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Testosterone Increases Amygdala Reactivity in Middle-Aged Women to a Young Adulthood Level

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Testosterone modulates mood and sexual function in women. However, androgen levels decline with age, which may relate to the age-associated change in sexual functioning and the prevalence of mood and anxiety disorders. These effects of testosterone are potentially mediated by the amygdala. In the present study, we investigated whether the age-related decline in androgen levels is associated with reduced amygdala activity, and whether exogenous testosterone can restore amygdala activity. Healthy young and middle-aged women participated during the early follicular phase of the menstrual cycle, and amygdala responses to biologically salient stimuli were measured with functional magnetic resonance imaging (fMRI). Androgen levels were lower in middle-aged than young women, which was associated with decreased amygdala reactivity. Endogenous testosterone levels correlated positively with amygdala reactivity across the young and middle-aged women. The middle-aged women received a single nasal dose of testosterone in a double-blind, placebo-controlled, crossover manner, which rapidly increased amygdala reactivity to a level comparable to the young women. The enhanced testosterone levels correlated positively with superior frontal cortex responses and negatively with orbitofrontal cortex responses across individuals, which may reflect testosterone-induced changes in amygdala regulation. These results show that testosterone modulates amygdala reactivity in women, and suggest that the age-related decline in androgen levels contribute to the decrease in amygdala reactivity.

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INTRODUCTION

Androgen levels in women decline with age, such that a woman has about half her early adulthood levels at age forty (Zumoff *et al*, 1995; Davison *et al*, 2005). Synchronously with the age-related decline in androgen levels, the prevalence of affective and anxiety disorders decreases (Kessler *et al*, 1994, 2003; Henderson *et al*, 1998). However, sexual functioning also declines in women (Hayes and Dennerstein, 2005; Hayes *et al*, 2007). Thus, the change in androgen levels might contribute to both the susceptibility for mood disorders and for sexual dysfunction. For example, the decrease in gonadal steroid hormone concentrations after oophorectomy is associated with a worsening of mood and sexual motivation, which improves with subsequent testosterone treatment (Sherwin and Gelfand, 1985; Sherwin *et al*, 1985; Shifren *et al*, 2000). In addition, testosterone treatment also improves well-being, mood, and sexual function in healthy premenopausal women with low libido (Goldstat *et al*, 2003). However, it remains unknown by which neural mechanism testosterone influences mood and sexual function, and whether this is related to the age-related decline in androgen levels.

Animal studies suggest that testosterone influences mood and sexual function by modulating amygdala activity, a brain structure implicated in mood and anxiety regulation (Drevets, 2003; Rauch *et al*, 2003) and sexual arousal (Karama *et al*, 2002; Hamann *et al*, 2004; Gizewski *et al*, 2006). Testosterone modulates functioning of amygdala neurons in male and female rodents (Kendrick and Drewett, 1979; Kendrick, 1981), and testosterone infusions into the amygdala increase sexual behavior in male rodents (Wood and Newman, 1995; Bialy and Sachs, 2002). Whereas these studies investigated the effects of prolonged testosterone exposure recent studies suggest, that testosterone influences anxiety and sexual behavior also rapidly (Aikey *et al*, 2002; James and Nyby, 2002). Moreover, a single testosterone administration already increases physiological arousal in response to affective and erotic stimuli in women (Tuiten *et al*, 2000; van Honk *et al*, 2001).

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This study investigated whether nasally applied testosterone rapidly increases amygdala reactivity in healthy, naturally cycling, middle-aged women, using a double-blind, placebo-controlled, crossover design. Amygdala reactivity to biologically salient stimuli was measured with functional magnetic resonance imaging. To investigate whether the diminished endogenous levels of androgens during middle adulthood influence amygdala activity, the placebo session was compared with the placebo session of young, healthy, naturally cycling women, who participated in an identical experiment with a different drug manipulation (van Wingen *et al*, 2007) and who were expected to have higher androgen levels.

MATERIALS AND METHODS

Participants

This study included two groups of participants. The experimental group consisted of 27 middle-aged, naturally cycling, premenopausal women. One participant was not available for the second study occasion, and data of one session of another subject were lost due to a technical failure. Therefore, the results are based on the data of the remaining twenty-five participants (mean age 42 years; range: 37–50 years). The reference group consisted of 17 young, naturally cycling women (mean age 23 years; range: 19–30 years) who participated previously in another study with the same experimental procedure but a different drug manipulation (van Wingen *et al*, 2007). All 44 participants were physically and mentally healthy as determined by a structured interview, were free of medication and hormonal contraceptives, and right handed. The study was approved by the local ethics committee (CMO Regio Arnhem-Nijmegen, The Netherlands). All participants gave written informed consent after the study had been explained to them.

Design and Procedure

All women participated two times during the early follicular phase (days 1–7) of different menstrual cycles, to ensure low endogenous hormone levels. The middle-aged women first completed a sexual arousal questionnaire (SAQ) (Tuiten *et al*, 2000), before the first venous blood sample was collected. Thereafter, a nasal dose of testosterone (0.9 mg; Noseafix®) or placebo was administered in a double-blind, crossover manner. Prior to scanning (30 min after drug intake), they completed the SAQ again and a second blood sample was drawn. About 140 min after drug intake, the final SAQ was administered, after which the participants completed mood (Mood Rating Scale; MRS) (Bond and Lader, 1974) and state anxiety (State Trait Anxiety Inventory; STAI) (Spielberger *et al*, 1970) questionnaires. The young women of the reference group completed a similar procedure, described in van Wingen *et al* (2007).

Behavioral Task

The experimental paradigm consisted of a blocked design, including an emotion condition, and a visuo-motor control condition. This paradigm has been used previously to investigate drug effects on amygdala reactivity (Hariri *et al*, 2002; Paulus *et al*, 2005; van Wingen *et al*, 2007). It robustly engages the amygdala, by contrasting the response to simultaneously present angry and fearful face stimuli (http://www.macbrain.org) with the response to ellipses (that consisted of scrambles of the same face stimuli). The results therefore do not show emotion-specific effects, but rather a general response to salient, biologically relevant stimuli.

Two emotion blocks were interleaved with three control blocks, and each 30 s block consisted of six 5 s trials. Each trial consisted of three simultaneously presented stimuli, with the cue stimulus presented above the target and distractor. In the emotion condition, an angry or fearful face was presented on top as cue, and subjects had to indicate by an appropriate button press, which of the bottom two faces (one angry and one fearful) matched the cue in emotional expression. The three simultaneously presented faces per trial were from different persons from the same sex. Half the trials presented faces of men and half of women, half of each target emotion (angry or fearful). In the control condition, a horizontally- or vertically-oriented ellipse was presented as cue above two ellipses (one vertical and one horizontal), and subjects had to select the identically oriented ellipse.

Magnetic Resonance Imaging Data Acquisition

MR data was acquired with a 1.5 T Siemens Sonata MR scanner (Siemens, Erlangen, Germany), equipped with a standard head coil. Seventy-six T2*-weighted blood oxygenation level-dependent (Bold) images were acquired using echo-planar imaging (EPI) with an echo time of 30 ms to reduce signal dropout, with each image volume consisting of 33 axial slices (3 mm, 0.5 mm slice-gap, TR = 2.290 s, 64×64 matrix, FOV = 224 mm, FA = 90°). In addition, a high resolution T1-weighted structural MR image was acquired for spatial normalization procedures (3D MP-RAGE, TR = 2250 ms, TE = 3.93 ms, TI = 850 ms, 176 contiguous 1 mm slices, 256×256 matrix, FOV = 256 mm).

Functional Magnetic Resonance Imaging Data Analysis

Image analysis was performed with SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). The first five EPI-volumes were discarded to allow for T1 equilibration, and the remaining images were realigned to the first volume. Images were then corrected for differences in slice acquisition time, spatially normalized to the Montreal Neurological Institute T1 template, super-sampled into $2 \times 2 \times 2\,mm^3$ voxels, and spatially smoothed with a Gaussian kernel of 10 mm FWHM.

Statistical analysis was performed within the framework of the general linear model (Friston *et al*, 1995). For each drug condition, the two experimental conditions were modeled as box-car regressors convolved with the canonical hemodynamic response function of SPM2. In addition, the realignment parameters were included to model potential movement artifacts and a high-pass filter (cutoff at 1/128 Hz). To account for various global effects, the EPI-data was proportionally scaled. Temporal autocorrelation was modeled with an AR(1) process and the parameter estimates were obtained by maximum likelihood estimation

(Friston *et al*, 2002) to allow departures from sphericity. Parameter images contrasting the emotion and visuo-motor control condition were obtained, and analyzed in subsequent random-effects analyses. Drug effects were assessed with paired *t*-tests, and independent samples *t*-tests were used to identify age effects. Statistical tests were family-wise error rate corrected for multiple comparisons across the whole brain. A small volume correction (Worsley *et al*, 1996) was used to correct for multiple comparisons across the search volume for regions of interest (ROI). As the basolateral amygdala is critically involved in sexual motivation (Everitt, 1990), the search volume for the amygdala was defined as a sphere with 7 mm radius around the probabilistic cytoarchitectonic center of the basolateral amygdala ([-26 -8 -18] and [28 -8 -18]), which approximates total amygdala volume (Amunts *et al*, 2005; Eickhoff *et al*, 2005). In addition, the face responsive region in the fusiform gyrus was defined as a sphere with 10 mm radius around previously reported Talairach coordinates (Kanwisher *et al*, 1997), that were transformed into MNI-space (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach; [-36 -64 -16] and [40 -56 -16]). The inverse transformation was used to convert the results from MNI to Talairach space, and all local activation maxima are reported in Talairach coordinates. In addition, ROI analyses were conducted to compare amygdala reactivity across the different conditions, for which the mean activity within amygdala search volumes was extracted (Brett *et al*, 2002). Effect sizes for these ROI analyses were calculated, and $r = 0.10$ indicates a small effect, $r = 0.30$ a medium effect, and $r = 0.50$ a large effect (Cohen, 1992).

Serum Analysis

The serum concentrations of total testosterone (T) were measured by liquid chromatography with tandem mass spectrometry detection after non-polar solvent extraction. Serum concentrations of dehydroepiandrosterone sulfate (DHEA-S), dihydrotestosterone (DHT), and 3α-androstanediol glucuronide (3α-diol) were measured by radioimmunoassay. The coefficients of variation for T were 4.3–14.8% for the inter-assay and 0.7–17.3% for the intra-assay precision, for DHEA-S 6.5–8.4% for the inter-assay and 3.6–6.9% for the intra-assay precision, for DHT 6.2–11.9% for the inter-assay and 2.2–18.1% for the intra-assay precision, and for 3α-diol 11–24% for the inter-assay and 8.0–23% for the intra-assay precision.

RESULTS

Age Effects on Androgen Levels and Amygdala Reactivity

Serum concentrations, questionnaire, and behavioral performance. Baseline serum concentrations of dehydroepiandrosterone sulfate (DHEA-S; $t(40) = 4.6$, $p < 0.001$), testosterone (T; $t(40) = 5.0$, $p < 0.001$), dihydrotestosterone (DHT; $t(19.7) = 7.3$, $p < 0.001$), and 3α-androstanediol glucuronide (3α-diol; $t(26.1) = 4.1$, $p < 0.001$) were significantly lower in middle-aged than young women, but sex hormone-binding globulin, and albumin (both $p > 0.15$) levels were not significantly different between the age groups (see Table 1). The age groups did not significantly differ in state anxiety as measured with the STAI after scanning (mean ± SEM; young: 35.5 ± 2.0; middle-aged: 33.1 ± 1.6; $p > 0.3$), but the younger women were more accurate in matching the stimuli in both experimental conditions (emotion and control condition) than the middle-aged women (young: 98.2 ± 0.9% correct, middle-aged: 91.5 ± 1.8% correct; $F(1, 35) = 8.7$, $p = 0.006$). In addition, the young women were faster than the middle-aged women in all responses (young: 1460 ± 59 ms, middle-aged: 1748 ± 92 ms; $F(1, 35) = 5.6$, $p = 0.024$), but no significant group × task condition interactions were observed.

Table 1 Baseline Serum Concentrations in Young ($N = 17$) and Middle-Aged ($N = 25$) Women in the Placebo Conditions, and Serum Concentrations in the Testosterone Condition Before and 30 min after a Nasal Testosterone Dose

	Young women		Middle-aged women					
	Placebo		Placebo		Testosterone			
	Baseline		Baseline		Baseline		30 min	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
3α-diol (pmol/l)	6183.7	727.8	2774.8	412.0	2732.1	354.0	2736.4	352.3
Albumin (g/l)	43.1	0.9	42.4	0.6	42.3	0.5	—	—
DHEA-S (nmol/l)	5847.1	536.9	3169.5	307.3	3173.9	311.9	—	—
DHT (pmol/l)	605.1	41.7	265.2	20.9	279.8	36.9	367.9	40.1
Estradiol (pmol/l)	—	—	186.5	33.2	144.8	18.3	146.4	18.9
SHBG (nmol/l)	79.1	6.4	100.0	11.9	95.8	8.3	—	—
T (pmol/l)	964.8	58.3	637.2	37.5	672.7	52.7	3398.1	404.5

Abbreviations: 3α-diol, 3α-androstanediol glucuronide; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; SHBG, sex hormone-binding globulin; T, testosterone.

Imaging results. In the young women, the emotion condition yielded larger responses than the visuo-motor control condition in the amygdala, ventral visual stream (ranging from the primary visual cortex to the fusiform gyrus), middle temporal gyrus, inferior prefrontal gyrus, midbrain, supplementary motor area, premotor cortex, and occipital gyrus (see Figure 1a and Table 2). In middle-aged women, the ventral visual stream, inferior prefrontal gyrus, and supplementary motor area were also significantly activated. In addition, the neural response in the temporal pole was larger during the emotion than control condition, but no significant activation was observed in the amygdala (see Figure 1b and Table 2). After lowering the statistical threshold, a non-significant activation within the amygdala was observed (peak voxel: [-20 -6 -13], $t(24) = 2.6$, $p = 0.008$, uncorrected), potentially indicating a weak or variable amygdala response.

Comparing neural responses between the young and middle-aged women revealed that amygdala reactivity was larger in the young than middle-aged women. In addition,

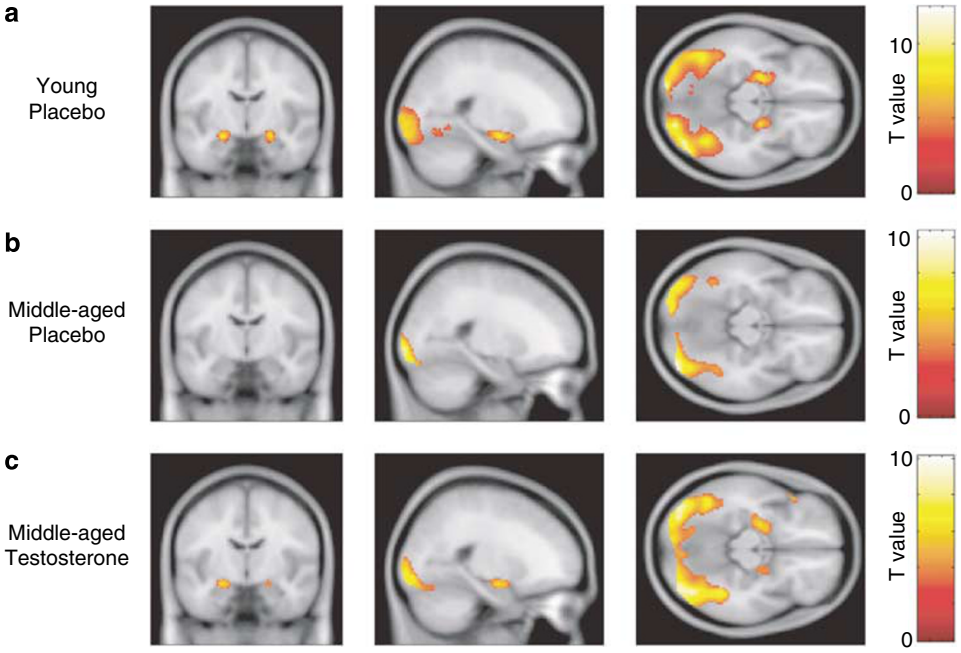

Figure 1 Amygdala reactivity to biologically salient stimuli in young and middle-aged women. (a) Significant amygdala activity in the placebo condition of young women ($N = 17$). (b) No significant amygdala activity in the placebo condition of middle-aged women ($N = 25$). (c) Significant amygdala activity in middle-aged women after a single nasal administration of testosterone ($N = 25$). All figures show coronal, sagittal, and axial planes at the amygdala peak ([-22 -8 -11]) at a statistical threshold of $p < 0.001$, uncorrected, cluster $\geqslant 20$ voxels ($T(16) \geqslant 3.69$ in a; $T(24) \geqslant 3.47$ in b and c).

the neural response to emotional faces was larger in the young than middle-aged women in the ventral visual stream ranging from the extra-striate visual cortex to the fusiform gyrus. Conversely, the neural response to emotional faces was larger in the middle-aged than young women in the superior temporal gyrus, superior frontal gyrus, anterior cingulate cortex, superior frontal gyrus, middle frontal gyrus, and operculum (see Table 3). After correction for task performance by including reaction times as covariate, the bilateral superior frontal gyri and right superior temporal gyrus responses remained significantly larger in the middle-aged than young women. Importantly, the pattern of results with significantly larger amygdala and ventral visual stream responses in the young than middle-aged women remained virtually identical. The differences in amygdala and superior frontal activity between the groups are therefore not related to differences in task performance.

To investigate whether these age differences in neural responses could be explained by differences in endogenous testosterone levels, a correlation analysis was performed. The testosterone levels of the young and middle-aged women during the placebo sessions were positively correlated with neural responses in the amygdala, fusiform gyrus, and insula across individuals (see Figure 2 and Table 4). Neural responses in the posterior cingulate cortex and superior frontal gyrus correlated negatively with testosterone levels (see Table 4). Although these correlations indicate that age differences in amygdala reactivity could be mediated by differences in testosterone levels, these associations are inherently confounded by age effects as testosterone levels were highly correlated with age ($r(42) = -0.53$, $p < 0.001$). Inclusion of age as covariate did not alter the pattern of results in the amygdala and fusiform gyrus, but these positive correlations did not remain significant after correction for multiple comparisons. To investigate whether testosterone indeed mediates amygdala reactivity, a crossover placebo-controlled design was used.

Rapid Effects of Testosterone on Androgen Levels and Amygdala Reactivity in Middle-Aged Women

Serum concentrations, questionnaires, and behavioral performance. No significant differences were observed in all serum measures of the baseline samples of the two study days (ie T, DHT, 3α-diol, DHEA-S, estradiol, progesterone, luteinizing hormone, sex hormone-binding globulin, and albumin; all paired *t*-tests $p > 0.2$). The nasal testosterone dose increased the T (drug $\times$ time interactions: $F(1, 24) = 48.1$, $p < 0.001$) and DHT ($F(1, 23) = 10.8$, $p = 0.003$) concentrations at 30 min after administration, but the 3α-diol ($p > 0.15$) and estradiol ($p > 0.3$) levels were not significantly increased (see Table 1). The testosterone level that was reached is within the upper range for healthy women (Zumoff *et al*, 1995; Davison *et al*, 2005). Testosterone did not influence sexual lust, bodily arousal, and genital arousal as measured with the SAQ (drug $\times$ time interactions; $p > 0.2$), state anxiety as measured with the STAI (mean $\pm$ SEM; placebo: 33.1 ± 1.6; testosterone: 33.1 ± 1.9; $p > 0.9$), and alertness (placebo: 322.4 ± 22.6; testosterone: 315.8 ± 25.6; $p > 0.8$), contentedness (placebo: 240.3 ± 9.9; testosterone: 247.0 ± 10.4; $p > 0.6$), and calmness (placebo: 110.7 ± 5.0; testosterone: 111.5 ± 6.9; $p > 0.9$) as measured with the mood rating scale. In addition, testosterone did not significantly modulate response accuracy (percentage correct; placebo: emotion condition $89.8 \pm 2.4\%$, control condition $93.2 \pm 1.8\%$; testosterone: emotion condition $91.0 \pm 2.8\%$, control condition

Table 2 Main Effects of Task for the Young ($N = 17$) and Middle-Aged ($N = 25$) Women in the Placebo Condition, and for Middle-Aged Women after a Single Nasal Testosterone Dose (ie Matching Angry and Fearful Faces > Matching Scrambled Face Stimuli)

	x	y	z	Cluster size	Z
Young women: placebo condition					
R inferior occipitotemporal cortex	28	−88	−2	4096	6.1
L inferior occipitotemporal cortex	−12	−95	−5	4186	6.0
L amygdala	−22	−8	−11	281	5.1
R amygdala	22	−8	−11	150	4.8
R middle temporal gyrus	46	−56	16	78	4.6
L inferior frontal gyrus	−42	9	27	532	4.5
R inferior frontal gyrus	44	11	27	721	4.3
Midbrain	4	−35	2	157	4.3
Supplementary motor area	−2	11	35	208	4.2
R premotor cortex	42	2	44	84	4.1
R occipital gyrus	38	−69	24	31	3.6
Middle-aged women: placebo condition					
L inferior occipitotemporal cortex	−26	−93	1	2262	6.3
R inferior occipitotemporal cortex	22	−95	−2	2638	6.3
R inferior frontal gyrus	48	22	21	501	5.1
L inferior frontal gyrus	−53	17	27	263	4.7
Supplementary motor area	2	12	47	368	4.6
L temporal pole	−30	5	−19	49	3.9
Middle-aged women: testosterone condition					
L/R inferior occipitotemporal cortex	18	−89	1	7642	6.3
R inferior frontal gyrus	48	9	33	1543	5.5
L inferior frontal gyrus	−38	11	27	851	4.7
L amygdala	−22	−8	−11	224	4.6
L orbitofrontal cortex	−48	23	−13	40	4.3
Medial superior frontal gyrus	4	27	39	124	4.1
Precuneus	0	−58	49	50	3.9
R hippocampus	22	−22	−6	49	3.9
R amygdala	20	−6	−11	56	3.5

Data are Talairach coordinates for cluster maxima at $p < 0.001$ with a cluster size $\geqslant 20$ voxels; Cluster size in number of significant voxels.

96.0 ± 1.6%; $p > 0.4$) or reaction times (placebo: emotion condition 2237 ± 102 ms, control condition 1260 ± 103 ms; testosterone: emotion condition 2205 ± 102 ms, control condition 1245 ± 113 ms; $p > 0.7$) during the behavioral task. These results indicate that the testosterone-induced differences in neural responses are unlikely explained by changes in mood states or behavioral performance.

Imaging results. In the testosterone session of the middle-aged women, the emotion condition yielded larger responses than the visuo-motor control condition in the ventral visual stream (ranging from the primary visual cortex to the fusiform gyrus), inferior frontal gyrus, orbitofrontal cortex, medial superior frontal gyrus, precuneus, and hippocampus. Importantly, significant bilateral amygdala activity was observed (see Figure 1c and Table 2). Direct comparisons showed that testosterone increased left amygdala reactivity in the middle-aged women (see Table 5). A similar effect was also observed in the right

Table 3 Effects of Age on Emotion Processing During the Placebo Conditions (ie Age × Task Interaction)

	x	y	z	Cluster size	Z	SVC *p*
Young > middle-aged women						
R inferior occipital cortex	32	−81	6	568	4.6	
L inferior occipitotemporal cortex	−22	−83	4	753	4.5	
L fusiform gyrus[a]	−42	−57	−7	61	4.3	0.001
R fusiform gyrus[a]	36	−57	−6	73	3.8	0.006
L amygdala[a]	−24	−6	−13	46	3.7	0.004
L lingual gyrus	−22	−58	0	47	3.5	
R amygdala[a]	28	−12	−11	1	3.2	0.017
Middle-aged > young women						
R superior temporal gyrus	67	−19	12	82	4.4	
L superior frontal gyrus	−24	41	37	63	4.0	
Anterior cingulate cortex	−6	31	0	73	3.9	
R superior frontal gyrus	16	20	48	158	3.9	
R middle frontal gyrus	28	56	−1	20	3.7	
L operculum	−65	−26	25	25	3.5	

Data are Talairach coordinates for cluster maxima at $p < 0.001$ with a cluster size $\geqslant 20$ voxels; Cluster size in number of voxels ($p < 0.001$, uncorrected); SVC *p* is small volume corrected *p*-value.
[a]Data from the search volume of the region of interest.

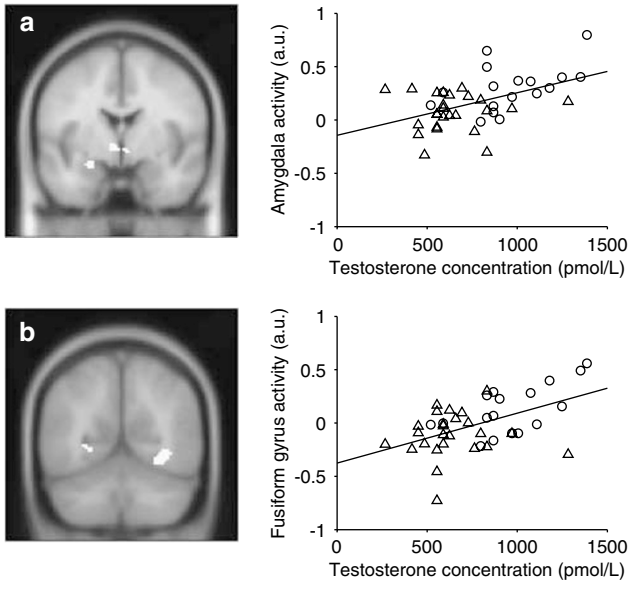

Figure 2 Baseline serum testosterone level correlates positively with amygdala (a; $y = -3$) and fusiform gyrus (b; $y = -58$) activity across young (circles) and middle-aged (triangles) women in the placebo sessions. Left figures show coronal planes (left = left) at the cluster maxima at a statistical threshold of $p < 0.005$, uncorrected, for illustration.

Table 4 Correlation Analysis Between Testosterone Levels and Neural Responses to Emotional Faces (ie Matching Angry and Fearful Faces > Matching Scrambled Face Stimuli) in the Placebo Session of the Young and Middle-Aged Women

	x	y	z	Cluster size	Z	SVC *p*
Positive correlation						
L insula	−40	−14	−6	53	3.8	
R fusiform gyrus[a]	32	−55	−6	26	3.2	0.041
L amygdala[a]	−24	−3	−13	1	3.3	0.015
Negative correlation						
Posterior cingulate cortex	−8	−33	35	145	3.8	
R superior frontal gyrus	24	31	43	32	3.4	

Data are Talairach coordinates for cluster maxima at $p < 0.001$ with a cluster size $\geqslant 20$ voxels; Cluster size in number of voxels ($p < 0.001$, uncorrected); SVC p is small volume corrected p-value.
[a]Data from the search volume of the region of interest.

Table 5 Effects of a Single Nasal Testosterone Administration on Emotion Processing in Middle-Aged Women (ie Drug × Task Interaction), and Correlations with Serum Testosterone Levels in the Testosterone Condition Across Participants

	x	y	z	Cluster size	Z	SVC *p*
Testosterone > placebo						
L amygdala[a]	−30	−12	−11	18	4.2	0.001
R inferior frontal gyrus	52	27	5	55	4.0	
L middle temporal gyrus	−48	0	−14	88	3.6	
R amygdala[a]	24	−12	−13	–	2.4	0.109
Placebo > testosterone						
Precuneus	−10	−32	50	22	3.4	
Testosterone condition: positive correlation with testosterone level						
L middle frontal gyrus	−30	21	30	86	4.6	
R superior frontal gyrus	12	33	48	31	3.8	
L supramarginal gyrus	−50	−49	28	80	3.8	
R precuneus	8	−60	42	25	3.4	
Testosterone condition: negative correlation with testosterone level						
R occipital gyrus	10	−91	−10	34	3.7	
R orbitofrontal cortex	28	22	−22	27	3.5	
L orbitofrontal cortex	−27	19	−17	46	3.5	

Data are Talairach coordinates for cluster maxima at $p < 0.001$ with a cluster size $\geqslant 20$ voxels; Cluster size in number of voxels ($p < 0.001$, uncorrected); SVC p is small volume corrected p-value.
[a]Data from the search volume of the region of interest.

amygdala, although at a less conservative statistical threshold ([24 -12 -13], $t(24) = 2.6$, $p = 0.008$, uncorrected). In addition, testosterone increased the neural response to emotional faces in the inferior frontal and middle temporal gyri. Conversely, testosterone reduced the neural response to emotional faces in the precuneus (see Table 5).

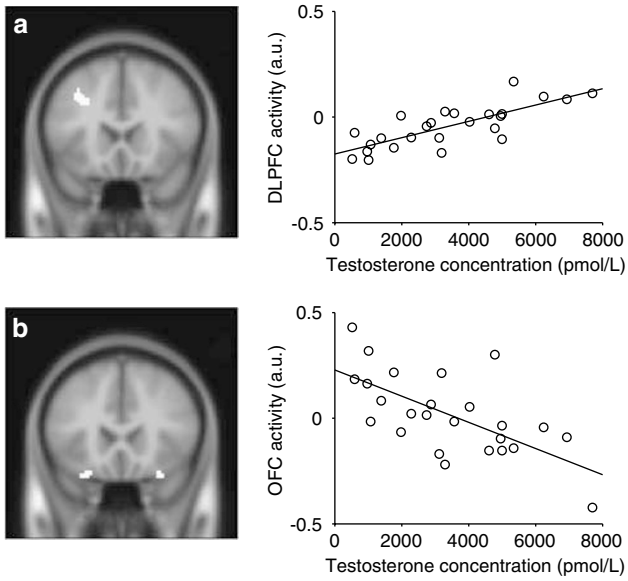

Figure 3 Serum testosterone level after a single nasal testosterone dose correlates positively with left dorsolateral prefrontal cortex (a; DLPFC, BA 9, $y = 21$) and negatively with orbitofrontal cortex (b; OFC, BA 47, $y = 22$) activity across middle-aged women. Left figures show coronal planes (left = left) at the cluster maxima at a statistical threshold of $p < 0.001$, uncorrected, cluster $\geqslant 20$ voxels.

A correlation analysis was performed to identify whether the enhanced testosterone levels were related to neural responses across individuals. Testosterone levels correlated positively with neural responses in the middle frontal gyrus (BA 9), superior frontal gyrus, supramarginal gyrus, and precuneus. Testosterone levels correlated negatively with responses in the orbitofrontal cortex (BA 47) and occipital gyrus (see Table 5 and Figure 3). Although testosterone reliably increased amygdala reactivity within subjects, no significant correlations with amygdala reactivity were observed across individuals, indicating individual differences in sensitivity to high testosterone levels.

To investigate whether testosterone increased amygdala reactivity of the middle-aged women to a level comparable to the young women, the data from the amygdala ROIs were extracted and averaged. The ROI analyses confirmed that amygdala reactivity was higher in the young than middle-aged women (left: $t(40) = 3.1$, $p = 0.004$, effect size: $r = 0.44$; right: $t(40) = 2.1$, $p = 0.043$, $r = 0.31$), and that testosterone increased left amygdala reactivity in the middle-aged women (left: $t(24) = 2.2$, $p = 0.035$, $r = 0.41$; right: $t(24) = 1.3$, $p = 0.19$, NS, $r = 0.27$). Importantly, testosterone increased amygdala reactivity in middle-aged women to a level comparable to that of young women (left: $t(40) = 1.2$, $p = 0.25$, NS, $r = 0.18$; right: $t(40) = 1.1$, $p = 0.26$, NS, $r = 0.18$; see Figure 4).

DISCUSSION

The present study investigated whether androgens influence amygdala reactivity in healthy, naturally cycling women. The results show that the age-related decline in androgen levels is associated with a decrease in amygdala reactivity.

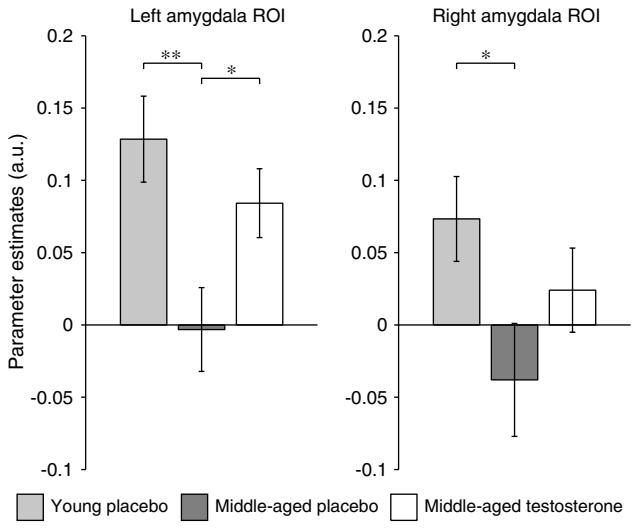

Figure 4 Testosterone increases amygdala reactivity to biologically salient stimuli in middle-aged women ($N = 25$) to a level comparable to young women ($N = 17$). The figure shows the mean (±SEM) amygdala response averaged across the amygdala ROI. $^{**}p < 0.01$, $^{*}p < 0.05$.

A double-blind, placebo-controlled, crossover study was applied to investigate whether exogenous testosterone modulates amygdala reactivity in premenopausal middle-aged women, who have about half their early adulthood androgen levels. The results show that a single nasal testosterone administration rapidly (within 45 min) increases amygdala reactivity in the middle-aged women to a young adulthood level.

The amygdala is part of a larger emotion circuitry, which is important for the identification of the emotional significance of stimuli, the generation of an affective response, and emotion regulation (Phillips *et al*, 2003). The endogenous testosterone levels were positively related to neural responses in the fusiform gyrus. This inferior temporal brain region is preferentially involved in face processing (Kanwisher *et al*, 1997) and in interaction with the amygdala important for emotional face perception (Haxby *et al*, 2002). The amygdala projects back to all levels of the ventral visual stream (Amaral *et al*, 2003), influencing emotional face perception (Vuilleumier *et al*, 2004). This suggests that the increased fusiform gyrus responses are a potential consequence of increased amygdala responses, indicating that testosterone may influence the perception of emotional stimuli. The enhanced testosterone levels were positively correlated with superior frontal cortex responses, and negatively with orbitofrontal cortex responses. Whereas the superior frontal regions are implicated in the controlled regulation of affect, the inferior frontal regions are thought to regulate emotional responses automatically via direct connections to the amygdala (Stefanacci and Amaral, 2002; Phillips *et al*, 2003; Ochsner and Gross, 2005; Stein *et al*, 2007). This suggests that the regulatory function of the orbitofrontal cortex decreases with increasing testosterone levels, potentially contributing to the observed amygdala disinhibition. In addition, the increased superior frontal cortex activity may reflect compensatory effort to regulate emotional responses.

The age-related changes in neural responses to faces between young adulthood and midlife reported in the present study are similar to the changes that have been reported previously in elderly. While amygdala and fusiform gyrus activity decreases, prefrontal activity increases in healthy older men and women (>60 years) in comparison to young adults (<30 years) (Iidaka *et al*, 2002; Gunning-Dixon *et al*, 2003; Mather *et al*, 2004; Tessitore *et al*, 2005). However, to our knowledge, amygdala reactivity during midlife has not been investigated previously. The results of the present study show that amygdala reactivity in naturally cycling women already decreases before menopause, when androgen levels have declined to half the early adulthood level. The correlation between decreasing endogenous testosterone levels and decreasing neural responses, together with the testosterone-induced amygdala reactivity increase, suggest that endogenous decreases in androgen levels may contribute to age-related decreases in amygdala reactivity.

Whereas the endogenous testosterone levels correlated positively with amygdala reactivity across individuals, the enhanced testosterone levels did not, even though testosterone reliably increased amygdala reactivity within subjects. This suggests that individual differences in amygdala sensitivity to high testosterone levels may exist. Furthermore, although the testosterone levels in the middle-aged women increased beyond the endogenous level of healthy young women, amygdala reactivity was similar. A recent study showed that a single testosterone administration also increases amygdala responses in healthy young women (Hermans *et al*, 2007). This suggests that testosterone increases amygdala reactivity within individuals regardless of baseline responses, but the absolute range of testosterone-potentiated amygdala responses may differ across individuals and age.

The rapid effect of nasal testosterone could be mediated by the rapid absorption of testosterone into the systemic circulation, but the nasal mucosa also provides a potential for pharmaceutical compounds to be available to the central nervous system directly (Hussain, 1998). Animal studies suggest that the timing of the testosterone-induced effect (within 45 min) indicates a nongenomic mechanism of action, potentially mediated by its rapid conversion to estradiol or its neuroactive steroid 3α-androstanediol. Studies in male mice have shown that changes in sexual behavior can be mediated by changes in estrogen levels, due to rapid changes in aromatase activity (Balthazart *et al*, 2006; Taziaux *et al*, 2007). In addition, testosterone also has anxiolytic properties, and its rapid anxiolytic action appears mediated by conversion to 3α-androstanediol, which potentiates inhibitory neurotransmission by modulation of the $GABA_A$ receptor (Aikey *et al*, 2002). This latter action may be similar to that of progesterone, which increases amygdala reactivity in healthy young women (van Wingen *et al*, 2007). Animal studies have demonstrated that the acute effects of progesterone are mediated by its neuroactive metabolite allopregnanolone that also exerts its action by binding to the $GABA_A$ receptor (Bitran *et al*, 1991, 1995), suggesting a common pathway by which neuroactive steroids could modulate amygdala function.

Testosterone did not influence self-reported sexual arousal, state anxiety, and mood in this study, which is in

line with previous studies (van Honk *et al*, 2001). However, testosterone may change subjective arousal after appropriate stimulation. For example, whereas testosterone increases physiological but not subjective arousal after a single exposure to erotic movies, repeated exposure to erotic movies also increases subjective sexual arousal (Tuiten *et al*, 2000, 2002). In addition, prolonged exposure to testosterone, or the prolonged withdrawal from testosterone, does influence the subjective experience. Mood and sexual function worsen after oophorectomy, when gonadal steroid hormone concentrations are chronically decreased. Moreover, subsequent testosterone treatment improves mood and sexual function in those women (Sherwin and Gelfand, 1985; Sherwin *et al*, 1985; Shifren *et al*, 2000). This suggests that although testosterone did not modulate self-report measures in this study, it may do so after appropriate stimulation or repeated administration.

This study shows that testosterone modulates amygdala activity in women, and animal research suggests that it may have a similar effect in men (Dimeo and Wood, 2006), for whom testosterone also modulates mood and sexual function (Schmidt *et al*, 2004). Also the female gonadal hormones progesterone (van Wingen *et al*, 2007) and presumably estradiol (Goldstein *et al*, 2005) modulate amygdala activity in women. The influence of these hormones in this study is likely limited, because the women were investigated during their follicular phase when progesterone and estradiol concentrations are lowest and similar to those in men. In addition, testosterone levels are also relatively low during the follicular phase, and increase during midcycle (Judd and Yen, 1973; Sinha-Hikim *et al*, 1998). Nevertheless, the sex hormones may interactively modulate amygdala activity. Furthermore, the amygdala is a sexual dimorphic brain structure with a relatively larger volume in men than women (Goldstein *et al*, 2001). The sex differences in amygdala structure and circulating hormone levels could mediate the sex differences in amygdala responsivity (Hamann, 2005; Cahill, 2006), and may thereby contribute to sex differences in the prevalence of psychiatric disorders (Kessler *et al*, 1994).

DISCLOSURE/CONFLICTS OF INTEREST

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