sex-related differential in stress response existed among study subjects. Any baseline differential would obscure distinctions between cognitive effects secondary to estrogen exposure and those potentially due to other sex-based differences in stress response. Multiple studies, for example, have reported disparate corticosterone release in male and female rats, and corticosterone augments the stress response in the PFC by down-regulating catecholamine transporters, augmenting the detrimental effects of NE α-1 receptor binding. Thus, if corticosterone appeared to exert independent effects on PFC function, it could prevent the effective isolation of estrogen exposure as an experimental variable. Clearly, however, the absence of a baseline differential does not preclude a contributory role of corticosterone in PFC impairment,

and synergism between estrogen and corticosterone represents an important object of future investigation.

Resultant data, then, support the role of elevated endogenous estrogen in impairing PFC function, but exclusively under conditions of acute stress.

Limitations of the Study

Interpretation of data from the present study must be rendered with consideration of several potential sources of error. Perhaps most important, the role of progesterone in the acquired results merits attention. Progesterone, too, has prominent albeit as yet poorly delineated activity in the brain. Furthermore, available data refute the simplistic conceptualization of progesterone as merely counter-regulatory balance for estrogen. Data from Tanabe et al (2004), for example, identify the capacity of progesterone replacement alone to restore spatial working memory after the development of scopolamine-associated cognitive deficits in rats (47). These data are consistent with prior studies suggesting both neuroprotective and antidepressant properties of progesterone. In the absence of scopolamine administration, however, data from the same study indicate that isolated progesterone replacement was not sufficient to generate improved spatial memory function, whereas such improvement was observed with either estrogen alone or estrogen and progesterone replacement. Additional studies of ovariectomized animals also demonstrate the ability of estrogen to independently effect changes in cognitive function and stress response (48). Therefore, for purposes of the present study, ample evidence exists to support the interpretation of resultant data as a

reflection of differential estrogen exposure. The concomitant role of progesterone, however, clearly merits further exploration.

A benefit of this study's methodology also poses a potential shortcoming. All data were gathered exclusively under exposure to endogenous estrogen, thus assuring physiologic accuracy of both hormone form and exposure levels. However, estrogen exposure consequently was not standardized across subjects, as opposed to studies of ovariectomized subjects with subsequent, exogenous estrogen administration. Therefore, the states of high and low estrogen exposure were relative constructs rather than absolute, quantifiable variables. Similarly, the degree of flux of endogenous estrogen exposure undoubtedly varied across subjects. To mitigate the effect of this variability on results, each individual served as its own control, with each subject tested under as many experimental conditions as possible. Furthermore, as the complete absence of estrogen does not characterize normal physiology, the methodology employed may better approximate naturally occurring interactions between variable estrogen exposure and acute stress response.

As with any experiment, limitations of subject number present a potential challenge to data interpretation. Each subject serving as its own control partially attenuated the low overall subject number; however, due to a variety of circumstances, not all female subjects underwent all six potential testing conditions. Though a conceivable weakness of the study, this would in fact be more likely to obscure significant, estrogen-mediated effects rather than to indicate a significant effect where none exists. The generation of significant findings, therefore, actually may reflect the strength of estrogen's role in prefrontal cognition and stress response rather than an insufficiency of subjects.

Subject habituation to restraint stress also may have impacted performance data. Habituation could occur in the short term, enabling some waning of an acute stress reaction over more prolonged periods of restraint. Working memory deficits evident across all subject groups after 2 hours of restraint, however, argue against this process. Conversely, under conditions of sufficient stress, elevated estrogen exposure may prove unappreciable, a nominally additive effect. Such a phenomenon might underlie the absence of a statistically significant difference in task performance between females in estrus and proestrus after 2 hours of restraint. Whereas significant impairment was observed exclusively in the proestrus group after 1 hour of restraint, cognitive performance suffered uniformly across subject groups subsequent to 2 hours of restraint.

Also meriting consideration, long-term acclimation to restraint may have affected resultant data; restraint stress might be expected to produce more severely impaired performance earlier in the study period when restraint constituted a novel stress. Conceivably, then, a decline in performance attributed to greater estrogen exposure actually would reflect the incidental testing of animals in proestrus sooner than in estrus. To address the potential confounding effects of long-term habituation, testing order was analyzed using an ordinal system, and no significant pattern of testing order was evident. Therefore, a substantial effect of habituation on testing outcome appears unlikely.

Finally, characterization of testing conditions as high or low endogenous estrogen exposure was predicated on histologic interpretation of vaginal lavage specimens. If epithelial cells were not definitively consistent with a particular estrous cycle phase, no assignment of cycle stage was made, and lavage was repeated on the following testing

day. Though an unlikely source of error, the identification of estrous cycle phase remained subject to inaccurate interpretation.

Despite these potential limitations of the study, resultant data nonetheless provide striking evidence of a detrimental effect on working memory associated with concomitant exposure to acute stress and elevated endogenous estrogen. A mechanistic understanding of estrogen's influence on brain neurochemisty is continually improving, with available animal data demonstrating direct relationships between estrogen and norepinephrine, serotonin, dopamine, acetylcholine, and corticosterone. Of critical importance will be the further delineation of estrogen's interaction with other neurotransmitters specifically within the PFC, as this region is most centrally implicated in the pathogenesis of MDD. Consequently, the elucidation of estrogen's role in the PFC requires a perspective of dynamism, the incorporation of a number of critical variables that will produce different functional or behavioral phenotypes. Contextual variables that merit discussion include the brain region subject to estrogen release; environmental factors, particularly with respect to stress as it affects neurotransmitter activity; and temporal variables, include both timing of estrogen exposure and stage of physiologic development. Importantly, exploration of these variables relies predominantly on animal studies, and, therefore, even an exhaustive discussion of research to date cannot address perhaps the most critical question, that of the degree to which these and similar data are applicable to human models of MDD and estrogen's role therein.

Brain Region Specificity

The data from the present study underscore the context-dependent nature of estrogen-mediated activity in the brain. Consequences of estrogen exposure first must be considered in terms of regional specificity. Particularly with regard to the hippocampus, estrogen produces neuroprotective and cognitive-promoting effects. In addition to data previously cited, Leuner et al (2004) found high doses of estrogen to confer improved associative memory in ovariectomized rats (49). Similarly, data from Bodensteiner et al (2005) showed that estrogen administration was associated with decreased perseveration and improved spatial memory in rats performing a Morris water maze task (50). Other examples of estrogen's cognition-enhancing effects specific to the hippocampus abound in the literature (51, 52, 53). In striking contrast, the present study indicates estrogen-induced impairment of working memory, a prefrontal cognitive modality, and estrogen moreover has been associated with diminished striatal cognition. In an effort to examine estrogen's role across cognitive strategies directly, Davis et al (2005) recently compared hippocampal and striatal learning in ovariectomized rats. Resultant data demonstrated estrogen-induced enhancement of place learning but not response learning, thus supporting a hippocampus-specific benefit of estrogen replacement (54). These results, then, provide direct evidence of the importance of regional context in understanding estrogen's effects on learning and memory. Further supporting this perspective, data from Korol et al (2004) demonstrate that estrogen exposure causes shifts in learning strategy in rats, with higher estrogen levels favoring hippocampus-mediated learning and lower estrogen levels favoring striatal learning strategies. Increased estrogen was associated with decreased GABA signaling in the hippocampus, and the authors posit that

the consequent, diminished inhibitory tone enhances place learning, a hippocampal cognitive function. These shifts in learning strategy were observed under both exogenous and endogenous variant estrogen exposure, occurring not only in ovariectomized rats with estrogen replacement but also in naturally cycling subjects; animals preferentially employed place learning during proestrus and response learning during estrus. Despite these shifts in strategy, however, no differences in absolute learning ability were observed (55, 56). From a purely teleological perspective, an estrogen-mediated effect on learning strategy appears adaptive, as learning and memory imperatives vary across the reproductive cycle with the respective demands of mating, pregnancy, and the post-partum.

An additional contextual variable that merits attention is degree of estrogen exposure. Many available data derive from studies employing exogenous estrogen administration, though no universal, ideal concentration of estrogen replacement has been established. Similarly, methodologies that involve endogenous estrogen exposure suffer from lack of standardized exposure across subjects. For the interpretation of these data, dopamine activity in the PFC may prove instructive. Dopamine receptor (DR) activation is characterized by an inverted "U" effect on working memory performance, with cognitive impairment evident at both extremes of DR-1 stimulation (57). Working memory may prove contingent on an inverted "U" model of ER activation, as well, so experimental outcomes may vary with the concentration of administered or endogenous estrogen.

Estrogen, Time, and Stress

The importance of temporality is highly relevant to estrogen exposure, as well, with regard to both the pattern of estrogen administration and the timing of exposure during the course of physiologic development. Work by Gulinello et al (2005) provides impressive evidence for the influence of timing on outcome of estrogen administration. Estrogen was delivered both acutely and chronically after precipitation of global ischemic brain injury in female rats. Acute estrogen administration was associated with increased survival of neurons in the CA1 region of the hippocampus but did not confer protective effects on spatial memory. In contrast, long-term estrogen therapy appeared to protect both spatial and visual memory. (58)

Data from Hodes and Shors (2005) further support the importance of time in estrogen-related effects with regard to developmental stage. The observed differential stress response in male and female rats was specific to adult animals and did not characterize pre-pubertal animals (59). Echoing this theme, estrogen was associated with deficits in both visual discrimination performance and spatial memory when administered to pre-pubertal rhesus monkeys (60). The promoting effects of estrogen on hippocampal cognition, then, appear particular to physiologically appropriate exposure, as premature exposure conferred detrimental effects. Of additional interest, the data from this study suggest that the acquired cognitive deficits endured beyond the period of exogenous estrogen exposure. Thus, estrogen is implicated not only as an agent of immediate influence on learning but also one responsible for long-term modeling of cognitive function. Importantly, these data pertain to the hippocampus rather than the PFC; however, they powerfully suggest the consideration of temporal variables in further

exploration of the relationship between estrogen exposure and the PFC. Critically, then, data from the present study must be understood as contextually pertinent to the adult, reproductive stage of life, with variable estrogen exposure that is physiologically appropriate as determined by the estrous cycle.

The influence of estrogen is not merely region-specific and time-dependent; a critical consideration, too, is the concomitant presence of stress and the timing, severity, and perceived uncontrollability thereof. The work of Shors et al (1998) lends support for the state-dependent importance of estrogen in learning and memory; although the data indicate that estrogen appears to promote associative learning in an unstressed state, estrogen exposure was correlated with impaired learning after an acute stress in female rats (61). A different relationship between estrogen and stress appears relevant to the PFC. In the present study, estrogen exposure alone had no significant effect on working memory. However, when estrogen and an acute stress response were confluent in the PFC, a substantial impairment in cognitive performance was observed. Thus, whereas stress appears to derail an otherwise enhancing effect of estrogen on hippocampal cognition, estrogen and stress effect a synergistic diminution in prefrontal cognitive ability.

Stress, however, cannot be viewed as a singular contextual variable, but rather must be characterized further with regard to pattern of stress exposure and the perceived degree of controllability of the stressor. Epidemiologic data specifically implicate chronic stress exposure and a high degree of perceived uncontrollability as risk factors for the development of MDD. Acute stress triggers catecholamine and corticosterone release in rats, and although these neurotransmitters may remain elevated even after the

removal of the stressor, they return to pre-stress levels soon thereafter (62). Correspondingly, in the present study, female subjects exhibited impaired working memory during proestrus immediately following restraint; however, on subsequent trials, these animals fully recovered their baseline performance. In contrast to acute stress, prolonged stress exposure has the capacity to effect long-term changes in neurotransmitter signaling; with sufficient time, stress can mediate remodeling processes associated with neuronal plasticity. These remodeling processes have been demonstrated clearly in the primary stress response. Recently, Bhatnagar et al (2005) published evidence of the effect of prenatal exposure to stress on long-term conditioning of the stress response, data that moreover demonstrated a sex-based differential in conditioning outcome (63). Indirect evidence supports a comparable plasticity in the hippocampus in response to chronic stress exposure, as well. Leuner et al (2004) found that under conditions of persistent, uncontrollable stress, female rats recovered performance of a hippocampus-mediated task with long-term fluoxetine treatment; recovery of baseline performance was not observed with acute treatment alone (64). The unique benefit of long-term treatment suggests the undoing of a remodeling process effected by chronic stress exposure. Of note, the same study demonstrated the profound significance of perceived uncontrollability in stress exposure, as the ability to control a stressor successfully mitigated the otherwise detrimental effect of stress on learning in female subjects. With regard to the present study, then, the question arises as to whether a sustained impairment of PFC function would be observed under conditions of chronic, uncontrollable stress, as with daily confinement of subjects to the restraint tubing. Such investigation would more closely approximate the posited relationship between chronic

stress and MDD; mood disorders tend not to emerge after a single, episodic stressor but rather under conditions of prolonged stress with the perception of uncontrollability. Time is essential not simply for stress to generate an instantaneous neurochemical milieu, but rather to potentiate differential responses to subsequent, stressful stimuli by means of neuronal network remodeling.

Again, such studies underscore the necessity of viewing the interaction between stress and cognition in a highly contextualized framework. Stress has been demonstrated both to improve and derail facets of cognition, with variable results contingent on the particular cognitive task and associated brain region, the timing and severity of the stressor, and the degree of perceived uncontrollability of the stressor.

Conclusion

The immediate application of animal data to humans is clearly inappropriate, but evidence derived from human studies does appear to recapitulate the basic themes emerging from animal research. Specifically, these data collectively support the interface of estrogen and the stress response in the PFC, with functional outcome variably determined by duration of stress, degree of perceived uncontrollability of the stressor, stage of physiologic development, and level of estrogen exposure. In conclusion, then, data from the present study offer further support for a model of differential susceptibility to mood disorders; such disorders certainly cannot be explained by simplistic models of derangements in hormone levels but rather develop from the intricate interaction of ovarian hormones and environmental exposures within a plastic neuronal framework. Despite its remarkable complexity, this model of differential susceptibility nonetheless

offers certain thematic interactions that may inform subsequent research. Among these fundamental themes are estrogen's region-specific activity in the brain, synergism between estrogen exposure and the stress response in the PFC, and the importance of variables of time, including duration of estrogen or stress exposure and stage of physiologic development. The present study provides substantial evidence of the functional importance of estrogen and the stress response when confluent in the PFC. Continued exploration of the impact of ovarian hormones on prefrontal cognition will undoubtedly promise further insights into the sex-related variance in the prevalence of MDD and other mood disorders.

References

1. Risch, N. 1990. Genetic linkage and complex diseases, with special reference to psychiatric disorders. *Genet Epidemiol*. 7(1):3-16.

2. Weissman, M.M., Bland, R.C., Canino, G.J., Faravelli, C., Greenwald, S., et al. 1996. Cross-national epidemiology of major depression and bipolar disorder. *JAMA*. Jul 24-31;276(4):293-9.

3. Rubinow, D.R., Hoban, M.C., Grover, G.N. 1987. Menstrually-related mood disorders. *Adv Biochem Psychopharmacol*. 43:335-46.

4. Kendler, K.S, Kuhn, J., Prescott, C.A. 2004. The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *Am J Psychiatry*. Apr;161(4):631-6.

5. Rodriguez, B.F., Weisberg, R.B, Pagano, M.E., Machan, J.T., Culpepper, L., Keller, M.B. 2004. Frequency and patterns of psychiatric comorbidity in a sample of primary care patients with anxiety disorders. *Compr Psychiatry*. Mar-Apr;45(2):129-37.

6. Hasin, D.S, Goodwin, R.D., Stinson, F.S., Grant, B.F. 2005. Epidemiology of major depressive disorder: results from the National Epidemiologic Survey on Alcoholism and Related Conditions. *Arch Gen Psychiatry*. Oct;62(10):1097-106.

7. Zubenko, G.S., Hughes, H.B., Maher, B.S., Stiffler, J.S., Zubenko, W.N., Marazita, M.L. 2002. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset, major depression. *Am J Med Genet*. Dec 8;114(8):980-7.

8. Tsai, S.J., Wang, Y.C., Hong, C.J., Chiu, H.J. 2003. Association study of oestrogen receptor alpha gene polymorphism and suicidal behaviours in major depressive disorder. *Psychiatr Genet*. Mar;13(1):19-22.

9. Perlman, W.R., Webster, M.J., Kleinman, J.E., Weickert, C.S. 2004. Reduced glucocorticoid and estrogen alpha messenger ribonucleic acid levels in the amygdale of patients with major mental illness. *Biol Psychiatry*. Dec 1;56(11):844-52.

10. Perlman, W.R., Tomaskovic-Crook, E., Montague, D.M., Webster, M.J., Rubinow, D.R., et al. 2005. Alteration in estrogen receptor alpha mRNA levels in frontal cortex and hippocampus of patients with major mental illness. *Biol Psychiatry*. Nov 15;58(10):812-24.

11. Choi, J.M., Romeo, R.D., Brake, W.G., Bethea, C.L., Rosenwaks, Z., McEwen, B.S. 2003. Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (Macaca mulatta). *Endocrinology.* Nov;144(11):4734-8

12. Granholm, A.C., Sanders, L., Seo, H., Lin, L., Ford, K., Isacson, O. 2003. Estrogen alters amyloid precursor protein as well as dendritic and cholinergic markers in a mouse model of Down Syndrome. *Hippocampus*. 13(8):905-14.

13. Gulinello, M., Lebesgue, D., Jover-Mengual, T., Zukin, R.S., Etgen, A.M. 2005. Acute and chronic estradiol treatments reduce memory deficits induced by transient global ischemia in female rats. *Horm Behav*. Aug 25;[Epub ahead of print]

14. Luine, V.N., Jacome, L.F., Maclusky, N.J. 2003. Rapid enhancement of visual and place memory by estrogen in rats. *Endocrinology*. Jul;144(7):2836-44.

15. Carrer, H.F., Araque, A., Buno, W. 2003. Estradiol regulates the slow Ca2+-activated K+ current in hippocampal pyramidal neurons. *J Neurosci*. Jul 16;23(15):6338-44.

16. Marriott, L.K., Korol, D.L. 2003. Short-term estrogen treatment in ovariectomized rats augments hippocampal acetylcholine release during place learning. *Neurobiol Learn Mem*. Nov;80(3):315-22.

17. Daniel, J.M., Hulst, J.L., Lee, C.D. 2005. Role of hippocampal M2 muscarinic receptors in the estrogen-induced enhancement of working memory. *Neuroscience*. 132(1):57-64.

18. Gibbs, R.B. 2002. Basal forebrain cholinergic neurons are necessary for estrogen to enhance acquisition of a delayed matching-to-position T-maze task. *Horm Behav*. Nov;42(3):245-57.

19. Daniel, J.M., Hulst, J.L., Lee, C.D. 2005. Role of hippocampal M2 muscarinic receptors in the estrogen-induced enhancement of working memory. *Neuroscience*. 132(1):57-64.

20. Daniel, J.M., Hulst, J.L., Lee, C.D. 2005. Role of hippocampal M2 muscarinic receptors in the estrogen-induced enhancement of working memory. *Neuroscience*. 132(1):57-64.

21. Rocha, B.A., Fleischer, R., Schaeffer, J.M., Rohrer, S.P., Hickey, G.J. 2005. 17 Beta-estradiol-induced antidepressant-like effect in the forced swim test is absent in estrogen receptor-beta knockout (BERKO) mice. *Psychopharmacology.* May;179(3):637-43.

22. Andrade, T.G., Nakamuta, J.S., Avanzi, V., Graeff, F.G. 2005. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT1A receptors. *Behav Brain Res.* Aug 30;163(1):18-25.

23. Korol D.L. 2004. Role of estrogen in balancing contributions from multiple memory systems. *Neurobiol Learn Mem.* Nov;82(3):309-23.

24. Shors, T.J., Lewczyk, C., Pacynski, M., Mathew, P.R., Pickett, J. 1998. Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport*. Feb 16;9(3):419-23.

25. Arnsten, A.F. 2000. Stress impairs prefrontal cortical function in rats and monkeys: role of dopamine D1 receptor and norepinephrine α-1 receptor mechanisms. *Prog Brain Res.* 126:183-192.

26. Levesque, J., Eugene, F., Joanette, Y., Paquette, V., Mensour, B., et al. 2003. Neural circuitry underlying voluntary suppression of sadness. *Biol Psychiatry.* Mar 15;53(6):502-10.

27. Drevets, W.C., Videen, T.O., Price, J.L., Preskorn, S.H., Carmichael, S.T., et al. 1992. A functional anatomical study of unipolar depression. *J Neurosci*. Sep; 12(9): 3628-41.

28. Mayberg, H.S. 1994 Frontal lobe dysfunction in secondary depression. *J Neuropsychiatry Clin Neurosci*. Fall; 6(4): 428-42.

29. Arnsten, A.F. 2000. Stress impairs prefrontal cortical function in rats and monkeys: role of dopamine D1 receptor and norepinephrine α-1 receptor mechanisms. *Prog Brain Res.* 126:183-192.

30. Birnbaum, S.G., Podell, D.M., Arnsten, A.F. 2000. Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug FG7142 in rats. *Pharmacol Biochem Behav.* Nov;67(3):397-403.

31. Bhatnagar, S., Lee, T.M., Vining, C. 2005. Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats. *Horm Behav.* Apr;47(4):430-8.

32. Bao, A.M, Hestiantoro, A., Van Someren, E.J., Swaab, D.F., Zhou, J.N. 2005. Colocalization of corticotrophin-releasing hormone and oestrogen receptor-alpha in the paraventricular nucleus of the hypothalamus in mood disorders. *Brain.* Jun;128(Pt 6):1301-13.

33. Berman, K.F., Schmidt, P.J., Rubinow, D.R., Danaceau, M.A., Van Horn, J.D., et al. 1997. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. *Proc Natl Acad Sci USA*. Aug 5;94(16):8836-41.

34. Kritzer, M.F., Kohama, S.G. 1998. Ovarian hormones influence the morphology, distribution, and density of tyrosine hydroxylase immunoreactive axons in the dorsolateral prefrontal cortex of adult rhesus monkeys. *J Comp Neurol*. May 25;395(1):1-17.

35. Luine, V.N., Richards, S.T., Wu, V.Y., Beck, K.D. 1998. Estradiol enhances learning and memory in a spatial memory task and affects levels of monoaminergic neurotransmitters. *Horm Behav*. Oct;34(2):149-62.

36. Becker, J.B. 2000. Oestrogen effects on dopaminergic function in striatum. *Novartis Found Symp*. 230:134-45. Review.

37. Birnbaum, S.G., Podell, D.M., Arnsten, A.F. 2000. Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug FG7142 in rats. *Pharmacol Biochem Behav.* Nov;67(3):397-403.

38. Garcia-Segura, L.M., Azcoitia, I., DonCarlos, L.L. 2001. Neuroprotection by estradiol. *Prog Neurobiol*. Jan;63(1):29-60. Review.

39. Karkanias, G.B., Li, C.S., Etgen, A.M. 1997. Estradiol reduction of alpha 2-adrenoceptor binding in female rat cortex is correlated with decreases in alpha 2A/D-adrenoceptor messenger RNA. *Neuroscience*. Dec;81(3):593-7.

40. Arnsten, A.F. 2000. Stress impairs prefrontal cortical function in rats and monkeys: role of dopamine D1 receptor and norepinephrine α-1 receptor mechanisms. *Prog Brain Res.* 126:183-192.

41. Shansky, R.M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C. et al. 2004. Estrogen mediates sex difference in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry*. May;9(5):531-8.

42. Ling, S., Jamali, F. 2003. Effect of cannulation surgery and restraint stress on the plasma corticosterone concentration in the rat: application of an improved corticosterone HPLC assay. *J Pharm Pharm Sci.* May-Aug;6(2):246-51.

43. Nakahara, D., Nakamura, M. 1999. Differential effect of immobilization stress on in vivo synthesis rate of monoamines in medial prefrontal cortex and nucleus accumbens of conscious rats. *Synapse*. Jun 1;32(3):238-42.

44. Mark, G.P., Rada, P.V., Shors, T.J. 1996. Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. *Neuroscience.* Oct;74(3):767-74.

45. Nakane, H., Shimizu, N., Hori, T. 1994. Stress-induced norepinephrine release in the rat prefrontal cortex measured by microdialysis. *Am J Physiol*. Dec;267(6 Pt 2):R1559-66.

46. Shansky, R.M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C. et al. 2004. Estrogen mediates sex difference in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry*. May;9(5):531-8.

47. Tanabe, F., Miyasaka, N., Kubota, T., Aso, T. 2004. Estrogen and progesterone improve scopolamine-induced impairment of spatial memory. *J Med Dent Sci*. Mar;51(1):89-98.

48. Shansky, R.M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C. et al. 2004. Estrogen mediates sex difference in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry*. May;9(5):531-8.

49. Leuner, B., Mendolia-Loffredo, S., Shors, T.J. 2004. High levels of estrogen enhance associative memory formation in ovariectomized females. *Psychoneuroendocrinology*. Aug;29(7):883-90.

50. Bodensteiner, K.J., Cain, P., Ray, A.S., Hamula, L.A. 2005. Effects of pregnancy on spatial cognition in female Hooded Long-Evans rats. *Horm Behav*. Sep 2;[Epub ahead of print]

51. Luine, V.N., Richards, S.T., Wu, V.Y., Beck, K.D. 1998. Estradiol enhances learning and memory in a spatial memory task and affects levels of monoaminergic neurotransmitters. *Horm Behav*. Oct;34(2):149-62.

52. Daniel, J.M., Hulst, J.L., Lee, C.D. 2005. Role of hippocampal M2 muscarinic receptors in the estrogen-induced enhancement of working memory. *Neuroscience*. 132(1):57-64.

53. Gibbs, R.B. 2002. Basal forebrain cholinergic neurons are necessary for estrogen to enhance acquisition of a delayed matching-to-position T-maze task. *Horm Behav*. Nov;42(3):245-57.

54. Davis, D.M., Jacobson, T.K., Aliakbari S., Mizumori, S.J. 2005. Differential effects of estrogen on hippocampal- and striatal-dependent learning. *Neurobiol Learn Mem*. Sep;84(2):132-7.

55. Korol D.L. 2004. Role of estrogen in balancing contributions from multiple memory systems. *Neurobiol Learn Mem.* Nov;82(3):309-23.

56. McElroy, M.W., Korol, D.L. 2005. Intrahippocampal muscimol shifts learning strategy in gonadally intact young adult female rats. *Learn Mem.* Mar-Apr;12(2):84-5.
57. Lidow, M.S., Koh, P.O., Arnsten, A.F. 2003. D1 dopamine receptors in the mouse prefrontal cortex: immunocytochemical and cognitive neuropharmacological analyses. *Synapse.* Feb;47(2):101-8.

58. Gulinello, M., Lebesgue, D., Jover-Mengual, T., Zukin, R.S., Etgen, A.M. 2005. Acute and chronic estradiol treatments reduce memory deficits induced by transient global ischemia in female rats. *Horm Behav*. Aug 25;[Epub ahead of print]

59. Hodes, G.E., Shors, T.J. 2005. Distinctive stress effects on learning during puberty. *Horm Behav*. Aug;48(2):163-71.

60. Golub, M.S., Germann, S.L., Hogrefe, C.E. 2004. Endocrine disruption and cognitive function in adolescent female rhesus monkeys. *Neurotoxicol Teratol*. Nov-Dec;26(6):799-809.

61. Shors, T.J., Lewczyk, C., Pacynski, M., Mathew, P.R., Pickett, J. 1998. Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport*. Feb 16;9(3):419-23.

62. Nakane, H., Shimizu, N., Hori, T. 1994. Stress-induced norepinephrine release in the rat prefrontal cortex measured by microdialysis. *Am J Physiol*. Dec;267(6 Pt 2):R1559-66.

63. Bhatnagar, S., Lee, T.M., Vining, C. 2005. Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats. *Horm Behav.* Apr;47(4):430-8.

64. Leuner, B., Mendolia-Loffredo, S., Shors, T.J. 2004. Males and females respond differently to controllability and antidepressant treatment. *Biol Psychiatry*. Dec 15;56(12):964-70.