Exogenous Testosterone Enhances Responsiveness to Social Threat in the Neural Circuitry of Social Aggression in Humans

Erno J. Hermans, Nick F. Ramsey, and Jack van Honk

Background: In a range of species, the androgen steroid testosterone is known to potentiate neural circuits involved in intraspecific aggression. Disorders of impulsive aggression in humans have likewise been associated with high testosterone levels, but human evidence for the link between testosterone and aggression remains correlational and inconclusive.

Methods: Twelve female participants underwent functional magnetic resonance imaging during three sessions while viewing stimuli differing in social threat value: angry and happy facial expressions. The first session served to establish associations between baseline hormone levels and neural activation. Participants were retested in a second and third session after placebo-controlled sublingual administration of .5 mg testosterone.

Results: Findings demonstrate consistent activation to angry versus happy faces in areas known to be involved in vertebrate reactive aggression, such as the amygdala and hypothalamus. Suprathreshold clusters were also found in the orbitofrontal cortex (Brodmann area 47), a region implicated in impulse control in humans. Baseline endocrine profiles of high testosterone and low cortisol were associated with stronger activation in subcortical structures. Neural responses in most activated regions were more persistent after testosterone administration than after placebo.

Conclusions: These data demonstrate that testosterone enhances responsiveness in neural circuits of social aggression. Based on animal literature, it is argued that actions of testosterone on subcortical reactive aggression circuits give rise to this effect. Implications for our understanding of the pathophysiology of disorders of impulsive aggression are discussed.

Key Words: Aggression, amygdala, fMRI, glucocorticoid, hypothalamus, testosterone

Intraspecific aggression in vertebrates, including humans, is thought to be controlled by an integrated molecular, neuroanatomic, and behavioral substrate that exhibits strong evolutionary stability (1). This system appears to underlie the establishment of hierarchies in social animals, often merely through ritualized, species-specific behavioral displays of hostile intent, such as angry facial expressions (2,3).

The hypothalamic–pituitary–gonadal (HPG) axis, through its end product testosterone, is an important agent in regulating this system. Across vertebrates, males are generally more physically aggressive than females, and gonadectomy reduces aggression (4,5). Moreover, animal research has unequivocally shown that testosterone elevation increases aggressiveness (6–8). In humans, there is correlational evidence for a link between testosterone and aggression within both sexes (9). Disorders characterized by impulsive, reactive aggression, such as antisocial personality disorder (APD) and borderline personality disorder (BPD), have likewise been associated with high testosterone (10–13). Pending conclusive causal evidence (see, e.g., 9), however, the mechanism through which testosterone may affect human aggression remains obscure.

From Helmholtz Institute (EJH, JvH), Utrecht University; F.C. Donders Centre for Cognitive Neuroimaging (EJH), Radboud University Nijmegen, Rudolf Magnus Institute of Neuroscience (NFR), University Medical Center Utrecht, the Netherlands.

Address reprint requests to Erno J. Hermans, M.A., F.C. Donders Centre for Cognitive Neuroimaging, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands; E-mail: erno.hermans@fcdonders.ru.nl.

Received April 26, 2006; revised April 4, 2007; accepted May 3, 2007.

Pathways controlling reactive aggression converge in amygdalar and hypothalamic regions. Animal research has shown that testosterone exerts its influence partly through interactions with arginine vasopressin (AVP) in the amygdala (14,15) and the (anterior) hypothalamus (16). Also, testosterone downregulates the hypothalamic–pituitary–adrenal (HPA) axis, resulting in chronically depressed cortisol (17). Hypocortisolemia in turn has also been associated with heightened aggression and social rank (18,19). In agreement, a recent study has demonstrated a significant positive relation between testosterone and overt aggression in delinquent adolescent males with low cortisol exclusively (20).

The capacity to inhibit reactive aggression, or impulse control, is generally attributed to the orbitofrontal cortex (OFC). Lesions to the OFC are known to result in socially aberrant behavior (21,22). Likewise, marked OFC hypometabolism has been observed in patients with personality disorders (23,24). As indicated by low cerebrospinal fluid levels of 5-hydroxytryptamine (5-HT) catabolites, this hypometabolism appears to originate from reduced functioning of the serotonergic system (4), and testosterone has been suggested to play a role in the etiology of this abnormality (5).

Human neuroimaging findings have most consistently identified the amygdala and (lateral) OFC in responding to angry facial expressions (25–29). Behavioral experiments have furthermore shown that affective responding to angry faces is positively related to testosterone levels (30,31) but negatively to cortisol levels (32).

In this study, 12 healthy female volunteers were tested using functional magnetic resonance imaging (fMRI) during three sessions. The reason for including only women was that women have lower endogenous testosterone levels and therefore presumably require a smaller dose to attain measurable effects (33). Moreover, there are no indications that effects of testosterone on affective responding to angry facial expressions may exhibit

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.05.013

qualitative gender differences (30). The first session examined the pattern of brain responses to angry versus happy facial expressions in relation to levels of testosterone and cortisol. As in previous studies (33), happy facial expressions were chosen as control stimuli because these provide for a comparison between a social threat and a social safety signal while controlling for unspecific activity due to face and expression perception. We scrutinized a hierarchical model (34) consisting of cortical inhibitory control, implemented in the lateral OFC (especially Brodmann area 47 [BA47]) and subcortical reactive aggression circuits (i.e., amygdaloid region [medial/central nuclei, stria terminalis], hypothalamus, and brainstem subnuclei [periaqueductal grey, or PAG]). In a second and third session, the same group of participants was retested in a placebo-controlled crossover design to test effects of testosterone administration on functioning of these circuits. We predicted that responding in subcortical reactive aggression circuits would be associated with high testosterone and low cortisol and that testosterone elevation would affect responding in a similar region-specific manner.

Methods and Materials

Participants

Participants were 12 healthy, adult female volunteers (aged 18–28; mean age 22.6). Exclusion criteria were history of endocrine or psychiatric disorder, left-hand dominance, habitual smoking, current pregnancy, history of closed-head injury, and presence of metal objects in the body. Use of (recreational) psychotropic drugs within 2 weeks of testing was not allowed.

Ten women used standard estrogen/progestagen oral contraceptives. For the other two women who did not use oral contraceptives, testing was restricted to the preovulatory phase to control for (minor) variations of androgen levels throughout the menstrual cycle.

All procedures were approved by the local institutional review board in accordance with the declaration of Helsinki, and each participant provided written informed consent. Participants were debriefed and received payment.

Materials and Apparatus

Stimuli were selected from two photosets: the Karolinska Directed Emotional Faces (35) and the Pictures of Facial Affect (36). Eight actors were chosen, and oval cutouts of two photographs of each, happy (H) and angry (A), were included in the set. All were gray-scaled and equalized in luminance and contrast.

Stimulus presentation was controlled by an x86 notebook PC and E-Prime (Psychology Software Tools, Pittsburgh, Pennsylvania). Stimuli were back-projected onto a screen near the participants' feet, which they viewed through a 45° angle mirror attached to the head coil. Participants were scanned in an ACS-NT 1.5-Tesla MRI scanner (Philips Medical Systems, Best, the Netherlands).

Drug Samples

Testosterone solutions consisted of .5 mg of testosterone, 5 mg of hydroxypropyl-beta-cyclodextrin (carrier), 5 mg ethanol, and .5 mL water. Placebo solutions differed only in absence of testosterone. Details concerning sublingual administration, pharmacokinetics, and efficacy time-course are available elsewhere (37). Identical procedures have been applied repeatedly in our laboratory (38).

Procedure

Participants were tested using fMRI on three separate afternoons (after 1:30 PM), the first of which did not involve drug administration. They were instructed not to eat within 1.5 hours before the appointment and to drink only water. Upon arrival, saliva samples (9 mL) were obtained.

On the second and third testing day, participants arrived at the laboratory 3.5 hours before scanning. After providing saliva samples, participants received the .5 mL drug sample and were instructed to keep it under the tongue without swallowing for a full minute. They were asked to return 3.5 hours later and avoid physically or psychologically straining activities in the meantime. Further procedures were equal during all testing sessions.

After standard MRI safety screening, participants were escorted into the scanner room. Scans with the following characteristics were obtained:

1. T2*-weighted blood oxygen level–dependent (BOLD) images (segmented three-dimensional echo planar imaging (EPI) with navigator echo, flip angle 9.5°, echo time/repetition time (TE/TR): 19.1/28.6 ms, filed of view (FOV): 256 × 256 × 120 mm, matrix 64 × 64 × 30, voxel size 4 mm isotropic, acquisition time 3.26 sec), with oblique angulation. This segmented short TE sequence was designed to minimize signal dropout and image distortion due to magnetic field inhomogeneity around the air-tissue interfaces. Details on 3D-EPI are described elsewhere (39).
2. Reference image: identical to functional images except flip angle: 30°, resulting in more T1-weighting. This image was used to facilitate registration of structural and functional images.
3. T1-weighted structural images (TE/TR 4.6/30 msec, flip angle 30°, FOV 256 × 180 × 208 mm, matrix: 256 × 256 × 150 mm, slice thickness 1.2 mm, voxel size 1 × 1 × 1.2 mm, duration: 8 min).

During the functional scan series, participants watched alternating 26.08-sec epochs. In each epoch, all eight stimuli were presented in random order and repeated seven times (total 56 stimuli). Each was presented for a duration of 200 msec with a stimulus onset asynchrony of 467 msec. Resting fixation epochs were also included (+). Epochs were presented in the following order: +AH+AH+AHHA+HA+HA+ (or A and H reversed, counterbalanced across participants), which precludes task covariation with linear drifts of BOLD signal (see Figure 1). After scanning, another saliva sample was collected.

Functional MRI Image Analysis

Preprocessing of fMRI data was performed with SPM99 (UCL London, United Kingdom). All functional scans were motion-corrected using transformation parameters, yielding a minimal sum of squared differences with the reference image. Registration of the reference and structural scans was achieved by estimating parameters resulting in maximum mutual information. All images were normalized to standard (Montreal Neurological Institute [MNI], Montreal, Quebec, Canada) space using affine transformations and nonlinear deformations and then smoothed with an 8-mm full-width at half maximum gaussian kernel. Normalized anatomic images were averaged over participants to serve as background for anatomic localization.

Statistical analysis steps started with fitting session-specific general linear models containing box-car functions with hemodynamic delay for both task conditions (angry vs. happy), movement correction parameters, and a discrete cosine transform high-pass filter with a cutoff of 1.17e-2 Hz. Proportional scaling was applied to each volume to remove unspecific global (drug) effects. Contrast images (three sessions × 12 participants)

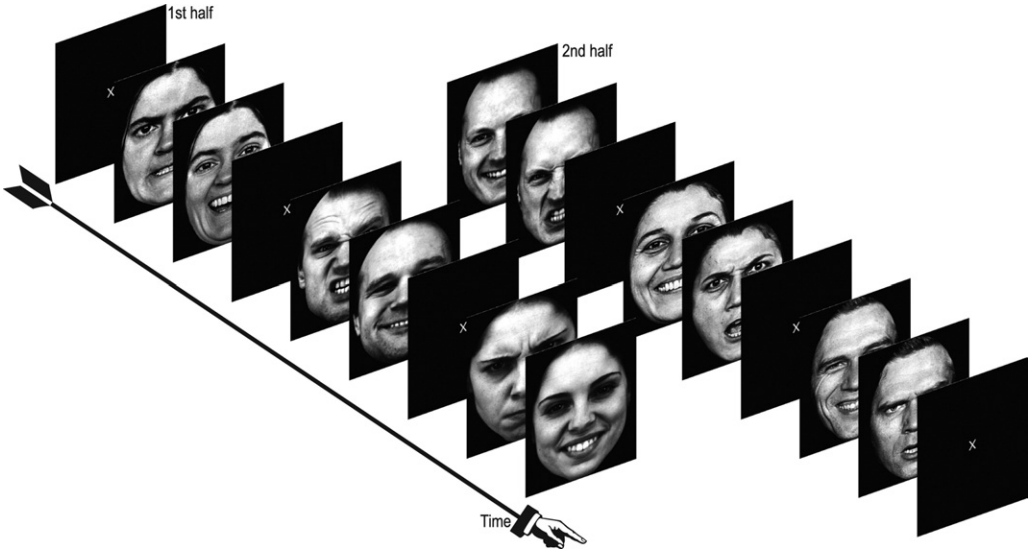

Figure 1. Order of epochs during the passive viewing task. Each epoch (fixation baseline, angry faces, or happy faces) lasted 26.08 sec, and during each target epoch, 56 stimuli were flashed onto the screen. The second half of the task is mirrored with respect to the first half to preclude covariation with linear signal drifts.

were calculated only for the main comparison of interest, the angry versus happy conditions, because this well-controlled contrast isolates the factor of interest (social threat). For the first session (no administration), a statistical map was subsequently calculated testing for the angry versus happy effect across participants using a statistical pooled variance approach (40). For our a priori regions of interest (ROI), bilateral BA47 in the OFC, bilateral amygdala, hypothalamus, and brainstem, the threshold for statistical significance was set at $p < .001$ uncorrected (i.e., $Z > 3.09$; one-sided). Adjacent voxels exceeding this threshold were clustered. Contrast parameter estimates for each participant and for each ROI were extracted from the original angry versus happy contrast images and averaged. These values were used for correlational analyses with hormone levels and between different areas. Significance threshold for voxels outside the ROIs was set using a Bonferroni correction for the whole brain, yielding a threshold of $\alpha = .05/24534 = 2.04e\text{-}6$; $Z = 4.61$.

For the second and third session, *Z*-maps were calculated for the angry versus happy effect and for the drug interaction, yielding areas in which the angry versus happy effect is positively larger in the testosterone condition. For visualization purposes, both statistical group maps were thresholded at $Z > 3.09$ one-sided and superimposed onto the averaged structural image.

Salivary Measurements

All saliva samples were stored in plastic vials and frozen at −20°C. Testosterone in saliva was measured after diethyl-ether extraction using a competitive radioimmunoassay employing a polyclonal antitestosterone antibody (J. Pratt, PhD, AZG 3290). [1,2,6,7-^{3}H]Testosterone (TRK402, Amersham, the Netherlands) was used as a tracer following chromatographic verification of its purity (see 41, for details). Testosterone levels in saliva samples taken after sublingual administration are not determinable.

Salivary cortisol levels were determined without extraction using a competitive radio-immunoassay employing a polyclonal anticortisol antibody (K7348). Following chromatographic verification of its purity, 1,2-3H(N)-Hydrocortisone (NET 185, NEN Dupont, Dreiech, Germany) was used as a tracer. The lower limit for detection is .5 nmol/l and reference values for adults are 4–28 nmol/L. Details concerning the validity and advantages of measuring cortisol in saliva have been published previously (42).

To increase reliability of measurements, all three baseline levels were averaged for cortisol and testosterone (yielding 11.16 nmol/L, SD = 2.80 and 79.28 pmol/L, SD = 22.33, respectively). Subsequently, both distributions were standardized to *t* scores (mean 50; SD 10) and individual testosterone/cortisol ratio scores were calculated.

Results

Endocrine Measures

Testosterone baseline levels of the three sessions did not differ significantly [$F(2,10) = 2.39$, *ns*). In the placebo condition, testosterone levels dropped slightly from preadministration to postscanning [$t(11) = 2.27$, $p = .044$].

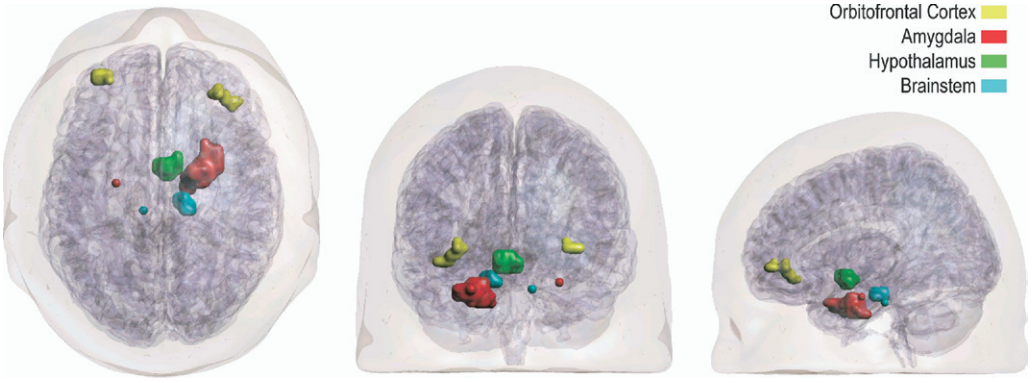

Figure 2. Three-dimensional rendering of the skin and the brain from a T1-weighted magnetic resonance imaging scan (top, frontal, and left views, respectively). Clusters of suprathreshold activity in response to angry versus happy facial expressions during the first session in the main regions of interest are color-coded.

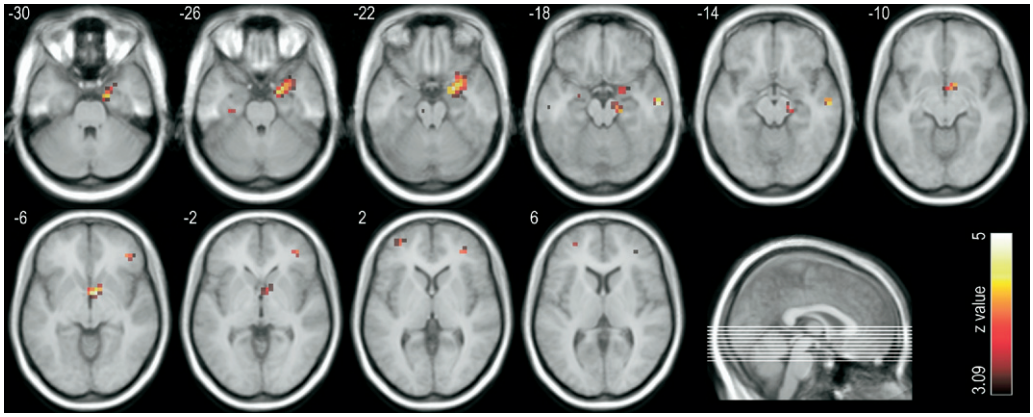

Figure 3. Ten axial slices at Z = −30 to Z = 6 in MNI space from the averaged, normalized, anatomic scans from all 12 participants (left hemisphere is left). All voxels exceeding the region of interest threshold of $Z > 3.09$ ($p < .001$) from the contrast angry versus happy from the first scan session are overlaid onto these slices. See Table 1 for additional data for each cluster.

Cortisol baseline levels were lowest on the first day of testing in comparison with the testosterone and placebo administration sessions [$F(1,11) = 11.35$, $p = .006$ and $F(1,11) = 9.01$, $p = .012$, respectively], which is explained by the fact that saliva samples were collected at a later time of day during the first session. Cortisol levels also dropped from the preadministration to postscanning samples for the second and third sessions [$F(1,11) = 39.26$, $p < .001$], but no interaction with drug administration was found [$F(1,11) = 1.45$, *ns*), indicating that exogenous testosterone elevation did not significantly decrease endogenous cortisol levels.

Functional MRI Results, First Session

Results for the main contrast of interest (angry vs. happy) are summarized in Table 1. The main regions of interest all showed evidence of suprathreshold activity. Amygdala responding was stronger in the right hemisphere. An additional focus of activity reaching a more conservative whole-brain corrected *Z* threshold of 4.61 was found in the inferior temporal gyrus. Moreover, two clusters of activity, one in the brainstem and one in the insular cortex, were more activated during the happy than during the angry face conditions. Figure 2 shows a three-dimensional rendering of clusters of activity in the main regions of interest. In Figure 3, all voxels exceeding the $Z > 3.09$ ROI threshold are overlaid onto axial slices of the averaged structural image.

Correlational Analyses

Averaged contrast parameter estimates for the angry versus happy comparison per suprathreshold cluster were used for correlational analyses with endocrine measures. Nonparametric statistics (Spearman's rho) were applied because of the small sample size. Results of these analyses are summarized in Table 2. Hypothalamic activity to angry versus happy facial expressions exhibited a negative correlation with baseline cortisol. Baseline testosterone levels correlated positively with activity in the inferior temporal gyrus only. The testosterone–cortisol ratio, however, proved more predictive: significant positive correlations were found with activity in the (predominantly right) amygdala, hypothalamus, brainstem, and inferior temporal gyrus. Scatterplots of the first two of these are shown in Figure 4.

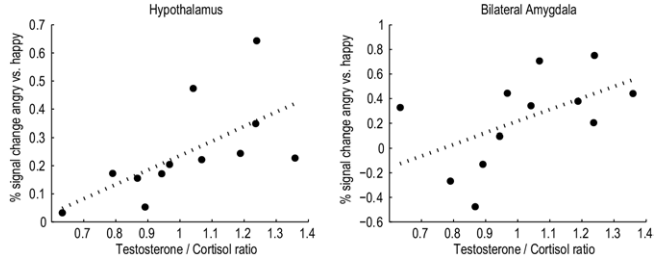

Figure 4. Scatterplots of the correlation between testosterone/cortisol ratio and the average magnitude of the blood oxygen level–dependent response to angry versus happy facial expressions in the hypothalamus (left) and bilateral amygdala (right).

Subsequently, interregional nonparametric correlations across participants were calculated between activated clusters (see Table 3). Activation during angry versus happy face conditions in the bilateral amygdala, hypothalamus, and combined brainstem clusters proved strongly interrelated. There was no evidence for a correlation between the lateral OFC and any of the other ROIs, however. Moreover, combined brainstem clusters responses were positively correlated with inferior temporal gyrus activity.

Functional MRI Results, Second and Third Session

Results of the drug administration sessions are summarized in Table 4. The angry versus happy contrast over these two sessions yielded a pattern of activated areas similar to the first session (i.e., bilateral BA47 in the orbitofrontal cortex, and the right amygdala), although not all regions reached significance. There was also evidence of stronger activity in the happy condition than in the angry condition in the brainstem (pons) and parahippocampal gyrus.

Drug interaction effects are shown in the lower half of Table 4 and Figure 5. As predicted, there were significant interaction effects in the greater part of the network specified in the first session with greater angry versus happy activity in the testosterone versus placebo condition. These effects were most pronounced in the amygdala and hypothalamus, but suprathreshold clusters were also found in the brainstem and lateral OFC (BA47). The peak location of the interaction effect in the amygdala appears to lie somewhat more medial than the main effects. Figure 6 shows the averaged activity in the angry (vs. happy) conditions in five clusters that exhibit the interaction effect. Further separate tests were performed on the contrast parameter estimates for angry versus happy conditions for the testosterone and placebo conditions separately to determine whether effects were carried mainly by activations in the testosterone conditions or by deactivations in the placebo condition. For these tests, contrast parameter estimates from all supratheshold voxels were averaged and tested using *t* tests. These analyses show that the testosterone effect in the left OFC is carried by a significant response to angry faces relative to happy faces. Drug interaction effects in the hypothalamus and brainstem appear to be carried

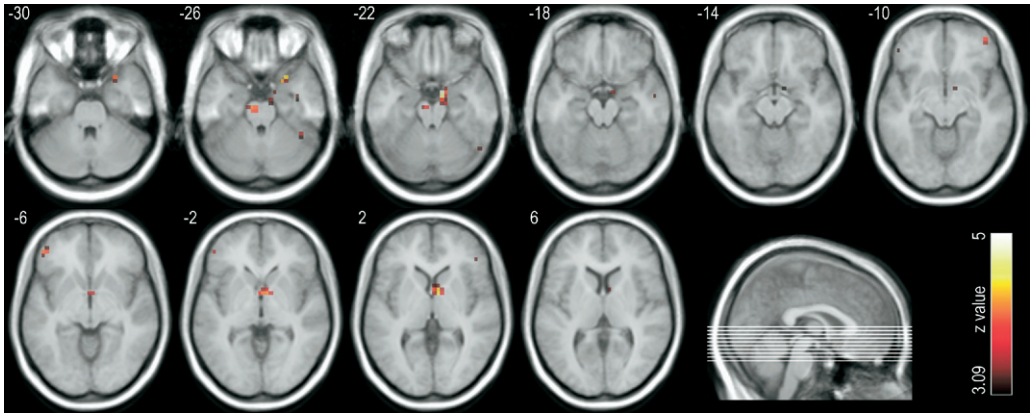

Figure 5. Ten axial slices at $z = -30$ to $z = 6$ in MNI space from the averaged, normalized, anatomic scans from all 12 participants (left hemisphere is left). All voxels exceeding the region of interest threshold of $Z > 3.09$ ($p < .001$) for the drug interaction (i.e., areas with a stronger angry vs. happy face effect in the testosterone compared with placebo session) from the second and third sessions are overlaid onto these slices. See Table 4 for additional data.

mainly by deactivations to angry faces relative to happy faces in the placebo condition [$t(11) = 2.72$, $p = .02$, and $t(11) = 2.45$, $p = .032$, respectively]. Other separate t tests did not reach significance.

Discussion

The purpose of this study was to gather insight into the neural substrates of human social aggression, in particular by investigating the regulatory role of steroid hormones. The main findings were, first, that cortical (the lateral OFC), as well as subcortical (the amygdala and its efferents), circuits implicated in aggression can be identified functionally using fMRI in healthy volunteers observing social threat stimuli: angry contrasted with happy facial expressions. Second, the degree to which these subcortical areas respond to social threat is associated with endocrine parameters, with strongest effects in participants with a profile of high testosterone and low cortisol. Third, in the two drug administration sessions, interaction effects were observed in most areas within the network that was activated in the first session, which suggest that overall the differential responses to angry and happy expressions are stronger, or more resistant to habituation, after testosterone versus placebo administration. Interaction effects were most pronounced in the amygdala and hypothalamus.

The medial and central nuclei of the amygdala, the bed nucleus of the stria terminalis, and efferent structures such as the hypothalamus and brainstem areas (e.g., PAG) have traditionally been characterized as a defensive circuit that choreographs autonomic, endocrine, and behavioral fight–flight responses to impending threat (43). Although this notion implies partly overlapping circuits for flight and fight, or fear and anger, contemporary human neuroimaging research has placed much more emphasis on the role of these pathways in fear than in anger. Neural responses to angry facial expressions are likewise sometimes interpreted in terms of fear. This study replicated the existing data by showing responses to angry faces in the bilateral amygdala (26–29), as well as in some of the expected efferent pathways of the amygdala, the hypothalamus and brainstem subnuclei. However, the main findings of this study place these responses in a different perspective.

Facial expressions of anger are a constituent part of socially aggressive behavior. Evolutionarily inspired theories emphasize that, over the course of evolution, selection pressures may have tended to moderate intraspecific conflicts over resources for survival and procreation, channeling them into ritualized dyadic exchanges of social signals of angry defiance (2). Nonverbal social behavior is therefore thought to be regulated by relatively closed, prewired, genetic programs (44) that integrate dedicated neural and molecular pathways with species-specific nonverbal behavior, the main vehicle of which is facial expression (45). Hence, angry facial expressions are presumed to play an important role in establishing and structuring social hierarchies (34),

Table 1. Summary of Suprathreshold Clusters of Activation to Angry versus Happy Facial Expressions in the First Session

Region	Side	X	Y	Z	Extent	Max z
Expression Main Effect: Activations						
Hypothalamus	R	8	0	−8	23	4.91[b]
Amygdala	R	24	0	−24	55	4.80[b]
Inferior temporal gyrus (BA20)	R	60	−16	−20	9	4.80[b]
Superior brainstem/posterior hippocampus	R	20	−24	−20	11	4.21[a]
Orbitofrontal cortex (BA47)	R	40	40	−8	11	4.00[a]
Orbitofrontal cortex (BA47)	L	−32	52	0	6	3.90[a]
Amygdala	L	−24	−8	−20	1	3.36[a]
Brainstem (Pons)	L	−8	−24	−24	1	3.14[a]
Expression Main Effect: Deactivations						
Brainstem	R	16	−20	−4	25	4.71[b]
Insular cortex	R	36	4	−4	51	4.70[b]

Coordinates are defined in Montreal Neurological Institute (MNI) space. BA, Brodmann's area.
[a]Activation significant at a $p < .001$ uncorrected threshold (one-sided and for regions of interest only).
[b]Activation significant at a $p < .05$ whole brain Bonferroni-corrected threshold. Extent indicates the cluster size of adjacent voxels with $p < .001$, uncorrected.

Table 2. Summary of Rank Order Correlations between Endocrine Parameters and Blood Oxygen Level–Dependent Responses to Angry versus Happy Facial Expressions in Activated Clusters during the First Session

	Cort		T		T/Cort Ratio	
Region	rho	*p*	rho	*p*	rho	*p*
Amygdala	−.32	.308	.12	.721	.68[a]	.015
Left	−.31	.319	−.09	.787	.51	.090
Right	−.28	.379	.22	.498	.69[a]	.013
Orbitofrontal Cortex (BA47)	−.20	.527	−.13	.688	.03	.914
Left	−.10	.746	−.34	.285	−.17	.587
Right	−.26	.417	.14	.664	.27	.391
Hypothalamus	−.58[a]	.048	.27	.397	.83[b]	.001
Brainstem	−.30	.342	.27	.397	.70[a]	.011
Superior (/post- hippocampus)	−.32	.308	.22	.498	.56	.059
Pons	−.21	.513	.09	.787	.50	.101
Inferior Temporal Gyrus (BA20)	.08	.795	.80[b]	.002	.67[a]	.017

BA, Brodmann's area; Cort, cortisol; T, testosterone.
[a]P < .05.
[b]P < .01

and evoke affective responses in the observer that vary as a function of social status (30,33,46).

Individual differences in functioning of the endocrine systems have proven to be reliably associated with social rank. The HPA and HPG axes exhibit mutually inhibitory functional interactions (17) with apparently opposite effects upon social dominance. Although aggressive episodes are accompanied by HPA-initiated phasic cortisol increases (47), a profile of low testosterone and chronically high cortisol is related to social submissiveness and low aggression in a range of species (19,48). In agreement, previous research has shown that various measures of affective responses to angry facial expressions in human volunteers are predicted by high levels of anger, dominance, drive, and testosterone levels (30,31,46,49), and are oppositely related to social anxiety and cortisol levels (32,46). Moreover, recent evidence indicates that cortisol mediates the relation between testosterone and aggression: a positive linear relation between overt aggression and testosterone was found in adolescent delinquents with low cortisol levels only (20). Hence, in our study correlations were calculated between BOLD responses within the activated areas and the (standardized) testosterone:cortisol ratio. In line with predictions, results show that individuals with a high testosterone:cortisol ratio respond more to angry (vs. happy) faces in the amygdala, hypothalamus, and brainstem areas. Note that this finding is at odds with an interpretation of activity in subcortical defense circuits solely in terms of fear because both animal (50) and human research (38,51) has shown that testosterone has fear-reducing properties.

Results from the second and third sessions of this study support this interpretation by showing that responses in amygdalar–hypothalamic regions persist more strongly after testosterone administration. This finding is consistent with research on rodents demonstrating that testosterone interacts with AVP in these regions to regulate aggression (15,16,52). As suggested by Figure 6, responses in these regions appear to habituate more strongly in the placebo condition, which is consistent with well-known habituation effects of amygdalar responding (53). Note that although the peak location of the drug interaction effect in the amygdala appears to lie medial with respect to the main effect, the underlying resolution of the statistical maps does not warrant inferences about amygdalar subnuclei.

In addition to interaction effects in subcortical areas, small clusters of increased activity to angry (vs. happy) facial expressions were found in the lateral OFC (BA47). Other than subcortical reactive aggression circuits, the role of the OFC in human social aggression appears to be inhibitory (34). Our data replicate earlier findings of responses in this area to angry facial expressions (25). Because the angry facial expression signals conspecifics to amend current behaviors, it has been argued that responding to angry faces recruits processes that are also implicated in response reversal and behavioral extinction, functions that have been ascribed to the OFC (34,54). These notions are supported by neuropsychologic observations of patients with OFC lesions. Often these result in impulsively aggressive, aberrant behavior (21,55). In agreement, neuroimaging studies have shown that impulsively aggressive individuals exhibit OFC hy-

Table 3. Summary of Nonparametric Cross-Correlations between Suprathreshold Clusters in the First Session

	1)		2)		3)		4)	
Region	rho	*p*	rho	*p*	rho	*p*	rho	*p*
1) Bil. amygdala								
2) Bil. OFC (BA47)	−.05	.88						
3) Hypothalamus	.60[a]	.04	.03	.91				
4) Brainstem	.76[b]	.004	−.18	.57	.57[a]	.05		
5) Inferior Temporal Gyrus (BA20)	.36	.26	−.33	.30	.21	.51	−.07	.83

BA, Brodmann's area; Bil, bilateral; OFC, orbitofrontal cortex.
[a]*p* < .05.
[b]*p* < .01.

Table 4. Summary of Suprathreshold Clusters for Expression Main Effects and Drug by Expression Interactions in the Second and Third Sessions

Region	Side	X	Y	Z	Extent	Max *Z*
Expression Main Effect: Activations						
Orbitofrontal Cortex (BA47/10)	L	−32	60	−4	1	3.59[a]
Orbitofrontal Cortex (BA47)	R	40	48	−8	1	3.42[a]
Amygdala	R	20	4	−20	3	3.39[a]
Expression Main Effect: Deactivations						
Parahippocampal gyrus (BA28)	R	20	−24	−9	24	6.51[b]
Brainstem (Pons)	L	−4	−17	−19	16	5.29[b]
Drug by Expression Interaction: Testosterone Activations						
Amygdala	R	12	−4	−24	15	4.94[b]
Hypothalamus	R	8	0	0	17	4.45[a]
Brainstem (Pons)	L	−8	−20	−28	7	4.05[a]
Orbitofrontal Cortex (BA47/46)	L	−48	44	−8	6	3.99[a]
Orbitofrontal Cortex (BA47)	R	44	52	−12	2	3.88[a]

Coordinates are defined in MNI space. BA, Brodmann's area.
[a]Activation significant at a $p < .001$ uncorrected threshold (one-sided and for regions of interest only).
[b]Activation significant at a $p < .05$ whole brain Bonferroni-corrected threshold. Extent indicates the cluster size of adjacent voxels with $p < .001$, uncorrected.

poactivity (56,57). Moreover, studies that used procedures to enhance emotional states in BPD found augmented amygdalar responding (58) in addition to reduced OFC activity (59), which fits into a picture of subcortical preeminence due to malfunctioning OFC impulse control. This OFC malfunctioning is associated with reduced serotonergic (5HT) neurotransmission (4). In agreement, selective serotonin reuptake inhibitors reduce impulsiveness in personality disorders (60), and reduce OFC hypometabolism (24). Consistent with findings of heightened testosterone in personality-disordered patients (10–13), testosterone has been shown to suppress 5HT systems (61–63). These effects, however, likely take place on a longer timescale. In our study, a subtle acute effect of testosterone on OFC reactivity was found. This effect may be taken to reflect an increased effort for inhibitory control over increased subcortical activation. In agreement, it has been argued that testosterone interferes with cortico–subcortical communication, thus reducing efficacy of OFC impulse control (64). Further research is needed to resolve this issue.

In conclusion, by showing a more persistent response to social threat conveyed through facial expressions after administration of testosterone, our findings shed light on the neural pathways through which gonadal steroids regulate social aggression in humans. Our findings are consistent with rodent models of reactive aggression but also with neuroanatomic models of human aggression regulation that imply an important role for the lateral OFC in impulse control. Moreover, these data suggest that testosterone plays a causal role in disorders of impulsive aggression. More research on this topic is warranted.

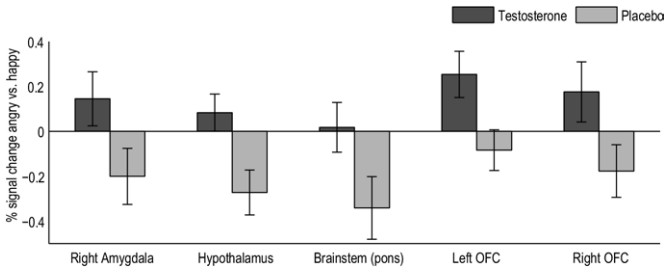

Figure 6. Bar graphs showing averaged contrast estimates for the angry versus happy facial expression comparison (with standard errors of the mean) in the testosterone and placebo sessions. Separate graphs depict the five regions that exhibit a significant drug interaction effect.

This research was supported by grants from the Netherlands Organization for Scientific Research (Grant No. 016-005-060) and the Research Institute for Psychology and Health. This work was performed while Erno J. Hermans was employed at Utrecht University. Erno J. Hermans, Nick F. Ramsey, and Jack van Honk reported no biomedical financial interests or potential conflicts of interest.

1. Nelson RJ, Chiavegatto S (2001): Molecular basis of aggression. *Trends Neurosci* 24:713–719.
2. Öhman A (1986): Face the beast and fear the face: Animal and social fears as prototypes for evolutionary analyses of emotion. *Psychophysiology* 23:123–145.
3. Blair RJ (2003): Facial expressions, their communicatory functions and neuro-cognitive substrates. *Philos Trans R Soc Lond B Biol Sci* 358:561–572.
4. Lee R, Coccaro E (2001): The neuropsychopharmacology of criminality and aggression. *Can J Psychiatry* 46:35–44.
5. Giammanco M, Tabacchi G, Giammanco S, Di Majo D, La Guardia M (2005): Testosterone and aggressiveness. *Med Sci Monit* 11:RA136–145.
6. Lumia AR, Thorner KM, McGinnis MY (1994): Effects of chronically high doses of the anabolic androgenic steroid, testosterone, on intermale aggression and sexual behavior in male rats. *Physiol Behav* 55:331–335.
7. Melloni RH Jr, Connor DF, Hang PT, Harrison RJ, Ferris CF (1997): Anabolic-androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. *Physiol Behav* 61:359–364.
8. Rejeski WJ, Brubaker PH, Herb RA, Kaplan JR, Koritnik D (1988): Anabolic steroids and aggressive behavior in cynomolgus monkeys. *J Behav Med* 11:95–105.
9. Archer J (2006): Testosterone and human aggression: An evaluation of the challenge hypothesis. *Neurosci Biobehav Rev* 30:273–436.
10. Virkkunen M, Rawlings R, Tokola R, Poland RE, Guidotti A, Nemeroff C, *et al.* (1994): CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. *Arch Gen Psychiatry* 51:20–27.
11. Stalenheim EG, Eriksson E, von Knorring L, Wide L (1998): Testosterone as a biological marker in psychopathy and alcoholism. *Psychiatry Res* 77:79–88.
12. Aromäki AS, Lindman RE, Eriksson CJ (2002): Testosterone, sexuality and antisocial personality in rapists and child molesters: A pilot study. *Psychiatry Res* 110:239–247.

13. Rasanen P, Hakko H, Visuri S, Paanila J, Kapanen P, Suomela T, Tiihonen J (1999): Serum testosterone levels, mental disorders and criminal behaviour. *Acta Psychiatr Scand* 99:348–352.
14. Ferris CF, Delville Y (1994): Vasopressin and serotonin interactions in the control of agonistic behavior. *Psychoneuroendocrinology* 19:593–601.
15. de Vries GJ, Miller MA (1998): Anatomy and function of extrahypothalamic vasopressin systems in the brain. *Prog Brain Res* 119:3–20.
16. Delville Y, Mansour KM, Ferris CF (1996): Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiol Behav* 60:25–29.
17. Viau V (2002): Functional cross-talk between the hypothalamic–pituitary–gonadal and –adrenal axes. *J Neuroendocrinol* 14:506–513.
18. Sapolsky RM (1990): Adrenocortical function, social rank, and personality among wild baboons. *Biol Psychiatry* 28:862–878.
19. Kalin NH (1999): Primate models to understand human aggression. *J Clin Psychiatry* 60 Suppl 15:29–32.
20. Popma A, Vermeiren R, Geluk CA, Rinne T, van den Brink W, Knol DL, *et al.* (2006): Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. *Biol Psychiatry* 61:405–11.
21. Blair RJ, Cipolotti L (2000): Impaired social response reversal. A case of "acquired sociopathy." *Brain* 123 (Pt 6):1122–1141.
22. Eslinger PJ, Damasio AR (1985): Severe disturbance of higher cognition after bilateral frontal lobe ablation: patient EVR. *Neurology* 35:1731–1741.
23. Goyer PF, Andreason PJ, Semple WE, Clayton AH, King AC, Compton-Toth BA, *et al.* (1994): Positron-emission tomography and personality disorders. *Neuropsychopharmacology* 10:21–28.
24. New AS, Buchsbaum MS, Hazlett EA, Goodman M, Koenigsberg HW, Lo J, *et al.* (2004): Fluoxetine increases relative metabolic rate in prefrontal cortex in impulsive aggression. *Psychopharmacology (Berl)* 176:451–458.
25. Blair RJ, Morris JS, Frith CD, Perrett DI, Dolan RJ (1999): Dissociable neural responses to facial expressions of sadness and anger. *Brain* 122 (Pt 5): 883–893.
26. Whalen PJ, Shin LM, McInerney SC, Fischer H, Wright CI, Rauch SL (2001): A functional MRI study of human amygdala responses to facial expressions of fear versus anger. *Emotion* 1:70–83.
27. Fischer H, Sandblom J, Gavazzeni J, Fransson P, Wright CI, Backman L (2005): Age-differential patterns of brain activation during perception of angry faces. *Neurosci Lett* 386:99–104.
28. Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR (2002): The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage* 17:317–323.
29. Sprengelmeyer R, Rausch M, Eysel UT, Przuntek H (1998): Neural structures associated with recognition of facial expressions of basic emotions. *Proc Biol Sci* 265:1927–1931.
30. Van Honk J, Tuiten A, Verbaten R, van den Hout M, Koppeschaar H, Thijssen J, de Haan E (1999): Correlations among salivary testosterone, mood, and selective attention to threat in humans. *Horm Behav* 36: 17–24.
31. Wirth MM, Schultheiss OC (2007): Basal testosterone moderates responses to anger faces in humans. *Physiol Behav* 90:496–505.
32. Van Honk J, Tuiten A, van den Hout M, Koppeschaar H, Thijssen J, de Haan E, Verbaten R (1998): Baseline salivary cortisol levels and preconscious selective attention for threat. A pilot study. *Psychoneuroendocrinology* 23:741–747.
33. Van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, *et al.* (2001): A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. *Behav Neurosci* 115:238–242.
34. Blair RJ (2004): The roles of orbital frontal cortex in the modulation of antisocial behavior. *Brain Cogn* 55:198–208.
35. Lundqvist D, Flykt A, Öhman A (1998): *The Karolinska Directed Emotional Faces*. Stockholm: Karolinska Institute.
36. Ekman P, Friesen WV (1976): *Pictures of Facial Affect*. Palo Alto, CA: Consulting Psychologists Press.
37. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R (2000): Time course of effects of testosterone administration on sexual arousal in women. *Arch Gen Psychiatry* 57:149–153.
38. Hermans EJ, Putman P, Baas JMP, Koppeschaar H, Van Honk J (2006): A single administration of testosterone reduces fear potentiated startle in humans. *Biological Psychiatry* 59:872–874.
39. van Gelderen P, Ramsey NF, Liu G, Duyn JH, Frank JA, Weinberger DR, Moonen CT (1995): Three-dimensional functional magnetic resonance imaging of human brain on a clinical 1.5-T scanner. *Proc Natl Acad Sci U S A* 92:6906–6910.
40. Ramsey NF, Tallent K, Van Gelderen P, Frank JA, Moonen CTW, Weinberger DR (1996): Reproducibility of human 3D fMRI brain maps acquired during a motor task. *Hum Brain Mapp* 4:113–121.
41. Dabbs JM, Jr. (1990): Salivary testosterone measurements: Reliability across hours, days, and weeks. *Physiol Behav* 48:83–86.
42. Kirschbaum C, Hellhammer DH (1994): Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 19:313–333.
43. Kling AS, Brothers LA (1992): The amygdala and social behavior. In: J.P. Aggleton (ed.). *The Amygdala*. New York: Wiley-Liss, pp 353–377.
44. Mayr E (1974): Behavior programs and evolutionary strategies. *Am Sci* 62:650–659.
45. Fridlund AJ (1991): Evolution and facial action in reflex, social motive, and paralanguage. *Biol Psychol* 32:3–100.
46. Putman P, Hermans EJ, Van Honk J (2004): Emotional Stroop performance for masked angry faces: It's BAS, not BIS. *Emotion* 4:305–311.
47. Summers CH, Watt MJ, Ling TL, Forster GL, Carpenter RE, Korzan WJ, *et al.* (2005): Glucocorticoid interaction with aggression in non-mammalian vertebrates: Reciprocal action. *Eur J Pharmacol* 526:21–35.
48. Blanchard DC, Sakai RR, McEwen B, Weiss SM, Blanchard RJ (1993): Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behav Brain Res* 58:113–121.
49. Van Honk J, Tuiten A, de Haan E, van den Hout M, Stam H (2001): Attentional biases for angry faces: Relationships to trait anger and anxiety. *Cogn Emotion* 15:279–297.
50. Aikey JL, Nyby JG, Anmuth DM, James PJ (2002): Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). *Horm Behav* 42: 448–460.
51. Van Honk J, Peper JS, Schutter DJLG (2005): Testosterone reduces unconscious fear but not consciously experienced anxiety: Implications for the disorders of fear and anxiety. *Biol Psychiatry* 58:218–225.
52. Koolhaas JM, Van den Brink TH, Roozendaal B, Boorsma F (1990): Medial amygdala and aggressive behavior: Interaction between testosterone and vasopressin. *Aggressive Behav* 16:223–229.
53. Fischer H, Wright CI, Whalen PJ, McInerney SC, Shin LM, Rauch SL (2003): Brain habituation during repeated exposure to fearful and neutral faces: A functional MRI study. *Brain Res Bull* 59:387–392.
54. Dias R, Robbins TW, Roberts AC (1996): Dissociation in prefrontal cortex of affective and attentional shifts. *Nature* 380:69–72.
55. Damasio H, Grabowski T, Frank R, Galaburda AM, Damasio AR (1994): The return of Phineas Gage: Clues about the brain from the skull of a famous patient. *Science* 264:1102–1105.
56. De La Fuente JM, Goldman S, Stanus E, Vizuete C, Morlan I, Bobes J, Mendlewicz J (1997): Brain glucose metabolism in borderline personality disorder. *J Psychiatr Res* 31:531–541.
57. Soloff PH, Meltzer CC, Becker C, Greer PJ, Kelly TM, Constantine D (2003): Impulsivity and prefrontal hypometabolism in borderline personality disorder. *Psychiatry Res* 123:153–163.
58. Herpertz SC, Dietrich TM, Wenning B, Krings T, Erberich SG, Willmes K, *et al.* (2001): Evidence of abnormal amygdala functioning in borderline personality disorder: A functional MRI study. *Biol Psychiatry* 50:292–298.
59. Schmahl CG, Vermetten E, Elzinga BM, Bremner JD (2004): A positron emission tomography study of memories of childhood abuse in borderline personality disorder. *Biol Psychiatry* 55:759–765.
60. Coccaro EF, Kavoussi RJ (1997): Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Arch Gen Psychiatry* 54:1081–1088.
61. Sundblad C, Eriksson E (1997): Reduced extracellular levels of serotonin in the amygdala of androgenized female rats. *Eur Neuropsychopharmacol* 7:253–259.
62. Martinez-Conde E, Leret ML, Diaz S (1985): The influence of testosterone in the brain of the male rat on levels of serotonin (5-HT) and hydroxyindole-acetic acid (5-HIAA). *Comp Biochem Physiol C* 80:411–414.
63. van de Kar L, Levine J, Van Orden LS, 3rd (1978): Serotonin in hypothalamic nuclei: increased content after castration of male rats. *Neuroendocrinology* 27:186–192.
64. Schutter DJLG, Van Honk J (2004): Decoupling of midfrontal delta-beta oscillations after testosterone administration. *Int J Psychophysiol* 53: 71–73.