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NAMS FELLOWSHIP FINDINGS

Effect of estradiol versus estradiol and testosterone on brain-activation patterns in postmenopausal women

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ABSTRACT

Objective: To determine the effect of estradiol and testosterone on brain-activation patterns in surgically postmenopausal women viewing erotic video clips using functional magnetic resonance imaging.

Design: Six women, who had undergone a bilateral oophorectomy and hysterectomy for benign disease, viewed erotic and neutral videos during functional magnetic resonance imaging while not on hormone therapy, while on estradiol therapy, and while on estradiol and testosterone therapy. Five similarly aged premenopausal women viewed the same videos. Areas of brain activation between the functional magnetic resonance imaging scans of both groups of women were compared to determine whether agonadal serum levels of sex hormones and administration of estradiol and testosterone impacted brain patterns of sexual arousal.

Results: When compared with premenopausal women, untreated postmenopausal women had significantly decreased areas of brain activation during both erotic and neutral stimulations. Administration of estradiol increased global brain-activation patterns during both visual stimulations, with erotic video viewing causing a limited increase in limbic system activation. Combined estradiol and testosterone therapy was associated with a greater activation of the central nervous system, with more limbic system activated during the erotic video. Brain-activation patterns of the postmenopausal women were similar to the premenopausal group only during the estradiol and testosterone treatment phase.

Conclusions: Agonadal serum hormone levels result in globally decreased brain-activation patterns in postmenopausal women while viewing neutral and erotic videos. Administration of both estradiol and testosterone increase global brain activation, and both sex steroids are independently associated with enhanced limbic system response during erotic visual stimulation.

Key Words: Postmenopausal – Sexual arousal – Estrogen – Testosterone – Functional magnetic resonance imaging – Brain.

Sexual dysfunction, including decreased sexual arousal, is reported by women of all ages, but prevalence increases in the perimenopausal and postmenopausal years.[1] The development of sexual dysfunction at this time is likely to be caused by lowered serum estradiol (E_2) and testosterone concentrations that occur with aging and the menopausal transition. Postmenopausal women have serum E_2

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levels that are approximately 15% of their premenopausal concentrations.[2] Serum testosterone levels are 50% lower in a woman in her 40s when compared with 20 years earlier, and levels decrease another 50% after bilateral oophorectomy or 15% with natural menopause.[3] Decreased serum E_2 concentrations result in reduced vaginal lubrication, diminished clitoral blood flow, and altered sensory perception from peripheral nerves located in the pelvic region.[4] Studies have shown that similar to E_2, testosterone impacts both genital and central nervous system (CNS) sexual responses.[5,6] Testosterone activates vaginal androgen receptors (AR) in the rabbit, promoting relaxation of smooth muscle and increasing sympathetic nervous system activity that occurs with female sexual arousal.[5,6] Use of hormone therapy (HT) in women with decreased serum concentrations of E_2 and testosterone who report sexual dysfunction is a common practice though clinical studies have yielded variable results.

Some of the variance in studies evaluating treatments for sexual problems has been due to the diverse modalities used to assess female sexual functioning. Clinical evaluation of sexual dysfunction has been determined mainly by subjective questionnaire such as the Female Sexual Function Index and the McCoy Female Sexuality Questionnaire.[4,7,8] Questionnaires can produce erroneous results owing to influences such as cultural or religious beliefs, socioeconomic background, and partner availability that may affect a woman's responses. Assessment of sexual arousal in experimental settings has included: vaginal photoplethysmography, which indirectly determines vaginal vasoengorgement; Doppler ultrasonography, which assesses clitoral blood flow; and magnetic resonance imaging (MRI) of the perineum, used to determine clitoral blood volume and size.[4,7,9] Although these methods provide a more objective measurement of genital arousal, they are not conducive to use in a routine clinical evaluation. In addition, sexual functioning studies have often found no correlation between the female participant's genital congestion and subjective sexual arousal.[10]

Researchers are now utilizing new advances in radiographic techniques to determine the cerebral basis for sexual response and ultimately the pathophysiology of sexual dysfunction.[11-13] Studies in both men and women have utilized positron emission tomography and functional MRI (fMRI) to determine the neurobiologic processing of sexually explicit images and subjective sexual arousal. Research in men has reported several areas of the brain activated by erotic visual images when compared with neutral films, specifically the limbic system, which includes the anterior cingulate gyrus, amygdala, hypothalamus, and thalamus.[11,13,14] The few studies in women have revealed gender differences in response to visual sexual stimuli, with premenopausal women having both decreased CNS signal strength, and thus neuronal activity, and limbic system activation when compared with men.[13,14]

The neuroendocrinology of sex steroids and their impact on sexual behavior in postmenopausal women can now be explored objectively and in real time using these radiographic techniques. Androgens and estrogens cross the blood-brain barrier and interact with receptors throughout the CNS, including the cortex, hypothalamus, thalamus, and amygdala.[15]

This pilot study was undertaken to determine whether the addition of E_2 and testosterone increased sexual arousal in surgically postmenopausal women as determined by brain-activation patterns with fMRI. Six bilaterally oophorectomized volunteers viewed erotic and neutral videotapes while not on HT, while on estrogen therapy (ET), and while on ET and testosterone supplementation. To differentiate the effects of age and hormone status on sexual arousal, brain scans of the postmenopausal women were compared with fMRI scans of five similarly aged premenopausal women who viewed the same videos. We hypothesized that ET improves sexual arousal in agonadal postmenopausal women, but that the addition of testosterone further enhances arousal as measured by fMRI.

METHODS

Study participants

Two groups of women participated in this study, which was approved by the University of California, San Diego (UCSD) Human Research Protections Program with written informed consent obtained from each participant before the study.

Postmenopausal Women

Six healthy women who had undergone a hysterectomy and bilateral oophorectomy at least 2 years before study for benign disease were recruited. All women had not been on HT for at least 12 weeks before recruitment. Participants' ages ranged from 40 to 56 years with a mean (± SEM) age of 49.8 ± 2.3 years, and all had normal liver function test results and fasting cholesterol and thyroid-stimulating hormone levels. Baseline blood work confirmed agonadal levels of total testosterone ($\leq$ 1.0 nM) and E_2

(≤ 127 pM). Serum levels of androstenedione (A4) and free testosterone were also measured.

The postmenopausal women were screened using the Brief Index of Sexual Function for Women (BISF-W) and Beck Depression Inventory (BDI) and had no evidence of sexual dysfunction or depression. All women had to be sexually active with at least one sexual encounter a week. Two additional questionnaires that were prepared by the UCSD Department of Psychology were also administered to ensure that the volunteers were receptive to viewing sexually explicit imagery.

Premenopausal Women

Five premenopausal women whose age ranged from 33 to 53 years, with a mean age of 42.6 ± 4.2 years, were initially recruited to confirm that the selected erotic images were an appropriate sexual stimulation. The women were screened with the UCSD Department of Psychology questionnaires to ensure they were not adverse to viewing sexually explicit material, but they did not take either the BISF-W or the BDI. Serum total and free testosterone, A4, and E_2 levels were measured to ensure that any variation in fMRI results was not secondary to hormonal deficiencies and all serum levels were in the normal premenopausal range.

Procedures

All 11 women underwent a baseline blood oxygen level–dependent (BOLD) fMRI study (hereafter referred to as fMRI) while viewing erotic and neutral videos (see description in "Materials and design"). Thereafter, the six postmenopausal women underwent two additional fMRI study sessions at 6-week intervals. The six postmenopausal participants were placed on transdermal E_2 at 0.05 mg/day for a total of 12 weeks. A repeat fMRI using the same erotic and neutral stimulations were performed at the end of 6 weeks of E_2 therapy. The postmenopausal women then added testosterone to their E_2 therapy in the form of 1.25 g of a 1% topical testosterone gel (AndroGel, Solvay, Marietta, GA) applied to their outer thigh daily. At the end of 6 weeks of combined E_2 and testosterone therapy, a third fMRI was performed with the identical erotic and neutral videotapes. Serum E_2, total and free testosterone, and A4 levels were measured after the second and third fMRI sessions. After the third fMRI session, all postmenopausal volunteers also completed a second BISF-W questionnaire.

Before starting the fMRI project, a pharmacokinetic study was performed with a different group of six agonadal women in order to determine the appropriate AndroGel dose for surgically postmenopausal women. Using a mathematical model that incorporated the daily testosterone production and metabolism of premenopausal women (kindly provided by John J. Brennan, PhD), two doses of testosterone gel were selected for study. The daily dose of 1.25 g testosterone gel for 4 weeks resulted in physiologic serum concentrations of testosterone in this group of women. None of the participants reported any androgen-related side effects and lipid levels were not affected.

Materials and design

Validation of the Erotic and Neutral Images

To assess the quality and appropriateness of the segments to be used in the fMRI study, the video clips were rated on several emotional parameters. A separate group of five women between the ages of 20 and 30 years was shown 40 one-minute sexually explicit video segments and asked to rate each vignette on an eight-point Likert-type scale (ranging from 0 = extremely low to 7 = extremely high). The scales were designed to assess positive and negative emotional reactivity. Each woman viewed the films sitting alone in a room. Eight 1-minute segments were selected that had the lowest levels of disgust, fear, sadness, and anger. All eight segments consisted of at least one man and one woman engaged in heterosexual activity. These women also screened and evaluated 20 one-minute neutral videotapes. Eight 1-minute segments were chosen that had the most neutral scores with regards to surprise, amusement, sadness, fear, anger, and disgust on an eight-point Likert-type scale. Neutral films included a vacuum infomercial and a clam-digging trip.

Functional Magnetic Resonance Imaging

Imaging was performed on a Seimens 1.5-T clinical MRI scanner in the UCSD Department of Radiology. BOLD-fMRI theory is based on the brain physiology and evaluates CNS activity by comparing the magnetic difference between deoxygenated and oxygenated hemoglobin. Areas of the CNS that are more active will have increased amounts of oxygenated blood and greater signal intensity on the fMRI scans.

To attenuate background noise generated from the scanner, circumaural headphones were used. These headphones also provided binaural audio delivery for both the neutral and erotic stimuli. During the first ten minutes of the scanning session participants relaxed while high resolution structural magnetic

resonance images were acquired. A total of 180 sagittal slices measuring 1 mm × 1 mm × 1 mm thick were taken from ear to ear. Functional MRI scans were obtained with whole-brain scanning consisting of contiguous slices from front to back each measuring 4 mm × 4 mm × 5 mm.

A blocked fMRI paradigm was used in this within-women design. The "on" epochs consisted of either erotic or neutral video clips with each clip lasting 1 minute. These stimuli alternated with the "off" epochs, a blank blue screen, lasting 1 minute. For the participants' comfort and to reduce the risk of head movement, the scanning sessions were divided into four experimental runs, each containing four 1-minute "on" cycles and four 1-minute "off" cycles. In each run, the first two volumes of collected data were discarded to allow for hemodynamic rise time. To completely differentiate the two stimulations and minimize projection of the erotic emotions onto the neutral paradigm, the two erotic runs were presented first. The two neutral runs were shown after the completion of the erotic films and a 2-minute delay. The length of time in the scanner was approximately 45 minutes for each testing, with none of the scanning sessions lasting longer than 1 hour.

Hormone assays

Blood samples were allowed to clot at room temperature for 10 minutes and sera were separated by centrifugation at 3,000 rpm for 15 minutes. Serum was stored at −20°C until assayed. Individual serum samples were analyzed in the same assay in duplicate. After ethylene glycol column extraction, serum concentrations of E_2, total testosterone, and A4 were measured by radioimmunoassay with intra- and interassay coefficients of variation (CV) less than 9% and 10%, respectively. Serum free testosterone was determined by a radioimmunoassay kit (Diagnostics Systems Laboratories, Inc., Webster, TX) with intra- and interassay CV of 6.2% and 9.7% respectively.

Statistical analysis

Serum hormone values of the premenopausal and postmenopausal women were compared with independent samples *t*-test. Effect of E_2 and E_2 plus testosterone treatment within the postmenopausal volunteers were analyzed using paired samples *t*-test. Results were considered significant if $P < 0.05$.

Functional MRI analyses were conducted using the Analysis of Functional Neuroimage software (AFNI; Version 2.5).[16] Motion correction was performed using AFNI's built-in automated alignment program, *3dvolreg*. The fMRI data from each individual participant were analyzed using a separate multiple regression analysis for each film condition. Nine parameters were entered into the regression analysis. One parameter was the estimated hemodynamic response function to the stimulus presentation. Six parameters were used to orthogonally remove effects of motion for rotation (roll, pitch, and yaw motion measured in degrees) and displacement (mm motion in the x, y, and z planes) and two parameters removed the effects of the global mean and linear trend within each film condition. The linear contrast weights for each film type resulting from the multiple regression analysis, which estimated the BOLD signal change for each film type relative to the null trial conditions, were converted to standardized *Z*-scores. The resulting *Z*-score activation maps were reassembled into Talairach-Tournaux (T-T) space, a brain atlas, using the AFNI hand land-marking procedure. Results were considered significant if $P < 0.05$.

The fMRI group analysis was conducted by submitting the *Z*-score activation maps from each participant in the four film conditions (two neutral and two erotic video segments) to a two-way repeated-measures mixed-model analysis of variance with film condition (fixed effect) and subject (random effect) as factors. The AFNI 3dANOVA2 program was used to conduct the analysis. A voxel-cluster threshold was used to correct for multiple comparisons resulting in an overall corrected level of significance, α of 0.05 (individual voxel $P \leq 0.005$, two-tailed; minimum cluster = 58 contiguous voxels [1,566 μL]; full width-half maximum autocorrelation estimate = 8 mm).[17]

The pre- and postmenopausal groups were analyzed separately. Identified brain regions in each group were compared with each other, but cross-group comparisons were not feasible due to the low number of women. Two independent analysts reviewed the functional image maps and all reported fMRI results had to pass the statistical threshold of pixel activation. The two analysts also independently calculated the volume of activation to help assess the intensity of brain activation.

RESULTS

No power calculation was performed to determine sample size as this research project was designed to be a pilot study.

The mean ages of the five premenopausal and six postmenopausal women were not significantly different. Five of the six postmenopausal women completed the entire study. One woman withdrew after

her E_2-treated scan because she moved out of the area. Another postmenopausal volunteer had only her baseline data used in the final analysis because her serum results at 6 and 12 weeks indicated noncompliance with the hormone regimen. Thus, six women were used in the baseline analysis, five in the E_2 treatment phase, and only four in the E_2 and testosterone treatment phase.

Because only the postmenopausal women were assessed using the BDI and BISF-W questionnaires, a comparison of responses to the premenopausal women was not possible. The average baseline BDI test scores, 3.5 ± 0.9, indicated no depression or dysthymia among the postmenopausal women. BDI values less than 10 are considered normal. The results of the BISF-W before HT were not significantly different from that obtained after the ET and testosterone treatment (40.7 ± 7.8 and 46.1 ± 4.8, respectively). For the BISF-W, a score of 33 is considered to be average for a sexually active woman with no sexual dysfunction, with lower numbers associated with increased sexual problems.[8]

Serum hormone levels

Baseline serum values of E_2, total testosterone, and A4 were significantly lower ($P < 0.05$) in the postmenopausal volunteers compared with the premenopausal women (Table 1). In contrast, free testosterone levels were not different between the pre- and postmenopausal women.

A significant rise in serum E_2 levels was seen in the postmenopausal women after 6 weeks of ET, with serum E_2 levels remaining unchanged from 6 to 12 weeks. There were no significant differences found in serum E_2 levels between the premenopausal women and postmenopausal women while on E_2 therapy, either at 6 or 12 weeks of treatment.

After 6 weeks of ET, serum total testosterone remained unchanged from baseline values. With the addition of 1% testosterone gel to the E_2 regimen, serum total testosterone levels increased significantly ($P < 0.05$). Testosterone gel administration resulted in serum total testosterone concentrations that were similar between the postmenopausal participants at 12 weeks and the premenopausal women.

Serum A4 was also measured to insure that adrenal androgen production in the postmenopausal women was stable and would not contribute to any brain-activation changes seen on fMRI. After 6 weeks of ET there was a significant difference between levels of A4 in the postmenopausal women compared with the premenopausal group ($P < 0.05$), but this significance was lost after addition of testosterone to ET. Although the addition of estrogens to HT in postmenopausal women has been shown to lower adrenal androgen production, the effect in this study was short-lived and the decrease was not enough to cause a statistical variation when serum A4 levels were compared within the postmenopausal group across all treatments.

Serum free testosterone levels in the postmenopausal women decreased significantly after E_2 therapy at 6 weeks ($P < 0.05$), and this decrease was consistent with an increase in sex hormone-binding globulin (SHBG). The use of oral ET is known to increase serum levels of SHBG, and this effect can still be seen even with transdermal ET. After additional treatment with testosterone for 6 weeks, free testosterone concentrations in the postmenopausal women rose; however, these concentrations were not significantly different from those observed at baseline or after 6 weeks of ET. Free testosterone levels were not significantly different between the premenopausal women and postmenopausal women at baseline and again during E_2 and testosterone therapy at 12 weeks.

TABLE 1. *Mean (± SEM) steroid hormone concentrations of premenopausal women and before and during HT in postmenopausal women*

Volunteers	E_2 (pmol/L)	Total T (nmol/L)	Free T (pg/mL)	A4 (nmol/L)
Premenopausal	239.0 ± 89.8[a]	1.01 ± 0.17[a]	1.54 ± 0.21	2.97 ± 0.4[a,e]
Postmenopausal				
Baseline	80.2 ± 11.8[a,b]	0.68 ± 0.09[a,c]	1.84 ± 0.14[d]	1.93 ± 0.20[a]
E_2 therapy (6 weeks)	140.8 ± 28.0[b]	0.64 ± 0.13	1.31 ± 0.19[d]	1.69 ± 0.16[e]
E_2 and T therapy (12 weeks)	153.5 ± 32.7	2.48 ± 0.92[c]	2.63 ± 0.70	2.07 ± 0.52

E_2, estradiol; T, testosterone; A4, androstenedione.
[a]Difference between premenopausal and postmenopausal women at baseline ($P < 0.05$).
[b]Increase in serum E_2 levels after E_2 therapy ($P < 0.05$).
[c]Increase in serum total testosterone levels after T therapy ($P < 0.05$).
[d]Decrease in serum free testosterone levels after E_2 therapy ($P < 0.05$).
[e]Difference between premenopausal and postmenopausal women after E_2 therapy.
($P < 0.05$).

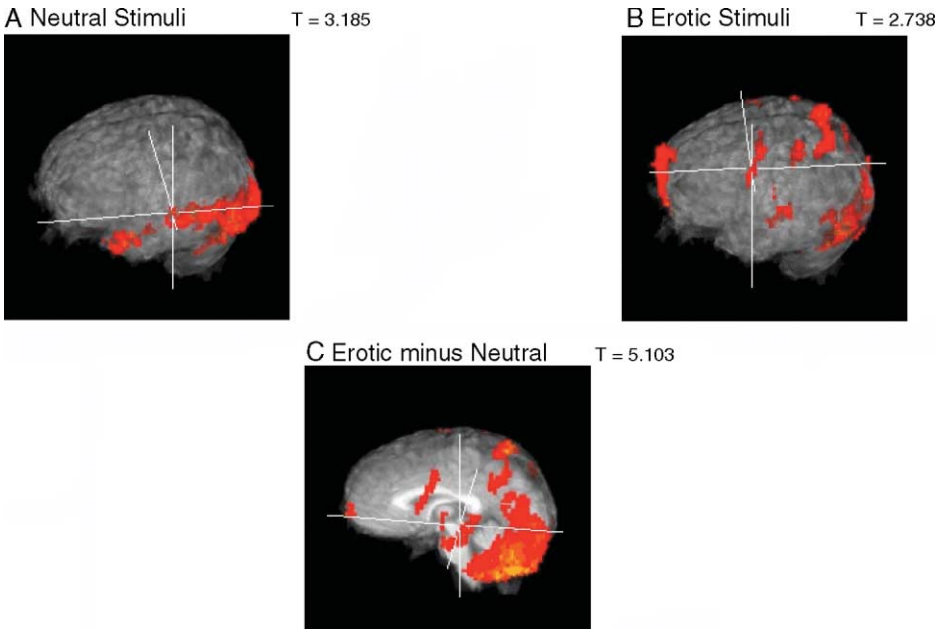

FIG. 1. Areas of brain activation in premenopausal women superimposed on Talairach-Tournaux (T-T) brain atlas. The T-T atlas is AC/PC (anterior commissure/posterior commissure) aligned with the numbers marking the distance in millimeters either right (R) or left (L), anterior (A) or posterior (P), and superior (S) or inferior (I) from the central axis. Activated areas are in red ($P < 0.05$) and intersection of the three lines mark a specific activated brain region. The threshold level for statistically significant brain activation is given by the T-values. A: Whole-brain picture with activation of left superior temporal gyrus [−52 (L), −29 (P), 5 (I)] during viewing of neutral video clip. B: Whole-brain image with left precentral gyrus [−45 (L), −2 (P), 44 (S)] activated during viewing of erotic stimuli. C: Activated areas of the brain that are unique to erotic stimuli after subtraction of the hemodynamic areas noted on neutral film viewing from that seen during erotic viewing seen on midsagittal slice of the brain. Lines intersect at the thalamus [−10 (L), −29 (P), −1 (I)].

In contrast to the other sex steroids, free testosterone was measured by radioimmunoassay directly from serum and not after ethylene glycol column extraction. Although the assay kit reports CVs that are less than 10% at the low free testosterone values seen in women, its precision does not seem to be satisfactory. Studies evaluating commercially available free testosterone kits have confirmed their inaccuracy in measuring this sex steroid at the low concentrations seen in both pre- and postmenopausal women.[18]

Functional MRI

All activated brain areas are significant ($P < 0.05$).

Premenopausal Women

The five premenopausal women had several predictable areas of brain activation that were common during the viewing of neutral and erotic visual stimuli: right inferior frontal gyrus, bilateral middle temporal gyrus, bilateral fusiform gyrus, and primary visual areas (Fig. 1A, B). These regions are activated during the processing of visual and auditory stimulations. When the hemodynamic activity that was measured in viewing the neutral films was subtracted from the activity observed in the erotic films, significant distinct areas were found. Namely, there was enhanced activity in many areas of the limbic system such as the left amygdala, left inferior parietal lobule, and bilateral precentral gyrus and thalamus (Fig. 1C).

Postmenopausal Women

At baseline, the six postmenopausal women demonstrated minimal areas of brain activation with both the neutral and erotic stimulation as compared with women in the premenopausal group. When viewing neutral visual stimuli, activation of the fusiform gyrus and the bilateral superior temporal gyrus was found (Fig. 2). Erotic video stimuli resulted in activation of bilateral anterior cingulate region, the bilateral superior temporal gyrus, and the left superior temporal gyrus (Fig. 2). In contrast to the premenopausal group, the only area of brain activation that was unique to the erotic stimulation was the middle frontal gyrus of the left hemisphere (Fig. 2). Untreated agonadal women had reduced overall signal activations when viewing both erotic and neutral stimuli and limited brain regions that were exclusive to the erotic visual stimulus.

After 6 weeks of ET, the postmenopausal volunteers all had a global increase in brain activation for both the neutral and erotic stimulations. The middle

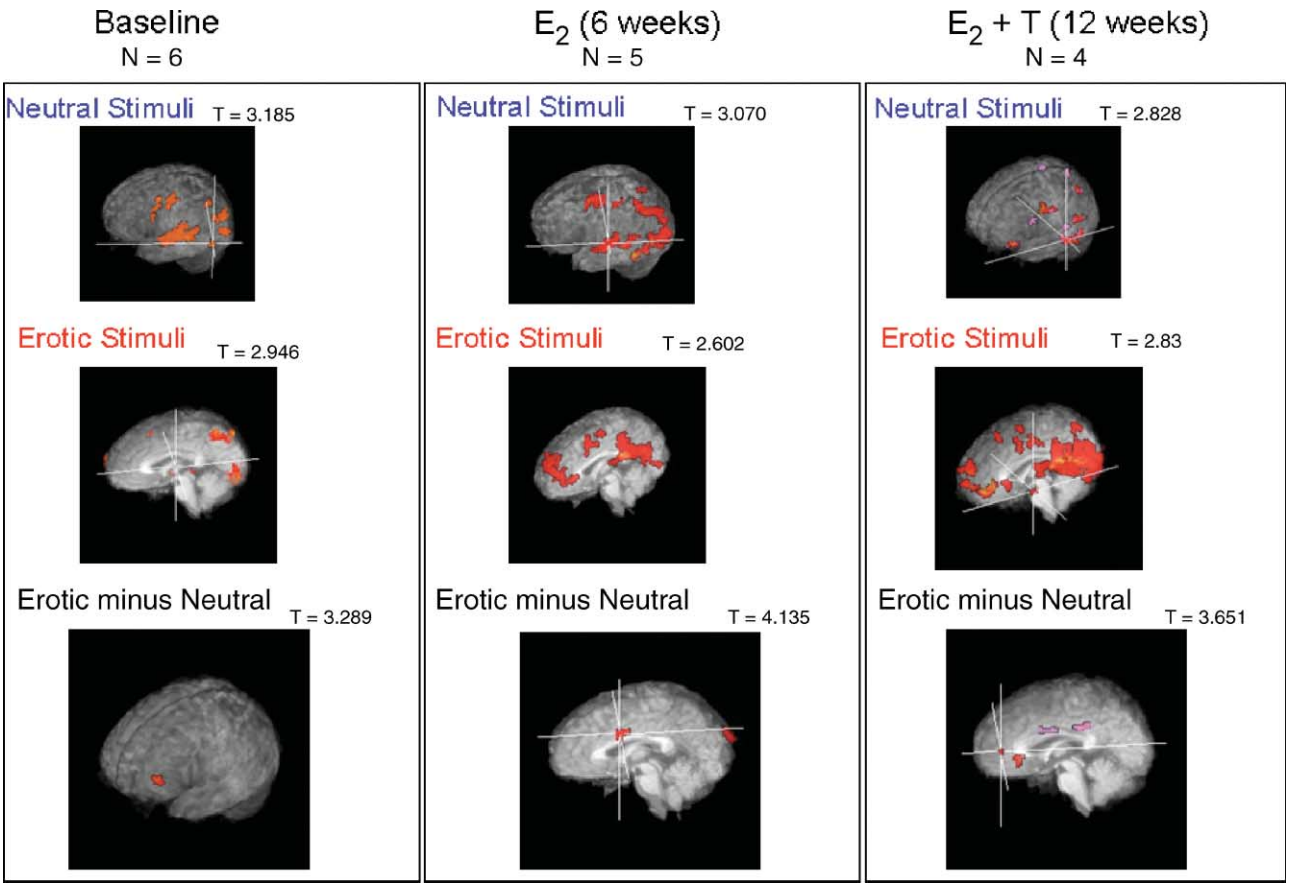

FIG. 2. Areas of brain activation in postmenopausal women superimposed on T-T brain atlas on whole-brain pictures or midsagittal slices of the brain. Activated areas are in red and purple ($P < 0.05$) and intersection of the three lines mark a specific activated brain region. Functional MRIs were done at baseline (six women), after 6 weeks of transdermal E_2 (five women), and after 12 weeks of transdermal E_2 combined with 6 weeks of topical testosterone gel (four women). The threshold level for statistically significant brain activation is given by the testosterone values.

Baseline: **Neutral stimuli; left fusiform gyrus [−41 (L), −73 (P), −14 (I)]. Erotic stimuli; no limbic system activation. Subtraction of neutral stimuli from erotic; left middle frontal gyrus [−38 (L), 43 (A), 12 (S)].** ***E_2 (6 weeks):*** **Neutral stimuli; left superior temporal gyrus [−53 (L), 19 (A), 3 (S)]. Erotic stimuli; activated areas in red include posterior cingulate and parahippocampal gyri. Subtraction of neutral stimuli from erotic; right cingulate gyrus [2 (R), 4 (A), 28 (S)].**

E_2 and testosterone (12 weeks): **Neutral stimuli; left fusiform gyrus [−46 (L), −40 (P), −20 (I)]. Erotic stimuli; parahippocampal gyrus [20 (R), −10 (P), −17 (I)]. Subtraction of neutral stimuli from erotic; bilateral medial frontal gyrus [Left: −9 (L), 44 (A), 14 (S)].**

and superior temporal gyrus and posterior parietal lobule were activated during the neutral films (Fig. 2). For the erotic films there was activation of the left superior parietal and fusiform gyrus as well as structures belonging to the limbic system. The midsagittal slice of the brain in Figure 2 shows the increased limbic system response noted when the activations from the erotic stimuli were subtracted from the neutral stimuli, specifically the left thalamus, medial frontal and right cingulate gyrus. In contrast, untreated

TABLE 2. *Limbic system activation with erotic stimulation in premenopausal women and before and during HT in postmenopausal women*

Volunteers	Thalamus	Amygdala	Medial frontal gyrus	Cingulate gyrus	Parahippocampal gyrus
Premenopausal	++	++	+		++
Postmenopausal					
Baseline					
E_2 therapy (6 weeks)	+		+	+	+
E_2 and T therapy (12 weeks)	++		++	++	++

E_2, estradiol; T, testosterone.

postmenopausal women had no limbic system activation during erotic stimulation.

After a total of 12 weeks of ET (with the last 6 weeks including testosterone treatment), the fMRI scans were repeated. Similar to the changes seen with ET, testosterone supplementation was associated with even more brain-activation areas during the viewing of both the neutral and erotic films. The left fusiform and middle frontal gyri were elicited during the neutral stimulation (Fig. 2). With the erotic paradigm there was not only increased areas of activation of the bilateral medial frontal and superior temporal gyri, but also greater limbic system response, specifically the parahippocampal gyrus and thalamus (Fig. 2). Only after E_2 and testosterone treatment did the postmenopausal women more closely mimic the premenopausal volunteers with regards to areas of the brain exclusively stimulated by the erotic video (Fig. 2 and Table 2).

DISCUSSION

This pilot study demonstrates that in untreated agonadal women, certain brain areas involved with sexual arousal, most noticeably the limbic system, are not activated while these women are viewing erotic films.[13] Though ET does increase areas of activation in agonadal postmenopausal women while viewing erotic stimuli, the addition of testosterone results in even greater CNS response. Specifically, testosterone therapy increases limbic system activation over that seen with ET alone. The limbic system, one of the more primitive areas of the cerebral cortex, is activated during fMRI studies investigating sexual arousal in men, and in studies exploring emotional processing.[11-13]

Prior research with men viewing films that depict heterosexual activity found several areas of the limbic system that activated exclusively with the erotic stimulus: the thalamus, hypothalamus, amygdala, cingulate gyrus, medial frontal gyrus, and occipitotemporal cortex.[11-13] Of the limited number of studies evaluating sexual arousal in premenopausal women, one found no gender differences and two showed decreased limbic system activation in women when compared with men.[13,14,19] In our study, similar areas of CNS activation with erotic stimulation were found (cingulate gyrus, medial frontal cortex, amygdala, inferior parietal lobule and occipitotemporal gyri) in premenopausal women and also in postmenopausal volunteers only when they were treated with E_2 and testosterone.[13,14,19]

Earlier research in sexual functioning has revealed a discrepancy between genital and subjective arousal, and neuroimaging studies have also shown a possible disconnect between activated CNS areas and participants' reports of sexual arousal.[10] An autonomic and thus unconscious response pathway to sexual stimulation has been noted in fMRI studies of sexual arousal.[10] This autonomic response has caused some consternation in evaluation of neuroimaging studies because researchers' interest has been more focused towards elucidating the conscious subjective sexual response. Both pathways employ the limbic system, but the subjective sexual response should be linked to higher cortical areas.[10] The medial frontal cortex is a pathway from the thalamus to higher brain areas and was activated during the erotic stimulation in the premenopausal women and in postmenopausal women treated with E_2 and testosterone, whereas the hypothalamus is more associated with autonomic control and was not activated. Also it seems unlikely that the areas activated by the erotic stimuli comprise an automated neurological pathway because they were not activated in the nontreated postmenopausal women.

The erotic stimuli activated the limbic system, which is rich in AR and ER. The increased activation seems to be a direct testosterone effect and not due to testosterone aromatization to E_2, because serum levels of E_2 remained stable between the 6 and 12 weeks of ET. The limbic area also expresses significant amounts of aromatase.[15,20] Aromatization of testosterone to E_2 does occur in the brain, and increased CNS levels of E_2 cannot be excluded during the ET and testosterone treatment compared with the ET-only phase.

Although the design of the study was to evaluate sexual arousal, it is possible that the increased limbic system activity generated by the erotic video might be more of an emotional response than a sexual one, because the limbic system is also involved in emotional processing. The erotic stimuli were selected to result in female sexual arousal; however, they might instead have caused a positive, or even a negative, emotional experience. In addition, ET and testosterone therapy in postmenopausal women have resulted in increased quality-of-life scores and sense of general well-being.[21] The increased limbic system activation might be a reflection of the volunteers' improved emotional state. This possibility is less likely because no limbic system activation was noted for the neutral film condition.

The neutral-film condition resulted in a very low level of activation in the postmenopausal group on no HT, which was not anticipated. The postmenopausal women viewed videos that would commonly be seen on television and had limited brain activity during this viewing exercise. Though cognitive function was

not addressed in this study, all six women were actively employed and volunteered no concerns about decreased cognitive abilities. The premenopausal women had a more robust activation with the neutral stimulation and because the ages of the women in these two groups were not significantly different, age-related changes are not likely to be the cause of the decreased activation. Treatment with E_2 and then testosterone in agonadal women resulted in significantly increased brain activation for the neutral stimuli and approached the results found in the similarly aged premenopausal women. Thus HT, specifically ET and testosterone treatment, could improve cognitive ability by increasing global brain activation.

Studies looking at E_2's effects on cognitive ability have shown conflicting results, with some indicating that ET improves memory and cognition and others reporting no change.[22,23] In 1999, Shaywitz et al[24] used fMRI to evaluate cognitive function by direct measurement of brain-activation patterns, and found both the inferior parietal lobule and superior frontal gyrus activated in postmenopausal women after ET. These CNS activation areas were also found in this study with E_2 treatment, and with even greater inferior parietal and superior frontal gyrus activation after testosterone was added to the ET. These results coincide with a possible role of testosterone in improving cognitive function, and several studies have shown a link between cognitive ability and testosterone supplementation in postmenopausal women.[20]

This study also points out the limitations of sexual questionnaires. The postmenopausal participants had no sexual difficulties as determined by the BISF-W and no significant scoring change after combined E_2 and testosterone therapy, though they all reported increased sexual thoughts and physical interactions with their partners. In contrast, the fMRI scans showed a significant increase in brain activation with erotic stimulation after E_2 and testosterone treatment. The accuracy of sexual questionnaires is often limited by recall bias, partner availability, partner's sexual functioning, and the participant's satisfaction with the relationship. Using fMRI to assess sexual response can reduce these confounding issues and focus more directly on an individual's sexual function and less on her partner's.

Unfortunately, a severe limitation of this study was the small sample size. Six postmenopausal women were recruited and only four women were analyzed at the 12-week phase. The statistically significant brain-activation patterns reported here have to be interpreted with a degree of caution. In addition, because of the low number of participants, a subset analysis of the postmenopausal women could only suggest differing results between the women more than 15 years from their surgical menopause and those who were fewer than 15 years from surgery. Those women closer to the menopause seemed to have increased brain activation with administration of both E_2 and testosterone when compared with women more removed from their oophorectomy. Histopathologic studies of postmortem brains have shown decreasing amounts of AR and ER with both increasing age and transitioning through the menopause.[25-27] If concentrations of functional AR and ER decrease with age and years from the menopause, then different results involving the effects of sex steroids on the CNS would be found in women of variable ages and years postmenopause. Additional fMRI research can help determine whether sex steroids have a CNS benefit in elderly women and whether the administration of E_2 to recently postmenopausal women lowers the risk of ER loss with age.

CONCLUSIONS

This pilot study found significantly increased areas of brain activation with ET and with ET and testosterone supplementation in postmenopausal women while viewing both neutral and erotic videos. Erotic stimuli resulted in increased limbic system activation, which is consistent with results reported in men viewing erotic films and is appropriate given the role of the limbic system in sexual functioning. Further research should help clarify the role of ET and testosterone in sexual arousal and cognitive function in postmenopausal women.

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REFERENCES

1. Graziotin A. The biological basis of female sexuality. *Int Clin Psychopharmacol* 1998;13:S15-S22.
2. Boothby LA, Doering PL, Kipersztok S. Bioidentical hormone therapy: a review. *Menopause* 2004;11:356-357.
3. Braunstein GD. Androgen insufficiency in women: summary of critical issues. *Fertil Steril* 2002;77:S94-S99.
4. Bachmann GA, Leiblum SR. The impact of hormones on menopausal sexuality: a literature review. *Menopause* 2004;11: 120-130.

5. Traish AM, Kim N, Min K, Munarriz R, Goldstein I. Role of androgens in female genital sexual arousal: receptor expression, structure, and function. *Fertil Steril* 2002;77:S11-S18.
6. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. *Arch Gen Psychiatry* 2000;57:149-153.
7. Rosen RC. Assessment of female sexual dysfunction: review of validated methods. *Fertil Steril* 2002;77:S89-S93.
8. Mazer NA, Leiblum SR, Rosen RC. The Brief Index of Sexual Functioning for Women (BISF-W): a new scoring algorithm and comparison of normative and surgically menopausal populations. *Menopause* 2000;7:350-363.
9. Deliganis AV, Maravilla KR, Heiman JR, et al. Dynamic MR imaging of the female genitalia using AngioMARK: initial experience evaluating the female sexual response. *Int J Impot Res* 2000;12:S45.
10. van Lusen RHW, Laan E. Genital vascular responsiveness and sexual feelings in middle women: psychophysiologic, brain, and genital imaging studies. *Menopause* 2004;11:741-748.
11. Mouras H, Stoléru S, Bittoun J, et al. Brain processing of visual sexual stimuli in healthy men: a functional magnetic resonance imaging study. *Neuroimage* 2003;20:855-869.
12. Stoléru S, Grégoire M-C, GérardD, et al. Neuroanatomical correlates of visually evoked sexual arousal in human males. *Arch Sex Behav* 1999;28:1-19.
13. Karama S, Lecours AR, Leroux J-M, et al. Areas of brain activation in males and females during viewing of erotic film excerpts. *Hum Brain Mapping* 2002;16:1-13.
14. Seo JJ, Kang HK, Ryu SB, et al. Functional MRI for evaluating cerebral centers of sexual arousal in men and women. *J Urol* 2001;165:222.
15. Sarrel PM. Psychosexual effects of menopause: role of androgens. *Am J Obstet Gynecol* 1999;180:S319-S324.
16. Cox RW, Hyde JS. Software tools for analysis and visualization of fMRI data. *Nmr in Biomedicine* 1997;10:171-178.
17. Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. *Magn Reson Med* 1995;33:636-647.
18. Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, Paulson RJ. Limitations of direct estradiol and testosterone immunoassay kits. *Steroids* 2003;68:1173-1178.
19. Hamann S, Herman RA, Nolan CL, Wallen K. Men and women differ in amygdala response to visual sexual stimuli. *Nat Neurosci* 2004;7:411-416.
20. Plouffe L, Simon JA. Androgen effects on the central nervous system in the postmenopausal woman. *Semin Reprod Endocrinol* 1998;16:135-143.
21. Bachman GA. Androgen cotherapy in menopause: evolving benefits and challenges. *Am J Obstet Gynecol* 1999;180:S308-S311.
22. Yaffe K, Grady D, Pressman A, Cummings S. Serum estrogen levels, cognitive performance, and risk of cognitive decline in older community women. *J Am Geriatr Soc* 1998;46:816-821.
23. Haskell SG, Richardson ED, Horwitz RJ. The effect of estrogen replacement therapy on cognitive function in women: a critical review of the literature. *J Clin Epidemiol* 1997;50:1249-1264.
24. Shaywitz SE, Shaywitz BA, Pugh KR, et al. Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA* 1999;281:1197-1202.
25. Tohgi H, Utsugisawa K, Yamagata M, Yoshimura M. Effects of age on messenger RNA expression of glucocorticoid, thyroid hormone, androgen, and estrogen receptors in postmortem human hippocampus. *Brain Res* 1995;700:245-253.
26. Ishunina TA, Kruijver FPM, Balesar R, Swaab DF. Differential expression of estrogen receptor α and ß immunoreactivity in the human supraoptic nucleus in relation to sex and aging. *J Clin Endocrinol Metab* 2000;85:3283-3291.
27. Ishunina TA, Fisser B, Swaab DF. Sex differences in androgen receptor immunoreactivity in basal forebrain nuclei of elderly and Alzheimer patients. *Exp Neurol* 2002;176:122-132.