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Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women

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Summary While cognitive changes and mood instability are frequent symptoms reported by menopausal women, the degree to which the decline in estrogen production is responsible is not yet clear. Several lines of evidence suggest that estrogen may produce its effects on cognition and mood through modulation of serotonergic function. To test this hypothesis, we used the tryptophan depletion (TD) paradigm to lower central serotonin levels and pharmacologically manipulated estrogen levels in healthy menopausal women. We examined the individual and combined effects of estradiol and serotonin on working memory, emotion processing and task-related brain activation. Eight healthy predominantly early postmenopausal women underwent TD or sham depletion followed by functional magnetic resonance imaging (fMRI) both before and after short-term transdermal estradiol 75—150 μg/d administration. There was an estradiol treatment by TD interaction for brain activation during performance on both the N-back Task (working memory) and Emotion Identification Task (affective processing). During the 2-back condition, TD attenuated activation prior to, but not after, estradiol treatment in the right and left dorsal lateral prefrontal and middle frontal/cingulate gyrus. During emotion identification, TD heightened activation in the orbital frontal cortex and bilateral amygdala, and this effect was attenuated by estradiol treatment. These results provide preliminary evidence that serotonergic effects directly mediate the impact of estrogen on brain activation during working memory and affective processing.

1. Introduction

The menopause transition is frequently accompanied by depressive symptoms and subjective declines in cognitive function (Shively and Bethea, 2004; Freeman, 2010; McVeigh, 2005; Schnatz et al., 2006). Unlike the well-characterized

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relationship between ovarian declines in estrogen production and vasomotor and urogenital symptoms, the role of hypoestrogenism in behavioral and cognitive disturbances during the menopause is less clearly established and frankly controversial (Schmidt et al., 2000; Henderson et al., 2003; Shumaker et al., 2003; Gibbs, 2006; Joffe et al., 2006; Krug et al., 2006; Kok et al., 2006; Soares et al., 2006; Morrison et al., 2006; Maki et al., 2007; Epperson et al., 2007; Luetters et al., 2007; Sherwin and Henry, 2008; Resnick et al., 2009; Coker et al., 2010; NAMS, 2010).

Estrogen has pronounced effects on numerous neurotransmitter systems (McEwen, 2002; Amin et al., 2005; Bethea et al., 2009) neurotrophins (Scharfman and MacLusky, 2006; Suzuki et al., 2009), and brain cytoarchitecture, structure and function (Hao et al., 2006; Kramár et al., 2009), all possible mechanisms by which estrogen exerts effects on neural systems involved in mood and cognition. In this study, we focus on the estrogen—serotonin interaction for several reasons. First, estradiol modulates serotonergic function from the level of neurotransmitter synthesis and degradation (Smith et al., 2004a; Sanchez et al., 2005) to the density of post-synaptic 5-HT type 2A (5-HT2A) receptors (Kugaya et al., 2003; Moses-Kolko et al., 2004) and sensitivity of the presynaptic 5-HT type 1A (5-HT1A) autoreceptor (Henderson and Bethea, 2008). Second, intact serotonergic function is important for healthy mood, learning and memory (Mendelsohn et al., 2009; Robbins and Arnsten, 2009), hence the heuristic importance of investigating this hormone—neurotransmitter interaction in menopausal women. Finally, central serotonin levels can be safely manipulated in human subjects using the tryptophan depletion (TD) paradigm, thus allowing investigators to probe the relative importance of intact serotonin function on behavior (Van der Does, 2001).

TD is achieved by administering an oral load of large neutral amino acids (LNAA) excepting tryptophan (Young et al., 1985). In addition to stimulating protein synthesis, these LNAAs compete with tryptophan already present in the blood for passage across the blood brain barrier. This dual process essentially depletes central nervous system (CNS) tryptophan levels. Without its precursor available, brain levels of serotonin plummet (Carpenter et al., 1998). Notably, neuroimaging studies suggest that the rate of serotonin synthesis declines more rapidly and to a greater degree during TD in women compared to men (Nishizawa et al., 1997).

TD can trigger depressive symptoms in individuals with selective serotonin reuptake inhibitor (SSRI)-remitted depression (Delgado et al., 1999), but also in healthy subjects who are vulnerable to affective disturbance secondary to a positive family history, serotonin transporter genotype, or female sex (Benkelfat et al., 1994; Ellenbogen et al., 1996; Klaassen et al., 1999; Neumeister et al., 2002). This phenomenon was not observed in menopausal women whose depression had resolved with estradiol treatment (ET) alone, ET plus SSRI or SSRI alone (Epperson et al., 2007). While there appears to be a modest sex difference in biochemical and behavioral response to TD, the impact of ET on TD-induced behavioral change in women has not been elucidated.

With respect to cognitive processes in healthy humans, TD impairs verbal learning (Park et al., 1994; Schmitt et al., 2000; McAllister-Williams et al., 2002; Amin et al., 2006), paragraph recall (Amin et al., 2006), response inhibition, decision-making and processing of reward cues (Park et al., 1994; Rogers et al., 1999, 2003; Murphy et al., 2002), and improves focused attention (Schmitt et al., 2000; Gallagher et al., 2003) and verbal fluency (Schmitt et al., 2000). It is less clear whether TD negatively impacts working memory, an aspect of cognition for which there is evidence of estrogen enhancement (Harrison et al., 2004; Riedel, 2004; Maki et al., 2001; Resnick and Maki, 2001; Shaywitz et al., 2003; Wolf and Kirschbaum, 2002; Duff and Hampson, 2000; Phillips and Sherwin, 1992; Henderson and Sherwin, 2007; Joffe et al., 2006).

Because estrogen has trophic effects on brain structures underlying important cognitive processes (Rapp et al., 2003) and potent serotonin-modulating properties, we hypothesized that estradiol administration would reverse the effects of TD on neural systems thought to underlie working memory and affective processing. Here we examined performance and brain activation in healthy, predominantly early postmenopausal women undergoing TD and sham depletions pre- and post-transdermal estradiol administration. Women underwent functional magnetic resonance imaging (fMRI) while performing a working memory (N-back Task) and an affective processing task (Emotion Identification).

2. Methods

2.1. Participants

Women ages 45—60 were recruited to a university-based women's behavioral health specialty research program by posted fliers, paid advertising, word of mouth and home mailings. Nine right-handed women without history of any Axis I psychiatric or substance use disorder according to the Structured Clinical Interview for Diagnosis-DSM-IV (SCID)-Non-Patient Version were enrolled. History of first-degree relatives with a substance use disorder, but not psychiatric disorder, was allowed. Women who had menstrual cycle irregularity or were within 15 years of their final menstrual period (FMP) and had follicular stimulating hormone levels of $\geq$20 IU/ml were eligible. All subjects were required to provide documentation of having a normal PAP smear, mammogram, and breast and pelvic examinations within the previous year. Women were in good health and without history of any significant medical problems including, but not limited to, cancer, unstable hypertension, known cardiovascular disease, thromboembolic disease, neurological disorders or previous head injury. All women were without hormone use for at least twelve months prior to participation. Subjects gave written informed consent to participate in this study, which was approved by the Yale University School of Medicine Human Investigations Committee. A total of 8 women with mean $\pm$ SD age of 53.4 $\pm$ 3.9 years completed all four test days and were included in data analyses. They were paid for each test day with an additional bonus for completing all four testing sessions.

2.2. Tryptophan depletion procedure

This study used a double-blind, placebo-controlled, crossover design with subjects undergoing an active and sham depletion prior to and after 3—8 weeks of transdermal

estradiol 75—150 μg/d (Vielle-Dot®, Novartis, Hamilton, New Jersey). On each test day, participants presented to the General Clinical Research Center at 7:30 am after an overnight fast and ingested 70 capsules containing either 31.5 g of amino acids without tryptophan (active depletion) or 31.5 g of lactose (sham depletion). The amino acid mixture consisted of L-isoleucine 4.2 g, L-leucine 6.6 g, L-lysine 4.8 g, L-methionine 1.5 gm, L-phenylalanine 6.6 g, L-threonine 3.0 gm and L-valine 4.8 g. This combination of amino acids reliably lowers plasma total and free tryptophan by 71—78% (Neumeister et al., 2004) and results in behavioral and/or brain metabolism change (Nugent et al., 2008; Neumeister et al., 2005, 2006). Blood was taken for free tryptophan analysis prior to and approximately 6 h after consumption of the amino acid mixture. Participants remained fasting and involved in quiet, sedentary activity until their fMRI scans.

2.3. Estrogen treatment

After completing two test days, subjects took transdermal estradiol 75—150 μg/d (Vivelle-Dot®, donated by Novartis Pharmaceuticals, Hamilton, NJ) for at least 3 weeks and then underwent their 3rd and 4th TD and fMRI test days. Dose of estrogen was adjusted from 75 μg/d to 150 μg/d after 2—3 weeks of treatment if estrogen levels were not above 50 pg/ml and at least twofold higher than baseline. For the five women with an intact uterus, this was followed by treatment with oral micronized progesterone (Prometrium® Abbott Laboratories, Abbott Park, IL) 200 mg/d for 10 days.

2.4. Tryptophan and hormone assays

Free plasma tryptophan levels were determined by direct injection of 5 μL of plasma ultrafiltrate on a high-performance liquid chromatographic—fluorometric system (Anderson et al., 1981). Tryptophan levels were measured with within-assay and assay-to-assay coefficients of variation of less than 5 and 10%, respectively, and with a detection limit of less than 0.01 μg/ml.

Estradiol levels were evaluated in the morning of each test day and measured by competitive immunoassay using a chemiluminescent substrate in a commercially available kit provided by Diagnostic Productions Corporation, Los Angeles, CA. The sensitivity for this kit is 15 pg/ml and the approximate coefficient of variability at ranges observed in this study is 11—13%.

2.5. Mood assessment

Clinician and patient ratings were performed the morning prior to and 6 h after ingesting the amino acid or lactose capsules. Ratings consisted of the 19-item Hamilton Depression Rating Scale (HDRS; Hamilton, 1960) and the Profile of Mood States (POMS; McNair et al., 1992). The HDRS is a clinician-rated instrument widely used to assess severity of depressive symptoms. The POMS requires that subjects rate themselves with respect to 65 different adjectives, such as 'friendly', 'listless', 'on edge,' which are then utilized to compute scores for 6 subscales; depression, tension/anxiety, anger/hostility, fatigue, vigor, and confusion/bewilderment.

2.6. Cognitive and affective task

2.6.1. N-back Task

The N-back Task is a working memory task that reliably activates the dorsal lateral prefrontal cortex (DLPFC) and medial frontal/cingulate gyrus (MF/CG) (Owen et al., 2005). Enhanced activation was reported in non-human primate studies of estrogen's effects on working memory (Rapp et al., 2003; Hao et al., 2006). Subjects completed a 2-back Task that was created using an E-Prime stimulus presentation program (Psychology Software Tools, Pittsburgh, PA). There were four versions of the task in order to minimize practice effects across scans. Order was counterbalanced across subjects. Subjects were instructed to press one of two buttons to indicate whether a stimulus presented on the screen is the same or not the same as the stimulus that was presented two trials earlier (2-back). A control condition (0-back) required the subject to press either button when a target stimulus was presented. The stimuli consisted of 4-letter non-words (e.g. "cnsy") without vowels. The two conditions (2-back and 0-back) alternated in 6, 18-s blocks, which were preceded by an instructions screen for 3 s. Blocks consisted of 12 trials of 1500 ms each.

2.6.2. Emotion Identification Task

The Emotion Identification Task probes emotional bias under various affective conditions and has been extensively studied in conjunction with the TD paradigm (reviewed by Mendelsohn et al., 2009). Estrogen may be associated with increased brain activation to positive affective stimuli (Amin et al., 2006), while TD is associated with bias towards negative affective stimuli (Evers et al., 2007, 2010; Klaassen et al., 2002). In addition, tasks involving emotional face identification are associated with amygdala and orbital frontal cortex (OFC) activation (Streit et al., 2003). TD has been found to modulate the OFC (Rubia et al., 2005) and enhanced serotonin transmission has been associated with attenuating the amygdala's response to negative emotional face stimuli (Del-Ben et al., 2005). Animal studies indicate that the amygdala has among the highest concentrations of estrogen receptors in the brain (Hirata et al., 1992; Merchenthaler et al., 2004; Mitra et al., 2003), highlighting the importance of the amygdala as a region of interest in this study.

The face processing task was also created using E-Prime. Subjects were randomly presented with images of 30 individuals with angry, happy and neutral facial expressions (a total of 90 face pictures). They were instructed to choose which emotion was being expressed. Face stimuli were obtained from a set of digital images which depicted approximately 40 individuals posing several different facial expressions (Tottenham, 2002). During a variable inter-stimulus interval (0.5—16 s) a fixation cross was randomly presented as a low level baseline. Each trial lasted 2500 ms, during which each face was presented for 150 ms followed by a choice screen lasting 2350 ms.

2.7. Image acquisition

Brain imaging was conducted on a Siemens 3.0 Tesla Trio system. For structural whole brain images, the first scan was a sagittal localizer, followed by a T1 scan oriented in the axial-oblique dimension, parallel to the anterior commis-

sure—posterior commissure (AC—PC) line (24 slices, RT = 300 ms, matrix size = 256 × 256, FOV = 220 × 220 mm). mm). A three dimensional high resolution spoiled gradient scan was conducted (176 slices, RT = 2530 ms, matrix size = 256 × 256, FOV = 256 × 256 mm). Functional whole-brain images were acquired using a gradient echo T2-weighted echoplanar imaging scan (RT = 1500 ms; echo delay = 30 ms; flip angle = 80°, FOV = 220 × 220 mm).

2.8. Image analysis

BOLD time series data were analyzed with FEAT (fMRI Expert Analysis Tool) Version 5.92, part of FSL 4.0, using standard image analysis procedures including brain extraction, slice time-correction, motion correction, high pass filtering (100 s), spatial smoothing (6 mm FWHM, isotropic), and mean-based intensity normalization. The median functional and anatomical volumes were coregistered, and the anatomical image was transformed into standard space (2 mm^3 T1 MNI template). Resulting transformation parameters were later applied to statistical images and the images were resampled (2 mm^3) before group level analyses.

Subject-level statistical analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Smith et al., 2004b). For the N-back Task, condition events (0-back, 2-back) were modeled using a canonical hemodynamic response function. The instruction period and motion correction parameters were included as nuisance covariates and the rest condition (fixation point) was treated as the unmodeled baseline. Similar procedures were used for Emotion Identification Task modeling the three condition events (happy, angry, and neutral) with fixation as baseline. To characterize the treatment (pre vs. post estrogen) by condition (sham vs. active depletion) effects for the N-back Task, mean percent signal change for the 2—0 back contrast was extracted from functionally defined regions of interest (ROIs) in the dorsolateral prefrontal cortex (DLPFC right and left) and medial frontal/cingulate gyrus (MF/CG). Similar procedures were applied to the Emotion Identification Task using atlas based ROIs for the amygdala (right and left) and orbital frontal cortex (OFC right and left). Mean percent signal change values were exported for analysis using procedures described below.

Region of interest masks for the DLPFC and MF/CG were functionally defined using the main effect of memory load (2—0 back) map derived from a whole brain repeated measures ANOVA of this sample (see Fig. 1). The activation map was cluster corrected at voxel threshold of $Z \geq 4.26$ and cluster probability of $p < 0.05$ (Woolrich et al.,2001, 2004). These procedures produced well-defined clusters in the right DLPFC, left DLPFC, and the MF/CG consistent with our previous studies (2005; Loughead et al., 2009, 2010). ROIs for the amygdala and OFC were anatomically defined using the Harvard-Oxford probabilistic atlas (Maximal Probability Threshold: 25%). ROI masks were transformed into native subject space using methods described above. Mean percent signal change values for each subject (from all ROIs described above) were entered as the dependent variable in a repeated-measures analysis of variance (ANOVA) with ROI, condition (active vs. sham depletion) and treatment (pre-estrogen vs. post-estrogen) as within-subject factors using SAS, version 9.2 (SAS Institute Inc., Cary, NC). The N-back and Emotion Identification Tasks were tested in separate ANOVAs.

2.9. Data analysis: behavioral measures and assays

Behavioral and hormone data were assessed for normality before analysis using normal probability plots and Kolmogorov—Smirnov test statistics. Variables were log-transformed as necessary. Analysis of plasma free tryptophan was performed with a linear mixed model, which included within-subjects effects of condition (active vs. sham depletion) and treatment (pre-estrogen vs. post-estrogen) and random subject effects. The interaction between treatment and condition was also fitted and explained by appropriate post hoc test. The best-fitting covariance structure was selected according to information criteria. Plasma estradiol, performance on the N-back and Emotion Identification Tasks, and mood assessments were analyzed using similar models as described above, with the exception that time (PM—AM) was included as a third factor in the analysis of HDRS and POMS data. Bonferroni correction was applied within but not between hypotheses.

3. Results

3.1. Tryptophan and estrogen assays

Mean number of months since FMP was 28.1 ± 10.9 months (range 12—41 months) in those women with intact uterus or

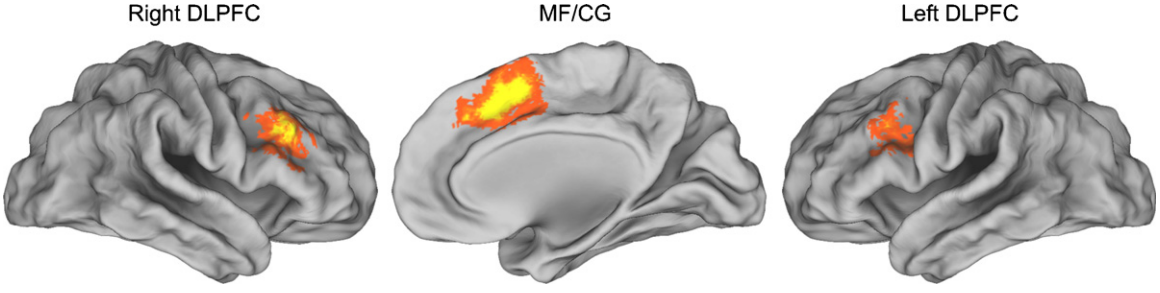

Figure 1 N-back brain rendering highlights regions of interest. N-back working memory task activation. Bilateral dorsolateral prefrontal cortex (DLPFC) and medial frontal/cingulate gyrus (MF/CG) regions identified by the main effect of working memory load (2 back vs. 0 back) in a whole brain repeated measures ANOVA ($p < 0.05$, corrected). Brain rendering performed with Caret (Van Essen et al., 2001).

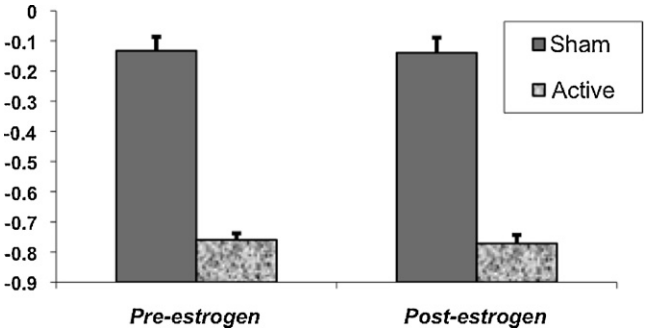

Figure 2 Change in free tryptophan levels during active and sham depletion. Free plasma tryptophan levels decreased by 64–90% from pre to 6 h post-ingestion of an amino acid mixture without tryptophan. Free plasma tryptophan levels decreased between 5% and 26% on sham depletion test days.

hysterectomy post FMP. Final menstrual period could not be confirmed for one 55 year-old subject who had a partial hysterectomy prior to menopause, but her FSH of 106 IU/ml confirmed that she was post-menopausal at the time of study participation. One other woman had hysterectomy with bilateral oophorectomy during the menopause transition.

All subjects ingested the 70 capsules within the allotted 45-min time frame and without vomiting or significant nausea. As expected, there was a main effect of TD on plasma free tryptophan levels ($F(1, 20) = 249$, $p < 0.0001$), with mean $\pm$ SD decreases of $75.9 \pm 0.06\%$ before and of $77.2 \pm 0.07\%$ after ET, but no significant interaction between estrogen treatment and TD condition ($F(1, 20) = 0.01$, $p = 0.95$) (Fig. 2). No significant changes in tryptophan levels were noted on sham test days either before ($-13.3 \pm 13.1\%$) or after ($-13.9 \pm 14.2\%$) ET.

There was no significant treatment (estrogen) by condition (TD vs. sham) interaction ($F(1, 21) = 0.05$, $p = 0.83$) in the model comparing estradiol levels. However, as expected estrogen treatment resulted in significantly increased plasma estradiol levels with mean $\pm$ SD being 26.3 ± 10.6 pg/ml during TD and 24.7 ± 9.6 pg/ml during sham depletion before estrogen treatment and 78.0 ± 27.5 pg/ml on active and 79.9 ± 43.1 pg/ml on sham tests days during treatment ($F(1, 21) = 25.7$, $p < 0.0001$) (Fig. 3).

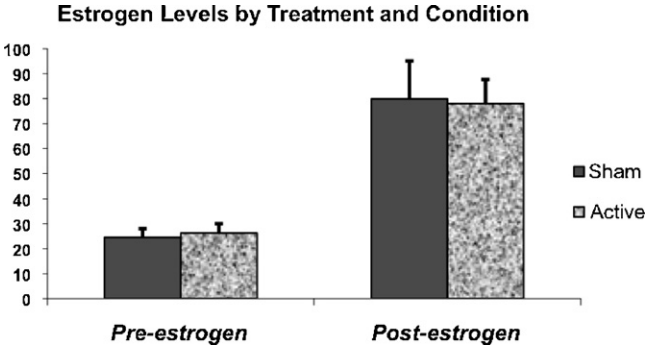

Figure 3 Estradiol levels pre and post estrogen treatment. Estradiol levels increased with estrogen administration but did not differ significantly between active and sham depletion test days after estrogen administration.

3.2. Mood assessment

Mean $\pm$ SD HDRS scores were all within the asymptomatic range of 1 ± 1 to the highest of 2 ± 3. Both HDRS and POMS subscale scores remained stable in all subjects across all test days. There was no significant treatment by condition interaction for the HDRS or POMS subscales (all $p > 0.05$) (data not shown).

3.3. Task performance

Reaction time during the 2-back Task on TD (695 ± 100 ms) and sham (712 ± 56 ms) test days before and after ET (TD; 681 ± 128 ms and sham; 681 ± 71 ms) did not differ (interaction: $F(1, 21) = 0.14$, $p = 0.71$). Similarly, there was no significant interaction between ET and condition with respect to errors made during performance of the 2-back ($F(1, 21) = 0.01$, $p = 0.95$) or the 0-back ($F(1, 21) = 0.09$, $p = 0.76$) blocks. Reaction time ($F(1, 19) = 2.49$, $p = 0.13$) and number of errors ($F(1, 19) = 3.11$, $p = 0.1$) during Emotion Identification Task performance were similar between treatments across conditions.

3.4. N-back region of interest analysis

The repeated measures ANOVA of mean percent signal change showed a main effect of condition ($F(1, 83) = 5.30$, $p = 0.024$), indicating reduced activation in the working memory regions for active depletion (vs. sham). There was no significant main effect of treatment ($F(1, 83) = 0.92$, $p = 0.34$) or ROI ($F(2, 83) = 0.92$, $p = 0.34$). However a significant condition $\times$ treatment interaction was observed ($F(1, 83) = 5.22$, $p = 0.025$), indicating that under the pre-estrogen treatment, mean percent signal change was reduced by active depletion (vs. sham) but post-treatment, TD did not modulate BOLD signal. The other 2-way interactions were removed from the model (as well as the 3-way interaction) as none were statistically significant. Although the ROI by condition by treatment ANOVA showed a significant ME (condition) and a 2-way interaction (condition by treatment), subsequent analyses in the individual ROIs revealed no significant effects (Fig. 4).

3.5. Emotion identification region of interest analysis

For the Face task, the repeated measures ANOVA on the mean percent signal change data showed a significant main effect of condition ($F(1, 114) = 14.53$, $p = 0.0002$), indicating increased activation for the active depletion (vs. sham) condition in the orbital frontal cortex and bilateral amygdala. There was a significant main effect of treatment ($F(1, 114) = 11.26$, $p = 0.001$), indicating reduced activation with estradiol treatment in both regions compared to pre treatment. There was no significant main effect of region ($F(3, 114) = 0.24$, $p = 0.872$). The condition $\times$ treatment 2-way interaction was found to be significant ($F(1, 114) = 7.07$, $p = 0.009$). In contrast to N-back, the Face task pre-treatment session showed increased mean percent signal change under active depletion and, as above, estrogen treatment negated TD effect on BOLD signal.

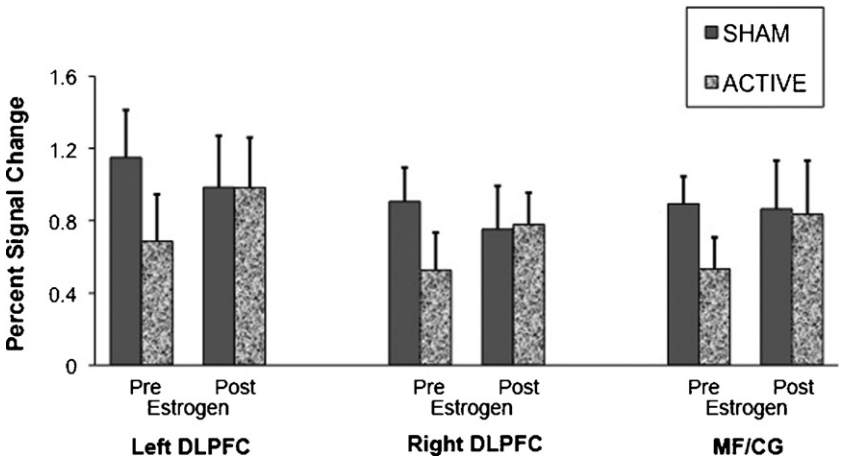

Figure 4 Mean percent signal change for the 2-back minus 0-back contrast calculated from functionally defined dorsolateral prefrontal cortex (DLPFC) and medial frontal/cingulate gyrus (MF/CG) regions of interest.

As with the N-back Task, other 2-way and 3-way interactions were removed from the model as none were statistically significant. The ROI by condition by treatment ANOVA showed significant main effects (condition, treatment) and a 2-way interaction (condition by treatment), however subsequent analyses in the individual ROIs revealed no significant effects (Fig. 5).

4. Discussion

This study is unique in that inclusion of estrogen treatment with the TD paradigm allows for the investigation of individual and interactive effects of estrogen and serotonin on brain activation and behavior. To our knowledge this is the first study to examine brain activation during TD in subjects undergoing both affective and cognitive processing tasks. That TD had a significant impact on brain activation during performance of both tasks in a group of postmenopausal women suggests that intact serotonergic function is crucial to the underlying neural networks mediating these behaviors. The direction of TD's effect on BOLD signal differed between tasks, providing evidence that the role of serotonin in affective and cognitive processes is disparate.

The individual effect of estrogen treatment on brain activation during performance of the Emotion Identification Task was significant, while there was only a modest effect of estrogen during performance of the N-back Task. An intriguing finding with respect to estrogen is that hormone treatment resulted in reversal of the effects of TD on brain activation during both tasks. Although preliminary in nature, these data provide novel evidence of an interactive effect of estrogen and serotonin in verbal working memory and processing of emotional faces. The direction of the estrogen–serotonin interaction differed between tasks, suggesting specificity of the interaction to the type of cognitive or affective task. Our failure to demonstrate a significant effect of either TD or estrogen administration on task performance is possibly due to power but is also consistent with findings from other studies (Dumas et al., 2010; Persad et al., 2009; Joffe et al., 2006; Allen et al., 2006; Shaywitz et al., 1999).

Previous studies of the effect of TD on brain activation during working memory tasks have been inconsistent, with some finding a reduction in activation (Cerasa et al., 2008) while others finding no significant effect (Gallagher et al., 2003; Allen et al., 2006). The effect of estrogen alone in the right and left DLPFC and MF/AC was not impressive, yet

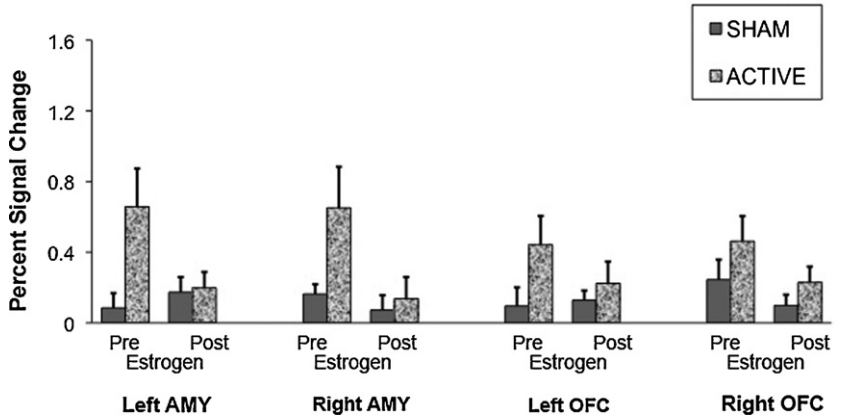

Figure 5 Mean percent signal change for the Emotion Identification Task effect calculated from anatomically defined amygdala and orbital frontal cortex.

non-human primate studies suggest estrogen-enhanced DLPFC function and performance in ovariectomized female rhesus macaques performing a DLPFC-dependent task (Hao et al., 2006). In addition, another study using PET receptor imaging before and after a dose and duration of estrogen similar to that used in this study showed a significant increase in 5HT-2A receptor density in a number of brain regions, including the DLPFC (Kugaya et al., 2003; Moses-Kolko et al., 2004). Including a sham depletion condition was essential in discovering the difference between the impact of TD before estrogen treatment vs. during estrogen treatment, and revealed clear evidence that both estrogen and serotonin contribute to verbal working memory. That effects of TD were seen prior to but not during estrogen administration suggests that serotonin plays a primary role in working memory, while estrogen has a moderating role by supporting healthy serotonergic function. This finding has clinical relevance considering that the use of estrogen treatment to promote working memory in healthy aging women is controversial, with some studies indicating a benefit (Duff and Hampson, 2000; Maki et al., 2001; Berent-Spillson et al., 2010) while others not (reviewed by Lethaby et al., 2008). These data imply that estrogen's beneficial effects on working memory may be limited to mid-aged and aging women with reduced serotonin function.

Our finding that women experienced an accentuation of amygdala and OFC activation with TD during Emotion Identification is consistent with previous fMRI studies of affective processing during TD (Cools et al., 2005; Fusar-Poli et al., 2007; van der Veen et al., 2007; Williams et al., 2007). Similar to our study, three of the above studies found no observable impact of TD on performance of Emotion Identification Tasks in healthy volunteers (Cools et al., 2005; Fusar-Poli et al., 2007; van der Veen et al., 2007). Three studies, which did not incorporate functional imaging, found that TD impaired recognition of fearful faces (Harmer et al., 2003; Marsh et al., 2006; Merens et al., 2008). This finding was limited to individuals who were 'at-risk' for depression as a result of having had a previous episode of depression (Merens et al., 2008) or being heterozygous for the short, and less efficient, allele of the serotonin transporter gene (Marsh et al., 2006). Subjects in the present study could be considered affectively resilient as they have come to mid-life with no history of depression or anxiety disorders, both twice as common in reproductive aged women compared to their male counterparts.

Several studies indicate that baseline serotonin function may impact an individual's behavioral or neural response to TD. Genetic variation in the gene encoding monoamine oxidase A, the enzyme for serotonin metabolism, predicts the degree of brain activation in the ventrolateral prefrontal cortex during performance of the N-back Task (Cerasa et al., 2008). Individuals heterozygous for the short allele of the serotonin transporter showed improved memory and attention (Roiser et al., 2007), but impaired fear face emotion recognition (Marsh et al., 2006) during TD compared to those heterozygous for the long allele. Individual characteristics such as threat sensitivity (Cools et al., 2005) and gender (Harmer et al., 2003) may also contribute to TD-induced changes in brain activation during emotion processing.

While the paradigm employed in this study is unique and the findings are of interest, cautious interpretation is warranted for several reasons. First, the sample size is small making it impossible to consider individual variables such as genotype or psychological sensitivities. Notwithstanding this limitation, all of our participants were postmenopausal, without previous personal or family history of psychiatric disorders and in good general health. Given the study paradigm, 8 subjects represents 32 day-long procedures culminating in an fMRI study. That there are multiple within-subject data points and each subject serves as her own control eliminates sampling error and limits, if not entirely negates, problems related to sample size. Although a placebo control group was not included in this study and would have been ideal, 4 different versions of each task were used and presented in randomized order across test days. The stability of performance across conditions and treatment suggests that our methods may have limited practice effects, although a placebo control would be needed to confirm our assertion of estradiol effects. Finally, time since FMP varied considerably in this small group. There is growing evidence that time since FMP may impact the effects of estradiol on brain function and a considerably larger sample would be required to include this variable as a covariate in our study design.

In summary, this is the first study to examine the effects of TD and estrogen administration on cognitive and affective tasks and brain activation in postmenopausal women. These data provide preliminary evidence that serotonin is important for verbal working memory and face emotion identification and that estrogen supports serotonin function under conditions of TD. That estrogen reverses the effects of acute TD on brain activation suggests that estrogen bolsters serotonergic functioning under conditions of reduced tryptophan and serotonin concentrations. Because serotonin reduction with tryptophan depletion is both dramatic and acute, the generalizability to slower and more chronic reductions in serotonin function seen with normal and pathological aging in humans is admittedly limited. Complementary work may be necessary in preclinical models.

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Conflict of interest

Dr. C. Neill Epperson has been a consultant to Eli Lilly and received research grant support for her research from Eli Lilly and Shire. Dr. Epperson or her dependent owns shares in Johnson and Johnson.

Dr. Gur declares that over the past three years he has received compensation as a consultant to Johnson and Johnson. He is or has been the recipient of Investigator Initiated Research Grants from Merck and from AstraZeneca.

All remaining authors have no conflicts of interest to report.

Contributors

C. Neill Epperson, M.D. designed the study and supervised all aspects of the study conduct and completion, the data analysis and interpretation and manuscript writing.

Zenab Amin, Ph.D. participated in the design of the fMRI procedures and cognitive paradigms. She was involved in recruiting subjects, managing and analyzing data. Dr. Amin participated in all stages of the manuscript preparation.

Kosha Ruparel, M.S.E. was responsible for fMRI data analysis and presentation. She participated in manuscript preparation.

James Loughead, Ph.D. supervised fMRI data analysis and participated in manuscript preparation.

Ruben Gur, Ph.D. advised Drs. Epperson and Loughead with respect to the data analytic plan and participated in manuscript preparation.

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