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Testosterone administration in women increases amygdala responses to fearful and happy faces

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Summary Data from both rodents and humans show that testosterone reduces fear. This effect is hypothesized to result from testosterone's down regulating effects on the amygdala, a key region in the detection of threat and instigator of fight-or-flight behavior. However, neuroimaging studies employing testosterone administration in humans have consistently shown increased amygdala responsivity. Yet, no study to date has investigated specifically how testosterone affects the amygdala response to fearful emotional expressions. Such stimuli signal the presence of environmental threat and elicit robust amygdala responses that have consistently been associated with anxious traits. In the present study, we therefore used functional magnetic resonance imaging combined with a single administration of 0.5 mg testosterone in 12 healthy women to assess testosterone's effects on amygdala responses to dynamic fearful (and happy control) faces. Our results show that both stimuli activate the amygdala. Notably, testosterone increased the amygdala response to both stimuli, and to an equal degree. Thus, testosterone appears not to reduce fear by attenuating the amygdala response toward signals of threat. Data further show that testosterone selectively increases activation of the superficial amygdala (SFA) and, to a lesser extent, the basolateral amygdala (BLA). No effect was found in the central nucleus, which is involved in the generation of autonomic fear responses. Both the SFA and BLA are considered input regions, and enhanced activation by testosterone might reflect the role of this hormone in adaptive responding to socially relevant stimuli. Furthermore, literature on the distinct roles of the SFA and BLA in fear processing show that increased activation of these subregions of the amygdala is consistent with a fear reducing effect of testosterone.

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Introduction

Over the last decade, knowledge on the role of the steroid hormone testosterone in human social and emotional behavior has greatly increased. Working from models based on effects of testosterone in mostly rodents, efforts have been made to investigate the effects of testosterone in humans (Eisenegger et al., 2011; Bos et al., 2012b). One of the results of these efforts is the finding that testosterone, known to reduce fear in rodents (Aikey et al., 2002), has comparable effects in humans (van Honk et al., 2005; Hermans et al., 2006, 2007). In these studies, a single administration of testosterone resulted in reduced startle potentiation in response to threat of mild electric shock (Hermans et al., 2006), reduced sympathetic autonomic nervous system responses to threatening pictures (Hermans et al., 2007), and reduced automatic attention for fearful faces, which signal environmental threat (Whalen et al., 2004; van Honk et al., 2005). However, the neural mechanism by which testosterone exerts these fear-reducing effects is currently unknown.

A key structure implicated in anxiety and the detection of salient environmental features including threat is the amygdala (Davis and Whalen, 2001). Through its target regions such as the hypothalamus and the periaqueductal gray (PAG), the amygdala is tightly linked to networks involved in fear responses and the initiation of fight-or-flight behavior (Price, 2003). Considering this role, it is not surprising this region is very sensitive to emotional facial expressions (Phelps and LeDoux, 2005). Dynamic facial expressions in particular have been shown to elicit robust amygdala activation (Sato et al., 2004; van der Gaag et al., 2007). Also, amygdala responses to fearful faces presented outside of focal attention are enhanced in highly anxious, but healthy, individuals (Bishop et al., 2004), and especially in females (Dickie and Armony, 2008). Therefore, dysregulation of the amygdala has been proposed to underlie the etiology of mood and anxiety disorders. Indeed, patients suffering from mood and anxiety disorders show exaggerated amygdala responses to emotional faces compared to controls (Drevets, 2003; Monk, 2008), in particular toward fearful faces (Labuschagne et al., 2010). Moreover, the amygdala is an important target of testosterone, as it is rich in androgen receptors (Sarkey et al., 2008), as well as in aromatase, which converts testosterone into estradiol (Biegon et al., 2010). Estradiol receptors are also abundant in the human amygdala, although they do not seem to be located in the central nucleus of the amygdala (Österlund et al., 2000). Finally, males show faster attenuation of the amygdala in response to fearful faces accompanied by lower autonomic stress responses compared to females, who have lower baseline testosterone levels (Williams et al., 2005). Based on these findings, it can be hypothesized that testosterone exerts its fear-reducing properties by reducing amygdala responses to environmental threat cues.

However, studies investigating the effect of testosterone on the amygdala performed so far show positive correlations between endogenous testosterone levels and amygdala responses to facial stimuli (Derntl et al., 2009; Stanton et al., 2009; Manuck et al., 2010). Also, studies employing a single administration of testosterone, which in contrast to correlational methods can give causal insight, show increased amygdala responses to faces after testosterone administration (Hermans et al., 2008; van Wingen et al., 2009). These findings are not in favor of the hypothesis that testosterone reduces fear by inhibiting the amygdala response. However, such findings may also be explained by the fact that the studies by Hermans et al. (2008) and van Wingen et al. (2009) did not use fearful faces as stimuli to elicit amygdala activity. For instance, Hermans et al. used angry and happy faces, while van Wingen et al. presented fearful faces simultaneously with angry faces, making it impossible to distinguish amygdala responses to these two expressions. Facial expressions of fear are prototypical innate human danger cues that are thought to evoke amygdala activity because they convey the presence of threat without providing information about its source, thus prompting sensory vigilance (Whalen, 1998; Davis and Whalen, 2001). Indeed, fearful faces have been shown to elicit stronger amygdala responses than angry faces (Whalen et al., 2001), which are less ambiguous because they directly represent the source of threat (Adams et al., 2003), which is social in nature (van Honk et al., 2001; Hermans et al., 2008). For these reasons, amygdala responses to the expression of fear are thought to be more suitable to probe neural processes underlying anxiety (Whalen et al., 2001; Bishop et al., 2004; van Honk et al., 2005). The present study therefore aimed to assess the effect of testosterone on the amygdala response specifically to fearful faces.

In a counterbalanced placebo-controlled crossover design, 12 healthy women were sublingually administered 0.5 mg of sublingual testosterone. Functional magnetic resonance imaging (fMRI) was used to measure neural responses to dynamic happy and fearful emotional faces, of which the happy facial expressions served as a control condition (van Marle et al., 2009). Happy and fearful faces were presented in separate blocks to investigate the responses in our a priori region of interest, the amygdala, to both emotional expressions. This design allowed us to answer the question of whether testosterone would reduce amygdala responses toward signals of environmental threat, thereby potentially decreasing fear.

Methods

Subjects

Twelve healthy, adult women with normal or corrected-to-normal vision (age range 18—25; mean age 20.4) were tested in a double-blind, placebo-controlled, fully counterbalanced crossover design. Participants were mostly students recruited from the university campus by advertisements. Only women were included because the dosage and temporal parameters of neurophysiological effects induced by a single sublingual administration of testosterone are unknown in men. Exclusion criteria were, for fMRI safety procedures: history of closed-head injury, current pregnancy, and presence of metal objects in the body. To reduce interindividual variability and avoid unwanted interactions with drug administration the following exclusion criteria were applied: history of endocrine or psychiatric disorders, irregular sleep patterns, use of (recreational) psychotropic drugs within 2 weeks of testing, left hand dominance, and habitual smoking. Nine women used standard monophasic estrogen/progestagen oral contraceptives. For the remaining three

women, testing was restricted to the pre-ovulatory phase (first ten days after the first day of the menstruation) in order to control for (minor) variations of androgen levels throughout the menstrual cycle (see also Hermans et al., 2008). Participants' trait anxiety was assessed using the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983). All scores were in the normal range (average 32.1; SD 8.2). Written informed consent was obtained from all participants and the study protocol was approved by the ethical review board of the University Medical Center Utrecht in accordance with the declaration of Helsinki. All participants received financial compensation.

Testosterone administration

The testosterone sample consisted of 0.5 mg of testosterone, 5 mg of the carrier cyclodextrin, 5 mg of ethanol, and 0.5 ml of water. The placebo sample was identical to the drug sample only without containing testosterone. Both testosterone and placebo were administered sublingually under supervision of the experimenter. Extensive prior research established that 0.5 mg of testosterone in young women, without exception, results in an approximate 10-fold increase in blood levels of testosterone 15 min after administration, and a return to baseline in 90 min (Tuiten et al., 2000). This 10-fold increase mostly reflects testosterone that is bound to sex hormone binding globulin (SHBG) and albumin, whereas the increase of the free fraction, which passes the blood brain barrier and exerts its effects in the brain, is increased more moderately. A study into dose-dependent effects of sublingual testosterone administration indeed shows that the increase of the free fraction by testosterone depends on the SHBG level in women (van Rooij et al., 2011). Behavioral and physiological effects of the administration, as demonstrated by enhanced vaginal pulse amplitude, peak 4 h after intake (Tuiten et al., 2000). Numerous studies using various cognitive, emotional, and neural measures have reported effects after this 4 h delay (for a review, see Bos et al., 2012b). Therefore, the same 4 h delay was used in the present study. For detailed description on how the administration relates to the physiological testosterone levels in females and the generalizability of the data to males, see Bos et al. (2010b).

Testosterone saliva measurements

Salivary sampling was based on Granger et al. (2004), which has been successfully applied in several previous studies (e.g. Bos et al., 2010b, 2012a). Testosterone in saliva was measured after diethylether extraction using a competitive radio-immunoassay employing a polyclonal antitestosterone-antibody (Dr. Pratt AZG 3290). [1,2,6,7-3H]-Testosterone (TRK402, Amersham Netherland B.V.) was used as a tracer following chromatografic verification of its purity. The lower limit of detection was 10 pmol/l and inter-assay variation was 16.1, 11.5, and 5.1% at 21, 100, and 230 pmol/l respectively (n = 4, 5, and 5).

Procedure

All participants were invited to the laboratory twice with an interval of at least 1 week and procedures were identical on both days. Appointments were scheduled 4 h before the scan sessions. All participants were instructed not to eat within 1.5 h prior to the appointment and were allowed only to drink water. After arrival, participants filled out the STAI-questionnaire and provided the saliva sample. After this, they received the 0.5 ml drug sample (or placebo), and were instructed to keep it under the tongue for a full minute. In accordance with previous studies (Bos et al., 2012b), they were allowed to swallow after this minute. They then received instructions on the scanner procedure. After this, participants were free to leave the laboratory and were requested to return 3.5 h later. They were instructed to avoid physically or psychologically straining activities within this time. After verification of compliance and standard MRI safety screening, participants were positioned in a supine position on the scanner bed. Ear plugs were used to attenuate scanner noise, and foam pads were placed inside the head coil to restrict movement. Instructions and task images were back-projected onto a translucent screen positioned near the participants' feet, which was visible through an angled mirror mounted onto the head coil. The scanner room was dimly lit throughout data acquisition. The participants first performed the dynamic facial expression task (DFET), and afterward a monetary incentive delay task which is reported elsewhere (Hermans et al., 2010).

Dynamic facial expression task (DFET)

The DFET consisted of blocks of facial stimuli which dynamically and rapidly changed from neutral to full expressions. There were eight blocks of happy expressions, eight blocks of fearful expressions, and eight blocks of baseline fixation. Each block lasted 26.7 s. Blocks were presented in a mirrored design avoiding covariation of task regressors with linear drift, in the following order: XFHXFHXFHXFHHFXHFXHFXHFX (X = fixation; F = fearful; H = happy), with blocks of F and H switched in a counterbalanced fashion across participants. Participants were instructed to passively view the stimuli. To control for attention, they were instructed to press a button each time the fixation cross appeared. Stimuli were grayscale ovals containing the faces from chin to forehead of 8 actors taken from a standardized set of faces controlled for luminance and contrast (Ekman and Friesen, 1976). Morphing sequences were computer generated animations consisting of four frames (at 55%, 70%, 85%, and 100% of emotional expression) with a total duration of 133 ms (created using WinMorph 3.01). Animations of each actor were repeated seven times within each block (i.e., $7 \times 8 = 56$ animations per block, 476 ms SOA). Prior studies using dynamic emotional faces have demonstrated robust amygdala activation (van der Gaag et al., 2007; van Marle et al., 2009), also when compared to static faces (Sato et al., 2004).

Scanning parameters

All scans were obtained using a 3 Tesla Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) with a Quasar dual gradient set and an eight channel phased array head coil. Whole brain T2* weighted 3D EPI images were acquired with two-dimensional SENSitivity Encoding (SENSE; see Pruessmann et al., 1999) acceleration. The following

parameters were used: TE/TR: 23/34 ms, FA 12°, FOV (anterior–posterior, inferior–superior, left–right): 224 mm × 256 mm × 128 mm, voxel size: 4 mm isotropic, volume acquisition time: 784 ms. Volumes were acquired in sagittal orientation (coverage ear-to-ear), with frequency encoding in the foot-head direction. SENSE was applied in anterior–posterior and in left–right directions with acceleration factors $R = 2$ and $R = 1.8$, respectively (see Neggers et al., 2008). This fast and short TE sequence was designed specifically to minimize signal dropout and image distortion due to magnetic field inhomogeneity around air-tissue interfaces. 816 such images were acquired for each session of the dynamic face task. For each scan session, one additional (geometrically identical) image was acquired with a larger flip angle (27°), which yields increased T1 weighting and hence higher gray–white matter contrast. This image was used as an intermediate to improve registration between functional and anatomical images. A T1-weighted high resolution fast field echo structural scan was obtained once for each participant using the following parameters: TE/TR: 2.3/25 ms, flip angle 30°, FOV 256 mm × 150 mm × 204 mm, voxel size: 1 mm isotropic, volume acquisition time: 6 min 42 s.

fMRI data processing and analyses

MR scans were analyzed using SPM8 (www.fil.ion.ucl.ac.uk/spm; Wellcome Department of Imaging Neuroscience, London, UK). Motion correction was performed on all functional scans using six parameter rigid body transformation and sum of squared differences minimization. To facilitate registration of functional images with structural scans, we first registered the functional scans to the 27° flip angle scan. This scan was subsequently used to perform cross-modal registration (six parameter rigid body transformation with mutual information maximization) with the T1 weighted structural image. Subsequently, all images were normalized into standard (MNI152) space using affine transformations and non-linear deformations, and resampled into 4 mm isotropic voxels using fourth degree B-spline interpolation. Finally, all images were smoothed using an 8 mm FWHM Gaussian kernel to accommodate residual between subjects variance in (functional) anatomy.

Statistical analysis of fMRI data was performed within the general linear model framework using multi-session models (testosterone and placebo) for each participant. Both face conditions (happy vs. fearful faces) were modeled separately as a box-car regressor for each session. These regressors described the onset and duration of the blocks with morphing faces, and were subsequently convolved with the canonical hemodynamic response function included in SPM8. High pass filter cut-off was set at 1/128 Hz. After estimating these models, parameter estimate maps for both regressors of interest (representing fearful and happy blocks) in the two sessions (testosterone and placebo) were subjected to second level random effects analyses using a factorial ANOVA involving emotion (fear versus happy) and drug (testosterone versus placebo) as within-subject factors. A covariate indicating order of administration was included initially as a between-subjects variable, but was removed from the model because it did not explain additional variance. Alpha was set at $P < 0.05$, corrected using random field theory based family wise error (FWE) corrections as implemented in SPM8. For our main region of interest (amygdala), a small volume correction (SVC) was used that was based on an anatomical mask of this region derived from the AAL template (Tzourio-Mazoyer et al., 2002). Outside of this ROI, only statistical differences exceeding a whole-brain FWE correction were considered significant. Statistical parametric maps resulting from these analyses were superimposed (using MRIcron: http://www.sph.sc.edu/comd/rorden/mricron/) onto a high resolution T1-weighted image of a single individual transformed into MNI152 space. For visualization purposes, thresholds for these images were set at $P < 0.001$, uncorrected. Inferential statistics are listed in Table 1.

Finally, we performed a post hoc analysis to give insight into the location of the testosterone-induced activation within subregions of the amygdala. Based on cytoarchitectonic probability maps, we made masks for the superficial amygdala (SFA), the basolateral amygdala (BLA), and the central-medial amygdala (CMA) (Amunts et al., 2005) by applying a 0.5 probability threshold. To avoid blurring of signal between these small subregions, we reran our first-level model estimation on data to which no smoothing was applied. We then extracted parameter estimates for each emotion, drug, hemisphere, and amygdala subregion, and averaged these values for each ROI. Resulting values were entered into a $3 \times 2 \times 2 \times 2$ repeated measures ANOVA with subregion (SFA, BLA, and CMA), hemisphere (left, right), emotion (fear, happy), and drug (testosterone, placebo) as within-subjects factors. A covariate indicating order of administration was again omitted because it did not explain additional variance. Additionally, post hoc repeated measures ANOVA's with the same factors (hemisphere, emotion, and drug) were performed in the three subregions separately to further specify the effects.

Finally, to see whether baseline testosterone affected our findings, testosterone values obtained from saliva (prior to administration) were entered as a covariate. Extracted data were analyzed using SPSS 20 with alpha set at 0.05.

Results

The contrast for the main effect of presentation of emotional faces (happy and fearful faces combined versus rest) across both sessions showed strong bilateral activation in a large part of the occipital lobe extending into the inferior temporal cortex, both involved in processing of visual information (see Table 1 and Fig. 1 left panel). The same contrast yielded bilateral activation in the amygdala, hippocampus, precentral gyrus, temporal pole, parts of the frontal cortex, and the triangular and orbital regions of the inferior frontal gyrus. Although there was strong activation for the main contrast of stimulus versus rest, no differential activation was found when comparing the two stimulus types. When looking at the effects of drug, a main effect of testosterone administration was observed in the right amygdala (right panel), which responded more strongly to the facial expressions after testosterone compared to placebo ($P < 0.05$; SVC). No other main effect or interaction of drug with stimulus type survived the threshold of $P < 0.05$, whole brain FWE-corrected, or SVC for the bilateral amygdala. Thus, no activation or deactivation was found other than reported above.

Table 1 Peak T values and corresponding MNI coordinates for significantly activated voxels.

Experimental effect		Peak voxel location			Peak *T*-values
Region		*x*	*y*	*z*	
Main effect: emotion > rest		41	−71	−7	
Lingual gyrus	R	−19	−95	5	18.09[a]
Middle occipital gyrus	L	−51	−7	53	15.41[a]
Precentral gyrus	L	53	−3	49	7.56[a]
	R	41	21	−35	7.04[a]
Temporal pole	R	−39	21	−35	6.90[a]
	L	−31	25	−23	5.51[a]
IFG, orbital part	L	37	37	−15	6.17[a]
	R	−15	17	65	5.58[a]
Superior frontal gyrus	L	45	17	25	6.14[a]
IFG, opercular part	R	17	−3	−19	5.97[a]
Amygdala	R	−19	−7	−19	5.69[a]
	L	−7	65	29	5.55[a]
Medial superior frontal cortex	L	−39	5	61	5.48[a]
Middle frontal cortex	L	−15	61	33	5.31[a]
Superior frontal cortex	L	−15	53	−11	5.29[a]
Superior orbitofrontal cortex	L	61	5	−19	5.29[a]
Middle temporal pole	R	−63	−11	33	5.27[a]
Postcentral gyrus	L	−55	25	9	5.23[a]
IFG, triangular part	L				5.17[a]
Main effect: testosterone > placebo		25	−7	−15	
Amygdala	R				4.16[b]

R, right; L, left; IFG, inferior frontal gyrus.
[a] Whole brain FWE corrected at $P < 0.05$.
[b] Small volume corrected at $P < 0.05$.

Additionally, we performed a post hoc analysis to investigate which subregions within the amygdala are most strongly affected by testosterone administration (see Fig. 2). Averaged parameter estimates extracted from unsmoothed data within each of the three anatomically defined subregions were entered into a repeated measures ANOVA. In agreement with statistical parametric mapping analyses reported above, this ANOVA yielded a main effect of drug ($F(2,10) = 4.55$, $P = 0.039$; $\eta^2 = 0.48$) and did not reveal any main effect or interaction involving the factor emotion. However, the main effect of drug was qualified by a drug by subregion interaction ($F(4,8) = 4.10$, $P = 0.043$; $\eta^2 = 0.67$), indicating that the effect of testosterone differed between subregions. Further post hoc testing within the subregions (see Fig. 2) revealed that the testosterone effect was most prominent in the SFA ($F(2,10) = 8.57$, $P = 0.007$; $\eta^2 = 0.63$). In the BLA there was no significant effect of drug, although there was a trend toward stronger responses after testosterone ($F(2,10) = 2.95$, $P = 0.098$; $\eta^2 = 0.37$). In the CMA, no effect of drug was found ($F(2,10) = 0.31$, $P = 0.74$; $\eta^2 = 0.06$). A significant main effect or interaction involving hemisphere was found in neither of the three subregions, indicating that the effect of testosterone was not lateralized.

Notably, BOLD responses to presentation of faces across drug conditions also varied between subregions (main effect of subregion: $F(4,8) = 17.42$, $P = 0.001$; $\eta^2 = 0.90$), with significant responses only in the SFA ($F(2,10) = 15.2$, $P = 0.001$; $\eta^2 = 0.75$) and BLA ($F(2,10) = 25.47$, $P < 0.001$; $\eta^2 = 0.84$), but not in the CMA ($F < 1$, n.s.). Finally, we tested whether testosterone baselines correlated with amygdala activation, or with the effect of testosterone, but testosterone baseline did not explain any variance in the data (all *P*-values > 0.05).

Discussion

In this study we attempted to investigate the mechanism whereby testosterone reduces fear in humans, and tested the specific hypothesis that testosterone would reduce amygdala activation in response to a prototypical innate human danger cue: the fearful face. Contrary to the hypothesis, testosterone administration increased the amygdala response to both fearful and happy faces, of which the happy faces served as a control condition. Additional analyses showed that this effect of testosterone was most pronounced in the superficial amygdala (SFA), extended marginally into the basolateral amygdala (BLA), but did not encompass the central-medial amygdala (CMA). Although these findings do not support our hypothesis, they are consistent with an earlier study that showed a positive correlation between endogenous testosterone levels and amygdala activation in response to (static) fearful faces (Derntl et al., 2009). Moreover, they correspond with previous testosterone administration studies that have shown increased amygdala responses to emotional faces (Hermans et al., 2008; van Wingen et al., 2009).

In the current study, we could not replicate previous findings of a stronger amygdala response to fearful than to happy facial expressions (e.g. Whalen et al., 2001). Our finding of comparably strong amygdala activation to fearful and happy faces is in line with recent literature showing

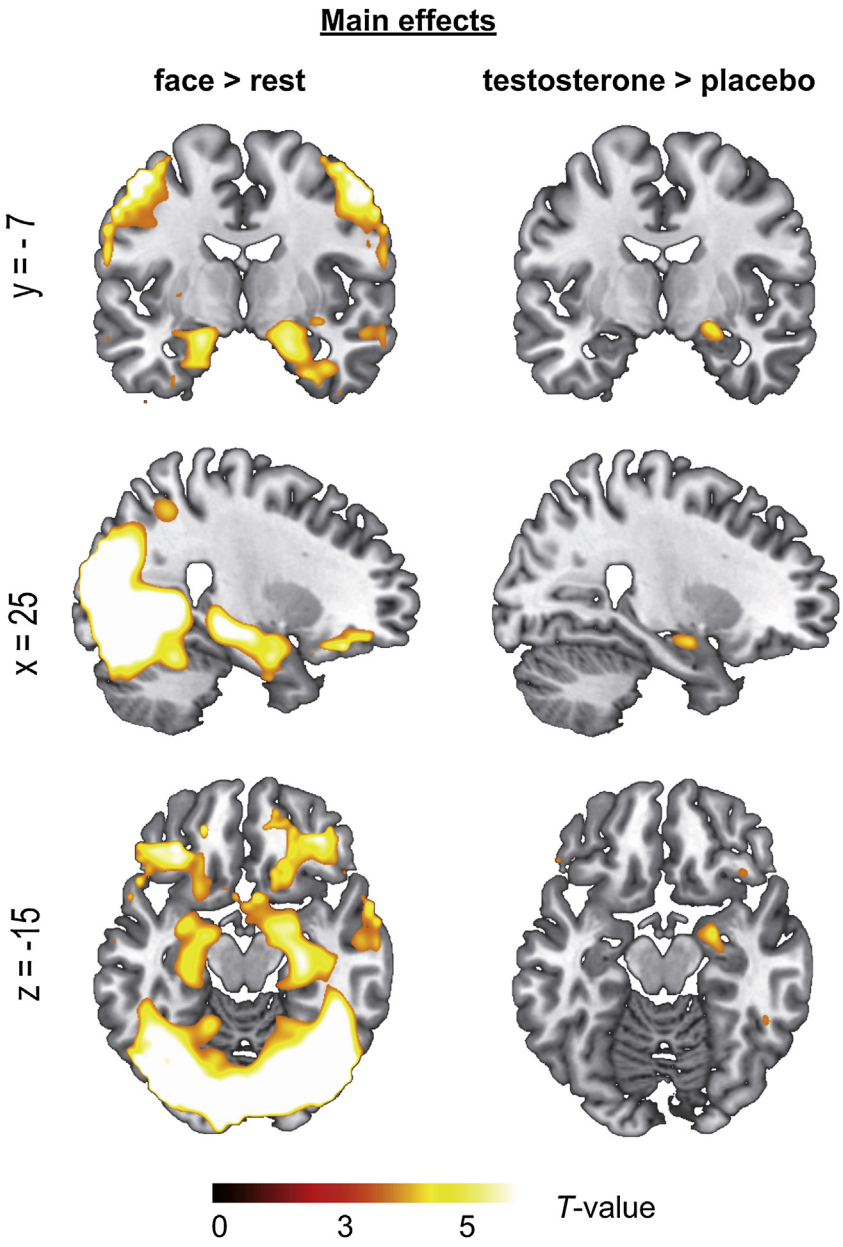

Figure 1 Coronal, sagittal, and axial slices with accompanying MNI coordinates of the *Y*-, *X*-, and *Z*-axis of the *t*-maps for the main effects of faces vs. rest and testosterone vs. placebo. The *t*-maps are thresholded at $P < 0.001$ uncorrected for illustration purposes and overlaid onto a T1-weighted canonical image.

non-specific amygdala responses to emotional faces (e.g. Sato et al., 2004; van der Gaag et al., 2007; Goossens et al., 2009). Such findings, together with those obtained in the present study, do not concur with the notion that the amygdala is a region that is specifically involved in fear processing. Instead, the emerging view is that the amygdala plays a more general role in detection and processing of salient information including facial expressions (Whalen, 1998; Davis and Whalen, 2001; Sander et al., 2003; Goossens et al., 2009).

Notably, our data also show that the amygdala response to facial expressions is mostly carried by the SFA and the BLA, but not the CMA. This finding corresponds with a number of previous studies showing that the SFA and BLA are especially sensitive to facial stimuli (Hoffman et al., 2007; Ball et al., 2009; Goossens et al., 2009). Moreover, effects of testosterone administration were also confined to these two subregions, although the potentiation of the BLA response was only significant at trend-level. Since the SFA and BLA are considered mostly as input regions, the increased activation of these subregions might reflect increased processing of socially relevant stimuli by testosterone. Studies in rats further show that the medial nucleus, which is adjacent to the SFA, is highly sensitive to androgens, and testosterone can structurally and functionally influence the medial nucleus (Cooke et al., 1999; Pitkänen et al., 2000; Johnson et al., 2012). In agreement, the human SFA is selectively enlarged in males, which might indicate sensitivity of this region to testosterone in humans as well (Kim et al., 2012). Furthermore, a functional neuroimaging study which inves-

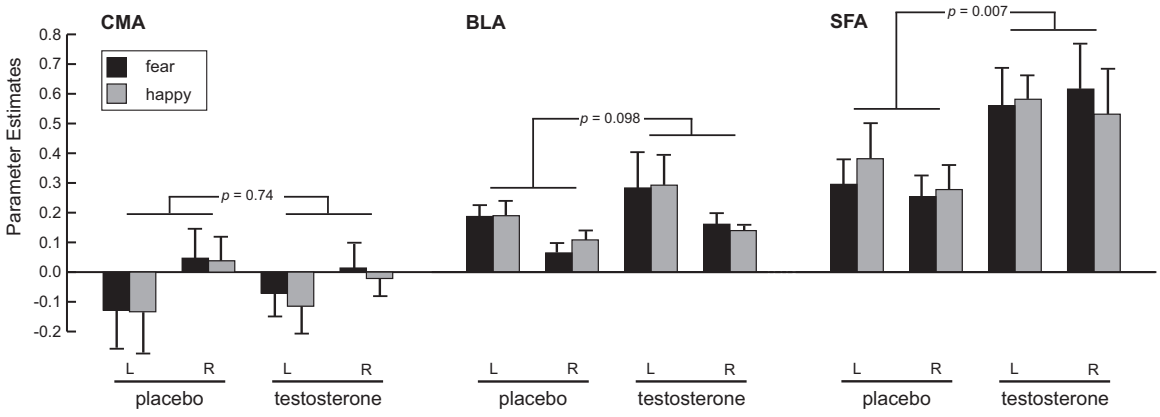

Figure 2 Parameter estimates extracted from the anatomically defined subregions of the bilateral amygdalae in the placebo and testosterone condition, for happy and fearful faces (L = left; R = right; CMA = central-medial amygdala; BLA = basolateral amygdala; SFA = superficial amygdala).

tigated the relation between testosterone levels and amygdala activation showed a positive correlation of testosterone baseline specifically with the dorsal part of the amygdala (Manuck et al., 2010). In this ventral/dorsal distinction of the amygdala, the dorsal part encompasses the CMA, the region where we did not find an effect of testosterone, but also parts of the SFA. In fact, the peak location for activation in the dorsal amygdala reported by Manuck et al. corresponds most strongly to the SFA in the cytoarchitectonic probability maps used in the present study (Amunts et al., 2005). It is therefore well possible that the effect reported by Manuck et al. is caused by testosterone acting on the SFA, which would correspond to our findings. Finally, also an indirect effect of estradiol, converted from testosterone, could be proposed, as these receptors in humans have been shown to be abundant in the amygdala, but not in the central nucleus (Österlund et al., 2000).

Overall, heightened sensitivity for socially relevant stimuli concurs with the challenge hypothesis, a framework which states that the function of testosterone is to deal with environmental challenges (Wingfield et al., 1990; Archer, 2006; Bos et al., 2012b). In such a challenge framework, testosterone facilitates any form of adaptive goal-directed behavior, such as increased sensitivity to socially relevant signals, more aggression in situations of conflict, and also a reduction of fear to immediate threat. Thus, activation of the SFA and BLA by testosterone may indicate more elaborate processing of social signals in the absence of a fear response.

The present data raise the question how testosterone, if not by reducing activation of the amygdala, reduces fear. One relevant aspect of testosterone with regard to this question was brought to light by the studies investigating the fear reducing effect of testosterone in humans. These studies showed that this hormone reduces phasic ''fear'' responses rather than tonic background ''anxiety'' (van Honk et al., 2005; Hermans et al., 2006). For instance, Hermans et al. (2006, 2007) showed that testosterone specifically reduces the cue-specific fear potentiation of the startle reflex, but does not affect baseline startle responses. This effect of testosterone is unlike that of anxiolytics such as benzodiazepines, which reduce baseline startle rather than fear potentiated startle (Baas et al., 2002; Grillon, 2002). Baseline startle is a measure that is proposed to relate to consciously experienced anxiety, which concurs with the common use of benzodiazepines in patients suffering from anxiety disorders. In contrast, fear potentiation of the startle reflex, which is targeted by testosterone, is a response that is triggered by a discrete threat cue. Moreover, in the study by van Honk et al. (2005) testosterone only reduced fear responses to fearful facial expressions presented below the threshold of conscious perception, while it did not affect conscious measures of anxiety. Together, these studies demonstrate that testosterone may reduce fear rather than anxiety, a distinction that might aid the interpretation of our current data.

A distinction between fear-related processes can also be found in the different subregions of the amygdala. As mentioned above, the SFA and BLA are mostly input regions for sensory information, while the CMA is an output region, projecting downstream to brainstem regions such as the PAG and hypothalamus (Pitkänen et al., 2000; Price, 2003). Thus, the CMA is most strongly involved in the generation of autonomic fear responses, while BLA responses are more related to fear learning (Davis and Whalen, 2001; Phelps and LeDoux, 2005). Also, the medial nucleus, which as described above is adjacent to the SFA and is sensitive to androgens, has in rodents been implicated in emotional stress responses, but not physical stress responses, while the CMA shows an opposite pattern (Ebner et al., 2004). Recent rodent data moreover show that the functions of the amygdala subregions are not only dissociated, but that the BLA is even capable of reducing CMA output (Macedo et al., 2007; Tye et al., 2011). Together, a selective effect of testosterone on the fear response and the distinct roles of amygdala subregions in fear processing imply that fear reduction induced by testosterone is consistent with increased activation of the SFA or BLA.

A limitation of the current study is that the paradigm used was specifically designed with dynamic emotional expressions to elicit robust amygdala activation. It is well possible that the strong amygdala activation obscured subtle differences between emotional expressions induced by testoster-

one administration that have been found with static faces in previous studies (Hermans et al., 2008). Also, the use of consciously perceived stimuli may have affected our data. Previous studies have often used stimuli presented outside of conscious awareness or focal attention to study neural responses related to fear and anxiety (Bishop et al., 2004; Whalen et al., 2004; Dickie and Armony, 2008). Since the testosterone-induced reduction of attention for fearful faces reported by van Honk et al. (2005) was specific to unconsciously presented faces, and since modulation of awareness can affect subregions of the amygdala differently (Lerner et al., 2012), a future study should combine testosterone administration with unconsciously presented faces to address this issue. Further suggestions would be to include a neutral condition, which is not feasible using dynamic facial stimuli, and a non-face condition to control for specificity to facial stimuli. Moreover, future research should assess autonomic nervous system measures of fear simultaneously with neural activation, for instance using threat of shock procedures (Hermans et al., 2006). Such a setup may give more direct insight into the question how increased responses in the SFA and perhaps BLA may relate to reductions in fear responses.

A final point of consideration is that this study was performed only in women, which raises the question whether these findings also apply to the other sex. Indeed, gender differences have been reported in mood disorders, with females being more prone to anxiety disorders (Piccinelli and Wilkinson, 2000). In agreement, numerous neuroimaging studies have reported sexually dimorphic responses during emotion processing (Cahill, 2006; Derntl et al., 2010). Females tend to have stronger amygdala responses that are more lateralized to the left hemisphere while males show the opposite pattern (Williams et al., 2005; Cahill, 2006; Schneider et al., 2011); but see Dickie and Armony (2008), and Etkin et al. (2004), for conflicting findings. Although in the current study there was no statistically significant difference between the testosterone effect in the subregions of the left and right amygdala, it appeared more pronounced on the right side of the brain, which is in line with previous testosterone administration studies (Bos et al., 2010a, 2012a; Hermans et al., 2010). Whether these findings relate to the reported sex differences remains a topic for further investigation, but based on correlational data in women we expect the effects of testosterone administration to be similar for males and females (van Honk et al., 1999, 2001). A more detailed discussion on generalizability, and the administration procedures in women can be found in Bos et al. (2010b, 2012b).

In this study, we tested the hypothesis that testosterone would decrease amygdala activation in response to fearful faces. In contradiction to this hypothesis, testosterone increased the amygdala response to both fearful and happy faces, suggesting that testosterone does not reduce fear by down regulating the amygdala response. Our finding that testosterone acted most prominently on input regions of the amygdala (i.e., SFA and to a lesser extent BLA) suggests that testosterone may promote preferential processing of salient stimuli in the context of social challenges. Finally, the literature on distinct roles of amygdala subregions in fear-related processes indicates that increased responses in SFA and BLA concur with fear-reducing properties of testosterone.

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Contributors

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Author JvH and EJH designed the study and wrote the protocol. Author PAB, NFR and EJH managed the literature searches and facilitated data analyses. Authors PAB and EJH undertook the statistical analysis, and authors PAB, JvH, DJS and EJH contributed to the writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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