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Estrogen treatment impairs cognitive performance after psychosocial stress and monoamine depletion in postmenopausal women

Paul A. Newhouse, MD,[1] Julie Dumas, PhD,[1] Heather Wilkins, BA,[1] Emily Coderre, BA,[1] Cynthia K. Sites, MD,[2] Magdalena Naylor, MD, PhD,[1] Chawki Benkelfat, MD,[3] and Simon N. Young, PhD[3]

Abstract

Objective: Recent studies have shown that women experience an acceleration of cognitive problems after menopause and that estrogen treatment can improve or at least maintain current levels of cognitive functioning in postmenopausal women. However, we have previously shown that the negative emotional effects of psychosocial stress are magnified in normal postmenopausal women after estrogen treatment. This study examined whether estradiol (E_2) administration can modify cognitive performance after exposure to psychological stress and monoamine depletion.

Methods: Participants consisted of 22 postmenopausal women placed on either oral placebo or 17β-E_2 (1 mg/d for 1 mo, then 2 mg/d for 2 mo). At the end of the 3-month treatment phase, participants underwent three depletion challenges in which they ingested one of three amino acid mixtures: deficient in tryptophan, deficient in phenylalanine/tyrosine, or balanced. Five hours later, participants performed the Trier Social Stress Test (TSST), followed by mood and anxiety ratings and cognitive testing. Cognitive measures included tests of attention, psychomotor function, and verbal episodic memory.

Results: E_2-treated compared with placebo-treated participants exhibited significant worsening of cognitive performance on tasks measuring attentional performance and psychomotor speed. Similar trends for impairment were seen in measures of long-term episodic memory compared with placebo-treated postmenopausal women. E_2-treated participants also showed a significant increase in negative mood and anxiety compared with placebo-treated women after, but not before, the TSST, although the worsening of both cognitive and behavioral functioning was not correlated. These effects were independent of tryptophan or tyrosine/phenylalanine depletion and were not manifested before the TSST or at baseline.

Conclusions: These data suggest that the relationship between estrogen administration and cognitive/behavioral performance in postmenopausal women may be more complex than initially appreciated and that the effects of psychosocial stress may influence whether hormone effects are beneficial.

Key Words: Estrogen – Menopause – Monoamines – Stress – Cognition.

Studies of the cognitive effects of estrogen or of women after menopause have strongly suggested that estrogen levels are directly relevant to cognitive function. Experimental studies of postmenopausal estrogen or estrogen treatment have, in general, tended to show positive effects on cognitive functioning.[1] The beneficial effects of hormone therapy (HT) on cognition after menopause have been confirmed in a number of studies showing that administration of estrogen to healthy postmenopausal women (PMW) improved visuospatial abilities, memory, and frontal lobe function,[2-10] although not all studies have not shown positive effects[11-15] and studies examining estrogen therapy specifically in older PMW have not shown significant benefit, including the large Women's Health Initiative study.[16-20] Meta-analyses[21,22] have demonstrated that HT shows cognitive benefit in younger women, but older women show less evidence of benefit or small negative effects.

Overall, studies support the hypothesis that estrogen helps to maintain aspects of attention, verbal, and visual memory[23-25] and may have positive effects on tasks mediated by the prefrontal cortex[26] and hippocampus,[27] especially in younger PMW, although in the recent Study of Women's Health

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From the [1]Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT; [2]Division of Reproductive Medicine, Baystate Medical Center, Tufts University School of Medicine, Boston, MA; and [3]Department of Psychiatry, McGill University School of Medicine, Montreal, Quebec, Canada.

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Address correspondence to: Paul A. Newhouse, MD, Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, 1 South Prospect St., Burlington, VT 05401. E-mail: Paul.Newhouse@uvm.edu

Across the Nation, perimenopausal women did not show the expected improvement with estradiol (E_2) treatment.[28] Certain estrogen receptor polymorphisms seem to be associated with the risk of developing cognitive impairment,[29] and estrogen reduces neuronal generation of β-amyloid peptides, which may be relevant to the onset of Alzheimer disease (AD).[30] PMW seem to be at higher risk for AD, particularly if they carry the APOE4 allele,[31] and there is considerable epidemiological evidence from both prospective and case-control studies that E_2 use in PMW may decrease the risk of the development and/or expression of AD,[32-36] with an overall odds ratio of 0.66.[24] In memory clinics, hormone users showed lower rates of dementia diagnoses versus mild cognitive impairment than did nonusers, who deteriorated more rapidly than did hormone users.[37]

In contrast to cognitive functioning, the increased vulnerability for depression seen in reproductive-age women declines after menopause[38,39] although perimenopause may be associated with increased vulnerability for both depressive symptoms and a diagnosis of new-onset depression.[40,41] Whereas some studies have supported positive mood effects of estrogen therapy or HT in PMW,[42-45] others have not.[46-48]

However, there are few studies regarding the interaction between mood effects and cognitive performance in PMW. A strong candidate for explaining the cognitive and mood alterations after menopause is the influence of declining levels of gonadal steroids on neurotransmitter systems and mood regulatory systems,[39] perhaps interacting with genetic vulnerability and life stress.[49] A potential hypothesis for how estrogen or its loss after menopause exerts effects on cognition and mood is through interactions with modulatory neurotransmitter systems. For example, significant work has been done on examining how estrogen interacts with cholinergic system activity to alter cognitive functioning in both animal models and humans (see Gibbs[50] and Dumas et al[51] for review). Recently, this laboratory has shown that E_2 seems to improve cognitive performance related to cholinergic function as measured by increased cognitive resistance to anticholinergic blockade in normal PMW.[52] This improvement may be dose and domain specific; that is, lower doses improve primarily attentional functioning, whereas higher doses may influence episodic memory.[53] The effects of E_2 on cholinergic function related to episodic memory may be age specific, with younger women showing benefit but older women showing no benefit or impairment (providing direct experimental support for the "critical period hypothesis"[53] of estrogen benefit after menopause). However, other monoamine neurotransmitters seem to have substantial modulatory roles on mood, anxiety, and cognitive performance and behavior. For example, estrogen shows effects on modulation of serotonin and dopamine receptor density,[54] dopamine release,[55] and potentiation of serotonin function.[56-59] PMW respond more briskly to serotoninergic antidepressants if taking estrogen.[60-63]

Catecholamine and indolamine systems can be investigated in humans using conceptually similar treatment-challenge models to those that have been used to investigate cholinergic system–hormone interactions. We have previously reported the effects of estrogen and monoamine depletion on mood after psychosocial stress.[64] As an extension of this study, we now report the effects of the same manipulation on cognitive functioning. The primary goal of the overall project was to test whether short-term administration of E_2 to normal PMW would alter mood reactivity and cognitive performance to experimental psychosocial stress and quantitatively change the behavioral responses to central nervous system catecholamine and/or serotonin depletion, pharmacological challenges that interact directly with central monoamine systems.

Estrogen levels vary within individuals and compared with premenopausal levels during perimenopause and postmenopause, and this variability is associated with physical and behavioral symptoms.[65,66] We reasoned that fluctuations in estrogen levels may lead to alterations in levels of monoamine neurotransmitters, which may influence mood reactivity and cognitive performance in response to external events. Thus, to probe the interaction of estrogen and monoamine neurotransmitters on cognition and mood, we used the technique of monoamine depletion and experimentally induced psychosocial stress. Acute tryptophan depletion (ATD) is a well-established technique for examining the role of serotonin systems in mood.[67,68] Acute tyrosine/phenylalanine depletion (ATPD) is a newer technique designed to examine the effects of reduced catecholamine synthesis and transmission on behavior and performance.[69] Tryptophan depletion can, in some circumstances, produce adverse effects on mood and behavior that are considered relevant to understanding the causes of affective illness.[68,70] Furthermore, central catecholamine depletion has been examined in normal premenopausal women and has been found to produce negative effects on mood under stress.[69]

In this study, women who were postmenopausal (>50 y) took a fixed dose of 17β-E_2 or placebo for 3 months and then participated in three challenges using monoamine depletion to briefly change the relative amounts of monoamine neurotransmitters in the brain (serotonin, dopamine, and norepinephrine). They then participated in a psychosocial stress paradigm to potentiate negative mood (Trier Social Stress Test [TSST])[71] that involves public speaking and has been shown previously to reliably produce mild-moderate psychosocial stress. We have reported previously on the mood effects of this manipulation,[64] in which we showed significant enhancement of negative mood effects after the psychosocial stress maneuver in the E_2-treated participants. Here, we report on the cognitive results from that study with additional participants.

We hypothesized that the psychosocial stress manipulation (TSST) would enhance any negative mood and cognitive effects of monoamine depletion and that estrogen administration would blunt or buffer the potential negative effects produced by the combination of the monoamine depletion and the stress test in a measurable and quantifiable way. Because estrogen has been noted to interact with both serotonergic and

catecholaminergic systems, it was hypothesized that depletion of either monoamine system would interact with estrogen treatment to alter cognitive performance.

METHODS

The basic design consisted of a double-blind parallel group design (each participant was randomly assigned to receive 3 mo of either E_2 or placebo), with each treatment group then undergoing acute depletion and social stress challenges. All participants signed fully informed consent forms after an explanation of all procedures, risks, and benefits. Participants received $100 as compensation for their time and a small gift pack after each study session. The study was approved by the University of Vermont Committee for Human Research in the Medical Sciences (institutional review board).

Participants

Participants were recruited through newspaper advertisement and health newsletters published by our medical center, public information sessions, newspaper advertisements, and random mailings. Study participants were first screened by telephone for eligibility. Participants consisted of 22 normal PMW aged 52 to 83 years (mean [SD], 64.3 [10.6] y). Participant demographics are described in Table 1.

Medical screening

Participants were without menses for at least 1 year, had a follicle-stimulating hormone (FSH) level greater than 30 mIU/mL, were nonsmokers, had a normal mammogram within the last year, and were without surgically induced menopause (bilateral oophorectomy). They were not taking HT or oral contraceptives and were at least 1 year without such treatment. Participants were physically healthy, had a body mass index of 34 kg/m^2 or lower, and had no cardiovascular disease other than mild hypertension. Participants with major concomitant illnesses were excluded on the basis of history, physical examination, and laboratory tests assessing hematopoietic, renal, hepatic, and hormonal function (complete blood cell, Chem 20, thyrotropin, urinalysis, and electrocardiogram). Participants were physically examined by a gynecological nurse-practitioner to establish general physical health and for specific physical contraindications for E_2 therapy (eg, adnexal mass and large uterine fibroids).

TABLE 1. *Participant demographic data (n = 22)*

	Mean ± SD	Minimum	Maximum
Estrogen treatment (n = 11)			
Age, y	65.18 ± 11.81	52.0	83.0
Body mass index, kg/m^2	25.83 ± 3.70	18.71	31.3
Years since menopause	14.57 ± 11.30	1.0	31.0
Baseline FSH	61.87 ± 16.11	42.8	99.0
Education, y	14.36 ± 2.58	9.0	18.0
Placebo treatment (n = 11)			
Age, y	63.45 ± 9.80	52.0	80.0
Body mass index	26.63 ± 5.29	20.44	33.27
Years since menopause	14.03 ± 10.24	1.3	37.0
Baseline FSH	69.60 ± 17.76	38.6	86.5
Education, y	15.54 ± 2.87	12.0	20.0

FSH, follicle-stimulating hormone.

Participants were excluded if they had specific contraindications for E_2 treatment or current or any past axis I psychiatric disorders. Specific criteria for exclusion for the E_2 treatment included contraindications for HT, including history of breast cancer or E_2-dependent neoplasia; blood pressure higher than 160/100 mm Hg (untreated); history of deep vein thrombosis or other thromboembolic disease; hepatoma; severe migraines or stroke on oral contraceptives; concurrent use of barbiturates, rifampin, insulin, carbamazepine, oral hypoglycemics, antidepressants, or lipid-lowering drugs; known intolerance to conjugated E_2; diabetes; untreated thyroid disease; clinical osteoporosis; and severe menopausal symptoms. All participants were taking no centrally active drugs. No participants were taking selective estrogen receptor modulators or herbal menopause preparations. A minimum of 14 days elapsed between discontinuation centrally active or psychoactive agents and participation in this study.

Cognitive/behavioral screening

All participants were cognitively and behaviorally assessed using standard tests designed to exclude participants with cognitive or behavioral impairment. Participants were evaluated using the Mini-Mental State Examination,[72] Brief Cognitive Rating Scale,[73] and the Mattis Dementia Rating Scale,[74] to establish a Global Deterioration Scale score, which rates the degree of cognitive impairment.[75] Participants were required to have a Global Deterioration Scale score of 1 to 2 and a Mini-Mental State Examination score of greater than or equal to 27. Participants were excluded if they scored below 123 on the Dementia Rating Scale[74] and were matched across the two groups in terms of educational background.

Behavioral screening consisted of a partial *Structured Clinical Interview for DSM-IV-TR*[76] to establish the presence/absence of present or past axis I major psychiatric disorders, particularly any present or past mood disorders. In addition, participants completed the Beck Depression Rating Scale[77] and a menopause symptom checklist modified from Sherwin[78] to detect subclinical depressive symptoms. An exclusion cutoff score of 10 was used for the Beck Depression Rating Scale.

Estrogen/placebo treatment

After screening and acceptance into the study, each participant was placed randomly and blindly on either oral placebo or 17β-E_2 (using identical pink capsules) for 3 months. There were 11 women in the E_2 group and 11 women in the placebo group. Women were initially placed on E_2 1 mg per day for 30 days, which was then increased to 2 mg per day. This was done because early pilot trials revealed that estrogen-related adverse effects (eg, breast tenderness or spotting) tended to be noticed by participants if the participant was begun on 2 mg of E_2 from the beginning. Using 1 mg of E_2 for the first 30 days helped to protect the blind. At the end of the 3-month treatment period, women participated in a series of challenge studies designed to examine differences in sensitivity to acute transmitter depletion and psychosocial stress. E_2 or placebo treatment continued throughout the challenge/stress studies. Twelve days of medroxyprogesterone acetate

(Provera) was given at the end of the study to produce shedding of the endometrial lining.

Acute depletion challenges

All studies took place at the University of Vermont General Clinical Research Center. Each participant underwent 3 test days, at least 7 days apart, in which they received each of the two amino acid (AA) depletion mixtures and the nutritionally balanced control mixture. The depletion sequence was determined by a random order procedure.

The procedure for the administration of the AA mixtures was the same as we have used previously.[69] Participants were placed on a low-protein diet for the evening meal before each study day. After an overnight fast, the study began at 8:00 AM with baseline testing and evaluation. Participants then ingested one of three AA mixtures: (1) a nutritionally balanced AA mixture, (2) a mixture deficient in tryptophan (ATD), or (3) a mixture deficient in phenylalanine and tyrosine (ATPD). The composition of the AA mixtures was that used in prior studies, adjusted for the generally lower weight of women. Mixtures consisted of AA suspended in water, with the worst tasting AAs (L-methionine, L-cysteine, and L-arginine) in capsules. The liquid suspensions were flavored with noncaloric, no-protein flavoring of orange, grapefruit, lemon, chocolate, or cranberry-lemon (participant's choice) to disguise the unpleasant taste. We have previously demonstrated the feasibility of administering 3 AA mixtures to female participants, with acceptable tolerability.[79] Testing concluded with a high-protein snack for repletion of AA levels.

Social stress test

Five hours after AA ingestion, participants performed a mildly stressful psychological task, the TSST.[71] The TSST consisted of three parts, a brief instruction period, a 10-minute anticipation period, and a 10-minute test period. For the brief instruction period, participants were taken to the TSST room, where three individuals were already sitting at a table and a visible video camera was set up. The participant was asked to stand on an "X" on the floor in front of the panel of people. The instructor presented the participant with one of three scenarios and asked the participant to prepare a 5-minute speech about the topic. Participants were told that the panel was especially trained to monitor nonverbal behavior and that a voice-frequency analysis of the speech would be performed. After being given the instructions, the participant returned to her room.

During the anticipation period, participants were asked to prepare the 5-minute speech. They were given 10 minutes to prepare and take notes in a separate room but were not allowed to use them during their speech. Participants presented their 5-minute speech followed by 5 minutes of arithmetic problems. The TSST was originally designed to be conducted one time per subject, using only the first speech scenario and arithmetic problem. To repeatedly confront the participant with the TSST on each of the three study visit days, the two additional scenarios and arithmetic problems were created. For each of the three scenarios, the participant was asked to take on a role within a given context and had to convince a panel to grant her a specific request: (1) role of a job applicant for the position of manager at a banking firm, (2) role of the director of a rehabilitation program for prisoners requesting a donation of a large sum of money to support the program, or (3) role of a building developer requesting a building permit to build a strip mall in a rural New England town. For the arithmetic problem portion of the test period, the problems consisted of serial subtractions of a two-digit number from a four-digit number, and upon every mistake, the participant was asked to begin again at the first number. Repeated exposure to the TSST has been shown to induce an equal physiological stress response.[80,81] Consultation with the creators of the TSST and their review of our scenarios produced general agreement that the repeated use of the TSST with our scenarios had precedent and would produce repeated equivalent stress (Schommer, personal communication, 2003).

Before the study began, participants were briefed about the general nature of the TSST and what was expected of them. This was done to equalize the anticipation of the TSST across the three study days. It should be noted that the actual performance of the participant during the TSST was not evaluated. The psychological stress induced was a product of the actual event of standing in front of a panel of strangers and delivering a speech; thus, the topic of the speech was less important. Regardless, the speech scenarios and arithmetic problems were judged to be equal in difficulty and equivalently controversial topics for the population being studied. Furthermore, the order of scenarios was randomized across participants, decreasing the possibility that differences in scenarios would produce different stress outcomes. Participants were debriefed at the end of the study regarding the mild deception in the stress test (ie, no actual monitoring of test performance).

Outcome measures

Cognitive

A cognitive testing battery was constructed to evaluate a number of cognitive domains potentially sensitive to monoamine depletion and psychosocial stress as well as affected by loss of and subsequent treatment with estrogen. These cognitive domains included tests of simple attention, complex attention, and verbal episodic memory. Each task is described in the section immediately below. The cognitive battery was performed once each study day, after the psychosocial stress maneuver. Participants were pretrained on the entire cognitive battery before study initiation to ensure stable asymptotic performance to ensure equivalent cognitive performance at baseline between the groups.

Simple attention. The Critical Flicker Fusion (CFF) task[82] and the Choice Reaction Time (CRT) task[83] from the Milford Test Battery were used as the measures of simple attention and were performed using the Leeds Psychomotor Device. During the CFF task, there were two different types of trials. In an ascending trial, the participant pressed a button that

indicated when the frequency of flashing lights had increased to the point that the lights appear to be no longer flashing but rather appear continuously on ("fused"). The lights began flashing at a rate of 12 Hz, and the frequency was increased to 50 Hz. In a descending trial, the participant pressed a button when the frequency of apparently fused lights was decreased such that lights began to appear to be flashing. The lights began flashing at 50 Hz and decreased to 12 Hz. The participant needed to respond before the frequency hit the upper or lower limit in each trial. The participant was presented with three of each trial type. Dependent measures for this task were the median detection frequency across all trials, as well as the median detection frequency on the ascending and descending trials separately. Lower frequency values are generally understood to reflect impaired attention and/or arousal.

The CRT task was a reaction time (RT) task in which participants kept their index finger on a "home" light sensitive diode (LSD) until one of six LCD lights arrayed in a semicircle, approximately 25 cm from the "home" key, was lit on the response box. The participant lifted her index finger and moved it to cover the LSD corresponding to the illuminated LCD. She then returned her finger to the "home" LSD. Three performance measures were obtained from the CRT. The first was the median total RT per trial. The second was the median recognition RT, the amount of time it took the participant to lift her finger off of the home LSD once the signal to respond appeared. The third measure was the median motor RT, the time it took the participant to move her finger and to cover the LSD corresponding to the illuminated LCD.

Complex attention. The measures of complex attention were the Digit Symbol Substitution Test (DSST)[84] and the Connors Continuous Performance Test (CPT).[85] In the DSST, participants were presented with nine numbers that corresponded to nine symbols. On the answer form, the participant was instructed to write the symbol that corresponded to each number and to complete as many as possible in 90 seconds. The dependent measure was the total correct completions.

In the computerized CPT task, individual letters appeared on the computer screen for 300 milliseconds with a response period of 2 seconds for 120 trials. Participants were instructed to press a button when they saw an "A" followed by an "X." The dependent measures were hits, errors of omission and commission, and hit RT.

Verbal episodic memory. The Buschke Selective Reminding Task (SRT[86]) and the Verbal Paired Associates (VPA) test (Wechsler Memory Scale III) were used as measures of verbal episodic memory. In the SRT, participants were read a list of 14 words, followed by an immediate recall trial. The experimenter then reminded the participant of any words she did not recall and she was instructed to recall all 14 words again. This process was repeated for eight trials. Three measures were obtained from this task: the total number of words recalled across all lists, the recall consistency from one trial to the next, and the recall failure from one trial to the next.

In the VPA, participants were read a list of eight pairs of words. Then they were read the first word in each pair and asked to recall the associate. The list was read and recalled a maximum of six times. If the participant recalled all words on the list within the first three trials, the test was discontinued after three trials. Four of the word pairs were strong associates (easy pairs), whereas the other four were weak associates (hard pairs). Dependent measures were number correct for the strong and weak associates after three and six trials.

The final test of verbal memory was a paragraph recall test.[87] Participants were read a short paragraph and then asked to retell the story from memory. The dependent measure was the number of information units correctly recalled from memory.

Cognitive tasks were performed in the following order: CFF, SRT, CRT, VPA test, CPT, DSST, and paragraph recall. This administration order was the same for all participants on all study days. A minimum of 10 equivalent versions of the testing forms were created so that a new version of each test was available for each of the testing days. These forms were counterbalanced across study days for all participants.

Behavior

The primary mood and anxiety measure was the participant-completed Profile of Mood States (POMS).[88] This scale is a 65-item adjective checklist that generates six bipolar factor–analytically derived factors (elated-depressed, composed-anxious, energetic-tired, agreeable-hostile, confident-unsure, and clearheaded-confused) or 12 unipolar factors, plus a total score. This scale has been used extensively in challenge study paradigms and is sensitive to the effects of psychotropic drugs and central nervous system state manipulations. It was administered three times during the experimental session: predepletion, postdepletion before TSST, and post-TSST. Participants completed a Beck Depression Index (BDI)[77] twice during the day: predepletion and postdepletion but before the TSST.

Neuroendocrine/physiological

Measures of E_2 and FSH were collected to assess compliance and the effectiveness of E_2 therapy. E_2 and FSH were measured with an ADVIA Centaur chemiluminescence competitive immunoassay (E_2) and an ADVIA Centaur two-site sandwich immunoassay (FSH) both using a labeled acridinium ester. Samples were collected at screening and on the first day of each challenge sequence. Blood was collected on each study day for measures of plasma total tryptophan, phenylalanine, and tyrosine to assess the adequacy of depletion. Samples were collected predepletion (−45 min) and at the end of the session (+400 min). Plasma phenylalanine and tyrosine concentrations were determined using a Beckman System Gold AA analyzer using gradient high-performance liquid chromatography with precolumn derivatization and fluorometric detection. Tryptophan was measured by isocratic high-performance liquid chromatography with fluorometric detection. Cortisol was measured by radioimmunoassay (Diagnostic Products Corporation).

Vital signs were recorded predepletion at −45 minutes, pre-TSST at +300 minutes, and post-TSST at +400 minutes.

Data analysis

The general approach was that of mixed-model repeated-measures analysis of variance using SAS PROC MIXED. Initial analysis of cognitive measures was a 2 (treatment; E_2 vs PLC) × 3 (depletion; ATD, ATPD, or Mock) mixed-model analysis of variance as an overall test of the effect of E_2 treatment and monoamine depletion effects on cognition after psychosocial stress. Treatment (E_2 vs placebo) was the between-participant factor and depletion (ATD, ATPD, and mock) was the within-participant factor. For the mood measures only, time was an additional factor as there was a predepletion, prestress maneuver mood assessment. Cognitive testing was performed once on each of the three experimental days, and thus, each participant performed cognitive testing under each monoamine depletion-psychosocial stress condition. Because the primary effect of interest in this study was the impact of E_2 treatment on cognitive performance after monoamine depletion and psychosocial stress, if no treatment-by-depletion effect was found, results were collapsed across depletions and the analyses were redone as an independent-samples t test. When there was a significant interaction (eg, treatment × depletion), nonorthogonal a priori contrasts were used to test for differences between treatments across depletions. Correlations between cognitive and mood measures were performed using Pearson product-moment correlations adjusted for multiple comparisons. The α level for rejection of the null hypothesis was set at $P < 0.05$.

RESULTS

Participants

Participants were matched for age, education, weight, and years since menopause (Table 1). The mean ± SD age of the participants was 64.3 ± 10.6 years. Body mass index averaged 26.23 ± 4.47 kg/m^2, and participants were an average of 14.3 ± 10.5 years postmenopause. This was a highly educated group, with an average of 14.9 years of education. Fifteen participants had had previous experience with HT (>1 y previously) and 7 did not. For those women who had previously used HT, the average duration of hormone use was 3.9 ± 4.9 years.

FSH, E_2, and cortisol levels

Mean pretreatment FSH level was 65.73 mIU/mL (menopausal level is considered >30-35 mIU/mL) and was not significantly different between treatment groups ($t_{18} = 0.98$, $P > 0.34$). After 3 months of treatment, the E_2-treated participants showed a significantly reduced mean FSH level of 29.6 mIU/mL compared with the placebo-treated participants, who had a mean level of 69.0 mIU/mL ($t_{17} = 5.39$, $P < 0.001$). Mean plasma E_2 levels after 3 months of treatment were significantly elevated at 135.36 pg/mL for the E_2-treated group compared with 18.3 pg/mL for the placebo-treated group ($t_{19} = 5.14$, $P < 0.001$). The levels of E_2 seen in the E_2-treated women are comparable with the late follicular phase levels in premenopausal women, whereas the level seen in the placebo-treated women is comparable with the early follicular phase. Cortisol levels were measured at baseline and at +420 minutes (post-TSST). Baseline levels (predepletion, pre-TSST) were higher ($P < 0.01$) in the E_2-treated participants but declined similarly across the experimental day in both treatment groups.

Amino Acid levels

Plasma concentrations of total tryptophan, phenylalanine, and tyrosine were measured at baseline (predepletion) and at +400 minutes (postdepletion; Table 2). After tryptophan depletion, plasma tryptophan levels declined by 76%. After tyrosine/phenylalanine depletion, both tyrosine and phenylalanine levels declined by 60%, suggesting that an adequate depletion was achieved.[69,89]

Clinical assessment of mood across treatment phase

A comparison of the clinical depression ratings (BDI) from screening to the end of the treatment phase for each participant revealed no significant time-by-treatment interaction ($F_{1,18} = 0.58$, $P > 0.45$). Furthermore, a comparison of the end-of-treatment BDI scores (the baseline BDI score on the first depletion challenge day) between treatment groups showed a small numerical difference (PLC, 2.91 ± 3.6; E_2, 4.55 ± 2.9) that was not significantly different between treatments ($t_{20} = 1.19$, $P > 0.25$). These data demonstrate that the treatment alone (E_2 or placebo) did not produce significant or clinically manifest negative changes in mood across the 3-month treatment phase nor did the groups differ before beginning the monoamine depletion challenges.

Cognitive performance

Cognitive testing results are presented in Table 3. Cognitive testing was performed only after the TSST. In general, significant impairment was seen across many cognitive measures in the E_2-treated group, particularly on attention and psychomotor measures.

Attention/psychomotor

CFF. Attentional performance as measured by the median frequency of all trials showed a significant main effect of

TABLE 2. *Amino acid levels (n = 22)*

	Predepletion, mean ± SD	Postdepletion, mean ± SD	% Change, mean
Total plasma tryptophan, μmol/L			
ATD	10.03 ± 1.46	2.49 ± 3.40	−76.06[a]
ATPD	9.90 ± 1.82	12.46 ± 4.37	33.56[b]
MOCK	9.90 ± 1.71	12.53 ± 3.78	31.09[a]
Plasma phenylalanine, μmol/L			
ATD	5.10 ± 12.6	85.34 ± 34.21	71.42[a]
ATPD	46.95 ± 5.82	19.51 ± 20.9	−58.69[a]
MOCK	48.70 ± 4.70	70.15 ± 34.34	42.42[a]
Plasma tyrosine, μmol/L[c]			
ATD	61.42 ± 23.09	177.41 ± 53.09	203.57[a]
ATPD	56.02 ± 10.52	21.6 ± 33.55	−59.98[a]
MOCK	58.72 ± 9.70	161.86 ± 64.31	184.54[a]

Predepletion time point is −45 minutes; depletion time point is 0 minutes; postdepletion time point is +420 minutes.
ATD, acute tryptophan depletion; ATPD, acute tyrosine/phenylalanine depletion; MOCK, mock (placebo) depletion.
[a] $P < 0.01$ for pre-post difference.
[b] $P < 0.05$ for pre-post difference.
[c] Tyrosine, n = 21.

TABLE 3. *Cognitive performance scores by treatment and depletion condition (n = 22; placebo, n = 11; E_2, n = 11)*

Cognitive construct	Task	Dependent variable	Treatment	Mock depletion	ATD	ATPD
Attention						
	CFF					
		Total, Hz[a]	Placebo	30.39 (0.87)	30.90 (0.85)	31.80 (0.73)
			E_2	27.90 (0.87)	28.45 (0.56)	27.39 (0.72)
		Ascending, Hz[b]	Placebo	28.71 (1.32)	29.91 (0.92)	30.45 (0.76)
			E_2	27.98 (1.32)	28.22 (0.94)	27.37 (0.75)
		Descending, Hz[a]	Placebo	31.95 (0.87)	31.78 (0.87)	31.95 (0.88)
			E_2	27.98 (0.87)	28.63 (0.88)	27.36 (0.87)
	CRT					
		Total, ms[b]	Placebo	702.12 (37.52)	684.70 (37.52)	734.21 (38.20)
			E_2	780.77 (37.52)	774.55 (38.94)	792.20 (37.50)
		Recognition RT, ms[b]	Placebo	382.40 (20.7)	391.84 (20.7)	412.61 (20.9)
			E_2	420.50 (20.7)	419.21 (21.1)	451.63 (20.7)
		Motor RT, ms	Placebo	311.87 (20.8)	324.31 (20.8)	311.08 (21.1)
			E_2	349.37 (20.8)	340.93 (21.5)	332.50 (20.8)
	CPT					
		Hits (proportion correct)[b]	Placebo	0.98 (0.06)	0.99 (0.05)	0.95 (0.07)
			E_2	0.86 (0.06)	0.89 (0.05)	0.84 (0.07)
		Errors of omission (errors)[b]	Placebo	0.64 (2.25)	0.18 (1.95)	1.99 (2.88)
			E_2	5.73 (2.25)	4.47 (1.98)	6.36 (2.81)
		Errors of commission (errors)	Placebo	0.45 (0.25)	0.64 (0.39)	0.57 (0.34)
			E_2	0.54 (0.25)	0.73 (0.39)	0.18 (0.33)
		Hit RT, ms[a]	Placebo	508.35 (24.79)	467.32 (24.79)	497.30 (25.36)
			E_2	400.67 (24.79)	407.35 (25.36)	433.46 (24.79)
	DSST					
		Number correct[b]	Placebo	58.82 (2.78)	60.18 (2.76)	60.98 (2.81)
			E_2	52.36 (2.78)	53.40 (2.81)	49.73 (2.78)
		Errors	Placebo	0.09 (0.15)	0.36 (0.22)	0.29 (0.14)
			E_2	0.27 (0.15)	0.26 (0.23)	0.18 (0.13)
Verbal memory						
	SRT					
		Total recall (number correct)	Placebo	83.36 (4.72)	81.18 (4.72)	79.31 (4.79)
			E_2	81.60 (4.95)	76.10 (4.95)	76.70 (4.95)
		Recall consistency (number correct)	Placebo	48.90 (5.23)	43.45 (5.23)	40.75 (5.32)
			E_2	48.60 (5.49)	40.70 (5.43)	40.90 (5.49)
		Recall failure (number of failures)	Placebo	11.82 (3.73)	11.82 (3.73)	13.9 (3.73)
			E_2	15.1 (3.91)	18.2 (3.91)	16.3 (3.91)
	PR					
		Proportion recalled (story units)	Placebo	0.43 (0.05)	0.38 (0.05)	0.37 (0.05)
			E_2	0.32 (0.05)	0.35 (0.05)	0.39 (0.05)
	VPA					
		Easy pairs (proportion recalled)	Placebo	0.95 (0.07)	0.95 (0.06)	0.94 (0.05)
			E_2	0.83 (0.07)	0.81 (0.08)	0.87 (0.05)
		Hard pairs[b]	Placebo	0.75 (0.08)	0.80 (0.08)	0.78 (0.08)
			E_2	0.59 (0.08)	0.59 (0.08)	0.64 (0.08)

Mock depletion indicates balanced amino acid administration. Values displayed are means (SE) except for CFF and RT values, which are medians.
ATD, acute tryptophan depletion; ATPD, acute tyrosine/phenylalanine depletion; CFF, Critical Flicker Fusion; CRT, Choice Reaction Time; CPT, Continuous Performance Test; DSST, Digit Symbol Substitution Task; SRT, Selective Reminding Task; PR, paragraph recall; VPA, Verbal Paired Associates; RT, reaction time.
[a] $P < 0.01$ for effect of E_2 treatment.
[b] $P < 0.05$ for effect of E_2 treatment.

treatment ($F_{1,20} = 8.66$, $P = 0.008$), with E_2-treated participants showing a reduced frequency compared with placebo-treated participants (Fig. 1), suggesting impaired attention. There was a strong trend for a treatment-by-challenge interaction ($F_{2,20} = 3.17$, $P > 0.06$), with women in the tyrosine/phenylalanine depletion condition showing a slightly poorer performance compared with women in the tryptophan depletion and mock depletion conditions after E_2 treatment. Falling trials showed a significant ($F_{1,20} = 11.61$, $P = 0.003$) main effect of E_2 treatment as well, producing a median frequency reduction, but rising trials did not ($P > 0.11$).

CRT. For the CRT, total median RT showed no treatment-by-challenge interaction. Collapsing the data across challenge conditions demonstrated a significant ($t_{20} = 2.68$, $P < 0.05$) effect of E_2 treatment, with the pattern of means showing that estrogen-treated participants performed slower across all depletion conditions than did placebo-treated participants (Fig. 1). The recognition component of the CRT showed a significant effect of challenge ($F_{2,37} = 10.04$, $P = 0.0003$) on median recognition RT, with women in the tyrosine/phenylalanine depletion condition showing a slower RT than did women in either the mock or tryptophan depletion conditions. However, there were no significant treatment or challenge-by-treatment effects. Analyses collapsed across challenge conditions revealed a significant effect of E_2 treatment ($t_{20} = 2.25$, $P < 0.05$), with RT significantly greater (slower) for E_2-treated participants. For the motor component of CRT, there were no significant treatment-by-challenge interaction

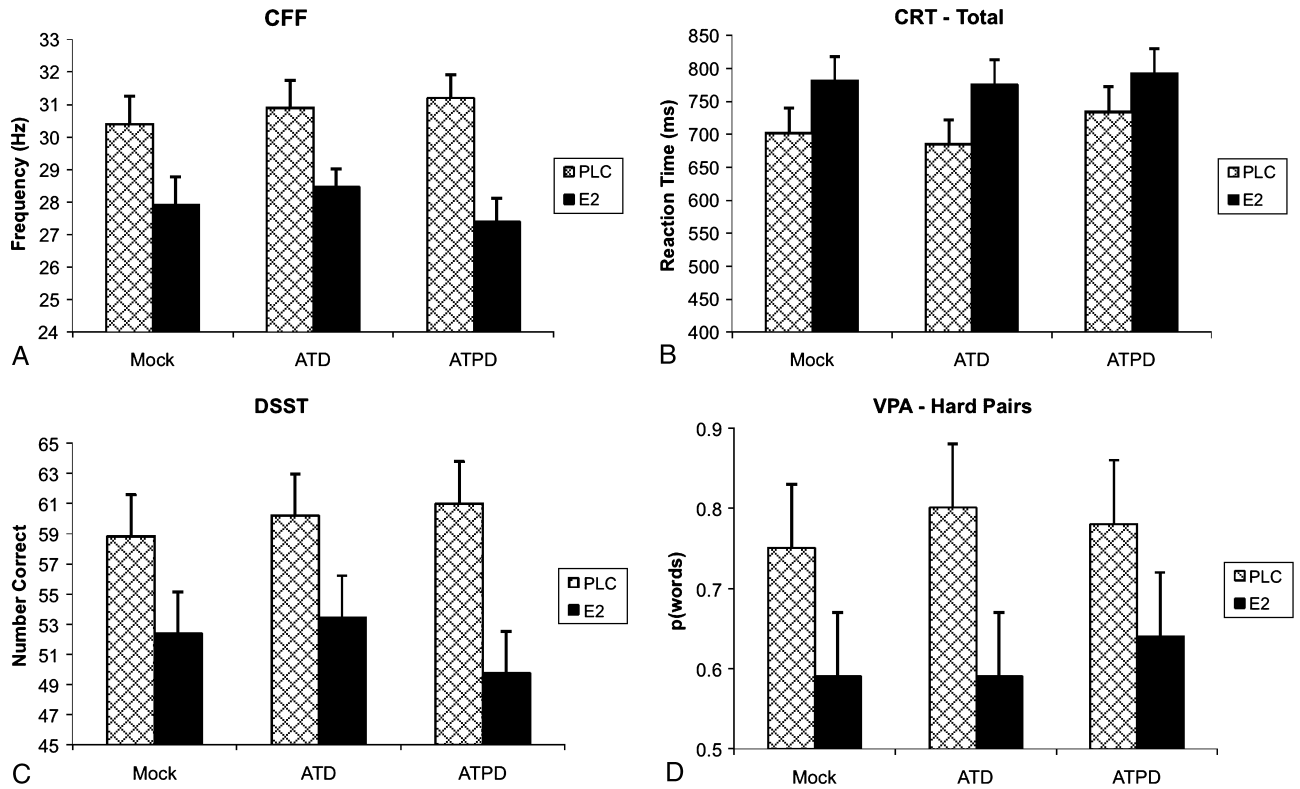

FIG. 1. Cognitive performance measures after the Trier Social Stress Test. Data are presented for E_2 and PLC treatment groups for each monoaminergic depletion: ATD, ATPD, Mock. Values shown are mean ± SEM scores for accuracy measures and median ± SEM times for reaction times. **A:** CFF task median detection frequency for all trials. **B:** CRT task median total reaction time. **C:** DSST number of correct completed items. **D:** VPA number of correct recalled for the hard word pair condition. E_2 treatment produced impairment compared with PLC treatment across depletion conditions on all four cognitive measures (all $P < 0.05$ for effect of E_2 treatment). E_2, estradiol; PLC, placebo; ATD, acute tryptophan depletion; ATPD, acute tyrosine/phenylalanine depletion; Mock, mock depletion; CFF, Critical Flicker Fusion; CRT, Choice Reaction Time; DSST, Digit Symbol Substitution Task; VPA, Verbal Paired Associates.

effects on median RT, but as with the other components, collapsing across challenge conditions revealed a significant ($t_{20} = 2.68$, $P < 0.05$) slowing effect of E_2 treatment.

CPT. As there were no significant challenge-by-treatment interactions, we examined treatment effects by looking at the data collapsed across challenge conditions. The proportion of hits showed a significant ($t_{20} = 2.65$, $P < 0.05$) negative effect of estrogen treatment, with E_2-treated participants demonstrating a reduced proportion of hits across all conditions. A similar significant pattern ($t_{20} = 2.65$, $P < 0.05$) was seen in errors of omission, with errors showing increases under all conditions for the E_2-treated participants. There were no significant or trend-level effects on commission errors, but the proportion of commission errors was very low.

By contrast, there was a significant ($F_{1,20} = 6.17$, $P = 0.02$) positive main effect of E_2 treatment on hit RT, with E_2-treated participants showing a faster RT (between 60 and 100 ms) across all depletion conditions. The contrast in these results suggests the possibility that E_2-treated participants demonstrated a speed-accuracy trade-off, becoming faster but less accurate. No other parameters showed significant effects.

DSST. There was a significant ($F_{1,20} = 4.63$, $P = 0.04$) main effect of treatment on the number of items correctly completed, with E_2-treated participants showing a significantly ($P = 0.001$) reduced number of items correctly completed compared with the placebo-treated participants (Fig. 1). In addition, there was a significant challenge-by-treatment interaction ($F_{2,38} = 3.88$, $P = 0.03$), with E_2-treated participants showing significantly ($t_{20} = 2.5$, $P < 0.05$) fewer correct completions after tyrosine/phenylalanine depletion.

Memory

SRT. There were no effects of E_2 treatment on this task. There was a trend ($P = 0.12$) for eight-trial recall to be reduced under both tryptophan and tyrosine/phenylalanine depletion conditions. In addition, recall consistency showed a significant main effect of depletion challenge ($F_{2,37} = 4.93$, $P = 0.01$), with consistency being significantly reduced under both tryptophan and tyrosine/phenylalanine depletion conditions. There was no interaction with E_2 treatment on this parameter. Recall failure showed a pattern of increased failure scores under E_2 treatment, but the effect of treatment was not significant.

VPA task. Whereas there were no significant challenge-by-treatment interactions, collapsing across challenge conditions,

there was a significant ($t_{20} = 2.15$, $P < 0.05$) effect of E_2 such that E_2-treated participants showed reduced recall of hard word pairs across all depletion conditions (Fig. 1). There was a similar trend ($P = 0.16$) for E_2-treated participants to show a similarly reduced recall for easy pairs of words.

Paragraph recall. There were no significant or a trend-level treatment, challenge, or interaction effects on this measure.

Mood effects

The effects of the hormone-monoamine depletions/psychosocial stress manipulation on mood were previously presented in detail in Newhouse et al.[64] We briefly review and update those findings here, focusing on the POMS results.

An examination of the entire model for the POMS total score and subscales revealed no significant interaction of treatment by depletion challenge. Thus, the analyses were redone, collapsing across challenge conditions to examine hormone treatment effects. The total mood disturbance score showed a significant interaction of hormone treatment by time ($F_{2,20} = 9.40$, $P = 0.001$), with E_2 participants showing a significant increase in total mood disturbance scores after the stress/monoamine depletion manipulation. Examining the subscale scores from the POMS revealed a similar interaction of hormone treatment by time for depression ($F_{2,20} = 4.50$, $P = 0.02$), confusion ($F = 3.93$. $P = 0.04$), and vigor ($F = 6.67$, $P = 0.003$), with E_2 participants showing significant score changes indicating worsening self-ratings after the stress/monoamine depletion manipulation compared with placebo treatment. In addition, on the tension subscale, there was a main effect of hormone treatment ($F_{1,20} = 6.84$, $P = 0.02$), with E_2-treated participants showing higher scores across time. The anger/hostility subscale showed only trivial significant effects of time and did not show any treatment or hormone treatment-stress manipulation effects.

Correlation with mood effects of psychosocial stress and monoamine depletion. We examined whether group differences in cognitive performance correlated with changes in self-rated mood after the psychosocial stress manipulation and monoamine depletion. We compared the POMS total score and subscale scores at the postdepletion rating in relationship to the performance measures that were obtained at the same time. The relationships were inconsistent, with some mood measures correlating with performance under placebo on some tasks and under estrogen on others.

There were small correlations between the tension subscale of the POMS and impaired performance on the SRT, the CRT, and the DSST; however, the pattern of treatment correlations was inconsistent as the tension subscale correlated with performance on placebo on some tasks and estrogen on others. A similar pattern was seen for the depression, fatigue, and confusion subscales. Furthermore, none of these correlations survived correction for multiple comparisons. Thus, it does not seem that the magnitude of the mood changes alone explained the estrogen treatment-related negative effects on cognitive performance.

We also had previously[64] examined effects of age, TSST scenario, and repeated administration of the TSST on mood dependent variables. Neither age, day, nor TSST scenario interacted with hormone treatment or depletion challenge. Moreover, there was no significant effect of repeated administration of the TSST and no significant interaction with hormone treatment or depletion challenge was found.

Vital signs

Only minor effects of hormone treatment and AA depletion were seen on vital signs. There was a trend for a treatment-by-challenge interaction on diastolic blood pressure ($P > 0.07$), with diastolic blood pressure showing a slight increase after tyrosine-phenylalanine depletion. No effects were seen on systolic blood pressure. Pulse showed significant main effects of challenge ($F_{2,33} = 3.77$, $P < 0.05$) and time ($F_{2,38} = 8.40$, $P < 0.001$), but no interactions with hormone treatment was found. The pattern of means showed that the mock depletion was not associated with an increase in pulse across the psychosocial stress maneuver compared with the ATD or ATPD that was associated with a reliable increase in pulse. Temperature showed a small significant ($F_{1,19} = 60.81$, $P < 0.001$) time-related change as expected but did not show any significant E_2 treatment–related effects or any systematic results of monoamine depletion. No clinically relevant changes occurred.

DISCUSSION

Postmenopausal women who were administered E_2 at a dose of 1 mg oral 17β-E_2 per day for 1 month, then 2 mg per day for 3 months generally exhibited poorer cognitive performance after a psychologically stressful event compared with placebo-treated women. This response was independent of the effects of monoamine depletion, which seemed to have only a small overall effect on the cognitive and emotional responses and did not interact with the effects of E_2. These effects did not seem to be secondary to baseline mood differences before depletion or the TSST, as participants' end-of-treatment depression scores (Beck) and predepletion mood scores (POMS) and depression scales were not significantly different between treatment groups. We expected that monoamine depletion and psychosocial stress together would produce negative cognitive and mood changes, as was seen by Leyton et al,[69] but that might be modified by the presence of E_2. Monoamine depletion produced only minor negative cognitive changes compared with mock depletion. By comparison, the effects of E_2 treatment on cognitive performance after social stress were larger and seemed to be largely independent of the monoamine depletion maneuvers.

The cognitive domains of impairment included both attention and, to a lesser extent, memory. Attentional impairment included simple speed measures that have generally been shown to be improved by E_2.[3,90] The current study reliably showed that E_2 treatment after psychosocial stress lengthened RT and decreased perceptual discrimination ability. Dumas et al[52] showed that these measures were improved by E_2 treatment after cholinergic challenge. However, E_2 had the opposite effect on these measures after psychosocial stress. In addition,

there was also partial impairment on some verbal episodic memory measures for the E_2 group relative to the placebo group. These data contrast with prior data by Maki et al[91] and Sherwin et al,[9] who showed that E_2 improved verbal episodic memory performance, although these studies were not done with a psychosocial stress or neurochemical stress maneuver. Thus, the psychosocial stress manipulation in this study seemed to interact with the E_2 treatment to generally impair cognitive performance, which was the opposite of what we originally hypothesized. We discuss further the implications for such an interaction.

The one exception to these findings was the hit RT measure during the CPT task, which improved in E_2-treated participants after the psychosocial stress/AA depletion maneuver, in contrast to the RT for other tasks such as the CRT, which showed significant slowing. On the CPT task, participants displayed a speed-accuracy tradeoff that interacted with the effects of estrogen treatment. The E_2-treated participants made fewer hits but had faster hit RTs compared with the placebo-treated group. In addition, differences in task specifics may explain these results. The CRT task is a sensory detection and motor task. By contrast, the CPT test requires a deeper level of stimulus processing to make an appropriate decision on whether to respond to particular stimuli and thus has greater test demands than does the simpler CRT task. Further studies should investigate whether task complexity or depth of processing changes the effect of stress- or hormone-related alterations on cognitive performance.

These results differ from prior published findings from our laboratory showing that 3 months of E_2 administration to PMW enhances cognitive performance after cholinergic blockade[52,53]; however, there were significant differences between the present study and our prior published work on E_2 and cognitive performance. Although the pattern of E_2 administration and the subject population were very similar to those of our prior studies showing cognitive enhancement, no psychosocial stress manipulation was used in our prior studies; rather partial cholinergic blockade provided the provocative stimulus. These prior studies showed that E_2 treatment reduced the sensitivity to cholinergic blockade and reduced the cognitive impairment associated with that blockade. Thus, E_2 seems to show evidence of enhancing cholinergic system–related cognitive function. By contrast, no cholinergic manipulation was used in the present study; rather the focus was on emotional stress and monoamine neurotransmitter manipulations. The impact of emotional or psychosocial stress on the ability of E_2 to enhance cholinergic-related cognitive performance remains to be examined.

How does psychosocial or emotional stress or stress hormones affect cognitive performance?

A series of studies have shown that psychosocial stress can induce certain cognitive deficits, including both working memory and retrieval deficits.[92-94] Chronic stress seems to have long-term negative effects on memory functioning and brain structure.[95] However, emotional arousal can result in enhancing as well as impairing effects on long-term memory. The directionality of this effect may depend on the baseline state of arousal, the type of emotion present, and the phase of the cognitive or memory process that is exposed to emotional arousal or stress. Significant sex differences exist in the neuronal circuitry involved in emotion-cognition interactions, suggesting the possibility that sex steroids may play a role in this process.[96] Kim and Diamond[97] have suggested that excess amygdala input and increased glucocorticoid secretion act to impair hippocampal plasticity and subsequent learning. Because estrogen levels can modulate hypothalamic-pituitary-adrenal axis activity in response to psychosocial stress[98] as well as the activity of limbic structures such as the amygdala response to negative emotional information,[99,100] it is probable that E_2 may directly affect the brain circuitry involved in an acute stress response and subsequent emotional learning.

Studies of the effects of E_2 on emotional perception are also few. Pearson and Lewis[101] have shown that recognition of emotional expressions is reliably altered across the menstrual cycle with the recognition of fear improving when E_2 levels were high. Protopopescu et al[100] have shown that specific subregions of the orbital frontal cortex changed their pattern of activity in reaction to negative emotional stimuli across the menstrual cycle. The authors interpreted these data as premenstrual enhancement of top-down modulation of limbic activity, with the accompanying suppression of sensory evaluative function. In a somewhat differently designed menstrual cycle study, Goldstein et al[99] showed that brain areas associated with negative emotional responses including the amygdala, anterior cingulate gyrus, and orbital frontal cortex showed lower activity during the mid-late follicular phase (when E_2 levels could be expected to be high) than during the early follicular phase (when E_2 levels would be lower). No such studies have examined the brain activity associated with emotional stimuli or emotional cognition in PMW or women undergoing HT. Alterations in cortical activity produced by differing circulating levels of hormones such as E_2 may thus play a role in regulating how the amygdala and other emotion-related structures respond to emotional stimuli and/or stressful events.[99,100,102] How the processing of emotional stimuli changes after menopause is, at this point, unknown. Thus, it may be that the steady administration of E_2 to PMW at a plasma level consistent with late follicular phase, as in this study, may have produced alterations in the cortical or subcortical processing of stressful or emotional experiences.

In two recent reviews, Phelps[103,104] has noted that the amygdala is responsible for the emotional contribution to declarative memory. Specifically, she suggests that the amygdala can modulate both the encoding and storage of hippocampal-dependent memories and that, bidirectionally, the hippocampus, by forming episodic representations of emotional significance, can influence the amygdala response when emotional stimuli are encountered.[103] Based on our data and neuroimaging studies of premenopausal women, it is not

unreasonable to suggest that sex hormone status may have a significant impact on cognitive processes after emotional stress in PMW. Our data suggest that exogenously manipulated E_2 levels may have a significant impact on both emotional reactivity and cognitive performance. It is therefore important in future studies to examine how menopause and postmenopausal HT may affect emotional reactivity and emotional memory.

PMW seem to show greater sensitivity than do premenopausal women in their physiological response to cognitive and speech tasks, with the difference being ascribed to both age and hormonal status.[105] Previous studies of the effects of hormones on experimental stressors have found that the various forms of estrogen seem to reduce some of the physiological effects of mild laboratory-induced stress (eg, solving arithmetic problems).[106,107] Lindheim et al[108] showed that the greater biophysical response of PMW after stress was reduced after 6 weeks of transdermal E_2 treatment. Estrogen treatment has been shown to enhance parasympathetic responsiveness to experimental stress, suggesting reduced sympathetic activation,[109] although in one study, the TSST was not found to provoke a differential effect on physiological measures in estrogen-treated women.[98] Kajantie and Phillips[110] conclude that there is an increase in sympathoadrenal responsiveness after menopause, which is attenuated during oral HT.

The lack of interaction of the E_2-induced effect on cognitive impairment after psychosocial stress with monoamine depletion suggests that other neurotransmitter systems may be involved in mediating these effects. Although the exact neurochemical mechanisms responsible for the negative cognitive and emotional responses seen here cannot be ascertained from this particular study, the lack of concomitant progesterone administration suggests that the impact of stress-related symptoms that would normally benefit from progesterone-derived neurosteroid–γ-aminobutyric acid A ($GABA_A$) receptor interactions may have had an impact on the effects seen in this study.

Limitations

Although the effects of estrogen on cognition after psychosocial stress in this study were large, caution is indicated in interpreting these results. Concerns regarding the repeatability of the TSST as well as the dose of E_2 are similar to those of our prior study.[64] The TSST was not originally designed for repeated administration, and repeated presentation may diminish the stressful response to the test. We examined this possibility in detail in our prior published work,[64] and although there were effects of the day and scenario, the magnitude was small, suggesting little habituation or sensitization in the current study. In addition, the negative effect of E_2 treatment remained across all depletion challenges. We also had to use a between-subjects design with regard to E_2 treatment because of limitations regarding how often participants can perform the depletion challenges and the TSST. The cognitive battery was performed only after the psychosocial stress maneuver, because the primary comparison of interest was between treatments, rather than within subjects. In addition, the length and difficulty of the entire experimental procedure were such that adding predepletion cognitive testing was felt to be too burdensome for participants. Thus, we do not have information about how the participants would have performed before the monoamine depletion and psychosocial stress. However, participants were extensively cognitively screened at baseline and trained on the cognitive battery before the initiation of the overall study, and thus, we are confident that cognitive performance was essentially equivalent between the two groups before estrogen or placebo treatment and AA/psychosocial stress challenge. In addition, the E_2 dose used in this study was somewhat higher than average clinical doses, although not beyond the normal clinical range. E_2 blood levels were not higher than is typically seen during the late follicular phase of a normal menstrual cycle. We have shown previously that E_2 levels in this range are beneficial in a cholinergic challenge model[52]; however, the interaction with psychosocial stress in the current study showed the opposite effects. Additional cortisol sampling beyond the two samples that we obtained would have been helpful to further characterize the magnitude of the stress response, but we were unable to do so in this study design. Finally, although this was a blinded study, we recognize that the subjective effects of E_2 may have been difficult to fully blind.

CONCLUSIONS

PMW who were administered E_2 for 3 months exhibited marked worsening of cognitive performance after a social stress test compared with placebo-treated PMW. These effects were generally independent of tryptophan or tyrosine/phenylalanine depletion and were not significantly correlated with negative mood changes. These data imply that the effect of E_2 on cognitive performance after menopause is not straightforward and may interact significantly with psychological stress or especially stressful events. The effects of the hormone-stress interaction on cognitive performance did not seem to be significantly modified via catecholamine or indoleamine mechanisms. Further research will be necessary to confirm and clarify these findings as well as explore underlying mechanisms. Replication without the depletion maneuver, the examination of the effects of different doses of E_2, combined E_2 and progestin therapy, and the examination of women during different phases of the menstrual cycle will help clarify these findings.

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