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The neural mechanisms by which testosterone acts on interpersonal trust

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ABSTRACT

Recently, we demonstrated that the steroid-hormone testosterone reduces interpersonal trust in humans. The neural mechanism which underlies this effect is however unknown. It has been proposed that testosterone increases social vigilance via neuropeptide systems in the amygdala, augmenting communication between the amygdala and the brain stem. However, testosterone also affects connectivity between the orbitofrontal cortex (OFC) and the amygdala, which could subsequently lead to increased vigilance by reduced top-down control over the amygdala. Here, in a placebo-controlled testosterone administration study with 16 young women, we use functional magnetic resonance imaging to get more insights into neural mechanisms whereby testosterone acts on trust. Several cortical systems, among others the OFC, are involved in the evaluation of facial trustworthiness. Testosterone administration decreased functional connectivity between amygdala and the OFC during judgments of unfamiliar faces, and also increased amygdala responses specifically to the faces that were rated as untrustworthy. Finally, connectivity between the amygdala and the brain stem was not affected by testosterone administration. Although speculative, a neurobiological explanation for these findings is that in uncertain social situations, testosterone induces sustained decoupling between OFC and amygdala by a prefrontal-dopaminergic mechanism, subsequently resulting in more vigilant responses of the amygdala to signals of untrustworthiness.

Introduction

Humans are a unique species, in that they much more than others animals, cooperate with and trust genetically unrelated and unfamiliar others. This human behavioral variation is thought to be a social adaptation, which underlies our evolutionary success (de Waal, 2010; Hrdy, 2009). However, trusting unfamiliar others can be hazardous, as humans also stand out in their capacities for deceiving and cheating on their own kind. For that reason, the readiness to believe the words of others, or what others pretend, or the failure to grasp their hidden motives may come with substantial economic and social costs. In sum, interpersonal trust apparently holds highly rewarding and vastly punishing properties, thus adaptive trusting behavior requests a refined sense of balance of knowing who to trust and who to be suspicious of.

Interestingly, recent evidence from social neuroendocrinology suggests that two hormones are in humans in opposite ways involved in setting the delicate balance for adaptive interpersonal trust. A hormonal promoter of interpersonal trust is oxytocin. It has been shown that this peptide hormone increases trust in unfamiliar others measured by facial trustworthiness evaluations (Theodoridou et al., 2009), and also via monetary transfer to anonymous others in an economic game (Kosfeld et al., 2005). Although it must be noted that these effects of oxytocin might not apply to all individuals, as a recent study showed that oxytocin can contrariwise hinder trust in people with borderline personality disorder (Bartz et al., 2011a). This finding stresses the importance of taking into account individual differences when studying the effects of hormones on behavior (Bartz et al., 2011b). Oxytocin's antagonist in this social domain, and thus the inhibitor of interpersonal trust is the steroid hormone testosterone. Recently, we reported that, in the opposite direction to oxytocin, testosterone administration induces reductions in trustworthiness evaluations of unfamiliar others (Bos et al., 2010b).

Although the neural mechanism whereby testosterone reduces interpersonal trust is unknown, it is proposed that testosterone might do so by acting on peptide circuits in the central amygdala (CA) (Bos et al., 2010b; Johnson and Breedlove, 2010). The general idea regarding this mechanism is that testosterone acts on vasopressinergic neurons in the medial part of the central amygdala (Bos et al., 2011; Huber et al., 2005). The expression of vasopressin, a peptide phylogenetically and structurally related to oxytocin, in the central amygdala (CA)

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indeed is androgen dependent (de Vries, 2008; Hermans et al., 2008; Koolhaas et al., 1990). By way of this amygdalar neuropeptide mechanism, testosterone could increase amygdala output to brainstem regions such as the periaquaductal gray, and in that way increase social vigilance (Huber et al., 2005; Kirsch et al., 2005).

Notably, there are other pathways by which testosterone can influence social behavior, as human neuroimaging data show that endogenous testosterone attenuates prefrontal–amygdala coupling (Volman et al., 2011), and that exogenous testosterone can reduce connectivity between the amygdala and the OFC (van Wingen et al., 2010). Thus, by reduced top-down control of the OFC over the amygdala–brainstem circuit, testosterone might then lead to an increase in social vigilance (Barbas et al., 2003; Bos et al., 2010b; Porges, 2001; Van Honk et al., 2011). Increased social vigilance would subsequently lead to reduced trustworthiness, protecting an individual from loss of status or resources (Bos et al., 2010b; Eisenegger et al., 2011). By which neurobiological mechanism testosterone reduces prefrontal–amygdala coupling is unknown, but it could be by augmenting dopamine synthesis or release (Aubele and Kritzer, 2011; Hermans et al., 2010; Kritzer and Creutz, 2008). Up-regulation of dopamine function in the prefrontal cortex (Aubele and Kritzer, 2011; Kritzer and Creutz, 2008) does alter connectivity between the PFC and the amygdala (Blasi et al., 2009), providing for an alternative route by which the hormone might increase social vigilance.

Irrespective of the exact mechanism, imaging data does indeed suggest that the amygdala is involved in the detection of facial trustworthiness (Engell et al., 2007; Said et al., 2009; Todorov et al., 2008a, 2008b; Winston et al., 2002), and that patients with bilateral amygdala damage show higher facial trustworthiness evaluations than healthy controls (Adolphs et al., 1998). The OFC is considered also to be importantly involved in (more elaborate aspects of) social–emotional evaluation (Kringelbach and Rolls, 2004), and the OFC can by way of the amygdala rapidly modulate subcortical activation patterns (Davidson, 2002; Mobbs et al., 2007; Wager et al., 2008). Other brain regions involved in the processing of social threat are the insula and anterior cingulate cortex (ACC) (Craig, 2009; Kober et al., 2008; Rushworth et al., 2007), but especially the amygdala seems responsive to signals of untrustworthiness (Engell et al., 2007; Todorov et al., 2008b).

In sum, testosterone might act on interpersonal trust via neuropeptide systems in the amygdala (Baumgartner et al., 2008; Hermans et al., 2008) and directly increase output to the brainstem, or by decoupling the amygdala from the OFC (Koolhaas et al., 1990; van Wingen et al., 2010), and thereby preclude inhibitory action of the OFC on amygdala–brain stem circuits (Barbas et al., 2003; Davidson, 2002; Mobbs et al., 2007). To get more insights into these issues, we use functional magnetic resonance imaging (fMRI) in a placebo-controlled, within-subject design. 0.5 mg of testosterone was sublingually administrated to 16 healthy young women (Bos et al., 2010b, 2011), who performed a validated trust task (Winston et al., 2002) during both the placebo and testosterone session. The participants were shown two matched sets of unfamiliar faces, one set of faces was judged on trustworthiness, while the other set was judged on age and functioned as control task.

Materials and methods

Subjects

16 right-handed healthy young women participated in the study; mean age 20.8 (SD 2.0). All participants were students recruited at the university campus of Utrecht University. We controlled for menstrual cycle-related hormonal fluctuations by including only women who used single phase oral contraceptives and did not perform scans during days of menstruation. Only women were included to maintain comparability with our previous study (Bos et al., 2010b) and because medical ethical approval is restricted to females (for further information see: Substance administration). Participants had no history of psychiatric, neurological, or endocrine abnormalities. Participants did not smoke and used no medication other than oral contraceptives. The experimental protocol was approved by the Ethics Committee of the University Medical Centre Utrecht and was in accordance with the declaration of Helsinki. Participants gave written informed consent prior to participation and received payment afterwards.

Substance administration

The testosterone sample consisted of 0.5 mg of testosterone, 5 mg of the carrier cyclodextrine, 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 bounding 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. Dose dependent administration of testosterone indeed shows that the increase of free fraction by testosterone depends on the SHBG level in women (van Rooij et al., 2011). The behavioral and physiological effects of the administration, as demonstrated by enhanced vaginal pulse amplitude, peaks 4 h after intake (Tuiten et al., 2000). Several studies using diverse emotional and cognitive measures have successfully replicated behavioral effects after this 4 h delay (for a review, see Bos et al., 2011). Therefore, the same 4 h delay was used in the present study. For detailed description on the pharmacodynamics of the testosterone administration in females and the generalizability of the data to males, see Bos et al. (2010b).

Testosterone saliva measurement/2D:4D ratio

Baseline testosterone levels were obtained since these have been shown to correlate with amygdala responses to faces (Derntl et al., 2009; Manuck et al., 2010) as well as with amygdala–prefrontal connectivity (Volman et al., 2011). Endogenous testosterone levels were obtained using saliva sampling according to Granger et al. (2004), which has been successfully applied in several previous studies (Bos et al., 2010b; Eisenegger et al., 2010; Hermans et al., 2008). 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 Nederland B.V.) was used as a tracer following chromatographic 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 ($n = 4,5,5$).

The ratio of the length of the second digit divided by the length of the fourth digit (2D:4D) is a marker for prenatal androgens in humans (Breedlove, 2010), and has recently been shown to predict the effect of testosterone administration on cognitive empathy (van Honk et al., 2011). 2D:4D ratio was measured from an image scan of the right-hand of the subjects, which is a valid method to measure finger lengths. Lengths of the second and fourth digits were measured from the ventral proximal crease of the digit to the fingertip using an Adobe® Photoshop tool (Breedlove, 2010).

Procedure

Participants were scanned at the same time of day on two separate days with an interval of at least a week. Before administration participants

were tested for pregnancy using a standard clinical urine HCG detection test, and were screened on a questionnaire for alcohol and drug use. Afterwards, the fMRI procedure was explained to the participants who were given the opportunity to ask questions. 4 h prior to fMRI data acquisition participants received testosterone or placebo administration due to the delay in physiological effects as explained above. After administration participants were instructed to refrain from physically and psychologically intensive tasks. Before asked to take place in the MRI scanner, participants filled out a shortened version of the profile-of-mood-states (POMS) questionnaire to obtain an index of their mood (Shacham, 1983). Afterwards, participants were screened using a MRI-checklist and a metal detector, and were instructed to position themselves on the scanner bed as comfortable as possible and to try to relax. Head movement was minimized by foam pads which were placed between the RF-coil and participants' head. Further instructions during the scan session were given by intercom. After the second session, participants were debriefed and given payment.

Experimental design

The experimental setup of this study follows a randomized, counterbalanced, cross-over, placebo-controlled, testosterone administration paradigm. The trustworthiness task was adapted from Winston et al. (2002), and consisted of two separate runs in both of which participants were shown 60 different unfamiliar facial pictures. During one of the two runs, participants had to indicate by pressing a button whether a face was trustworthy or untrustworthy. During the control run subjects had to indicate whether a face was younger or older than 30 years old. Of the 120 stimuli in the experiment, 75 were taken from a set used by Adolphs et al. (1998), and 45 were taken from the Psychological Image Collection at Stirling (PICS: http://pics.psych.stir.ac.uk/) based on trustworthiness ratings in a previous experiment (Baas et al., 2008). In the supplemental material consistency ratings obtained in the current study of the individual faces are provided. The stimuli were presented using Presentation software (Neurobehavioral systems, Albany, CA). The facial pictures where shown on a gray background and for 1 s and followed by an intertrial interval of 2 s consisting out of a gray background with a black fixation cross in the middle of the screen. Participants were asked to press the button indicating their judgment after the face had disappeared. The task also contained 60 null events of 3 s which were identical to the intertrial intervals. The stimuli and null events were presented in random order. The trust task and control task were thus identical, only differing in the instruction given to the participant.

Data acquisition

Scanning was performed on a 3 T Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands). Blood oxygen level dependent (BOLD-) response was measured with functional T2*-weighted sagittal whole-brain images obtained throughout the task. A 3D PRESTO sequence (Neggers et al., 2008; Ramsey et al., 1998) was used with the following parameters: 23 ms echo time, 16 ms repetition time; 224×224×136 mm field of view; flip angle of 9°. 2 runs of 450 scans with a volume acquisition time of 0.813 s were obtained, each comprising 39 sagittal slices; voxelsize 3.5 mm isotropic. Combining 3D scanning with echo shifting and SENSE (sensitivity encoding) in two directions allows very fast whole-brain scanning to increase the number of observations, yielding improved contrast-to-noise (Neggers et al., 2008). The scan sequence was constructed specifically to reduce signal loss in regions susceptible for artifacts (e.g. ventral prefrontal areas, amygdalae), and for that purpose achieved a short echo time (reducing signal loss), short echotrains (reducing image distortion) and short volume acquisition times (reducing within-volume acquisition motion artifacts). For this scan, optimal use was made of SENSE (Pruessmann et al., 1999) and 3D segmented K-space acquisition (van Gelderen et al., 1995).

Subsequently a high resolution T1-weighted anatomical scan with the following parameters was acquired for co-registration and normalization purposes: 4.7 ms echo time, 9.5 ms repetition time, 240×221×160 mm field of view, 266 sagittal slices, flip angle of 8.0°, voxelsize 0.6 mm isotropic.

Data analyses

Preprocessing and subsequent analyses were performed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Functional scans of the two runs in both sessions were motion corrected to the first dynamic scan by using a least squares approach and six parameter rigid body spatial transformations. Functional volumes were then coregistered to the anatomical scan by the same six parameter rigid body transformations based on maximization of mutual information. Brain extraction was performed on the individual anatomical scans within SPM5 by segmenting gray and white matter and applying the gray and white matter maps as a mask for the anatomical scans. Extracted brains were used in further preprocessing steps leading to better fit during coregistration. Subsequently, functional volumes were normalized to a standard brain template (MNI) using 12 parameter affine transformations and subsequent non-linear deformations and were resliced at 2 mm isotropic voxelsize. Smoothing with an 8 mm full width at half maximum Gaussian kernel was applied to the normalized functional volumes.

A general linear model (GLM) was applied to both runs of the two sessions to investigate the effects of trustworthiness assessment compared to age assessment, and the interaction with drug administration. For both sessions, neural responses to the presentation of the stimuli in the trust run and the control run are modeled using 1 s boxcar function convolved with a hemodynamic response function (hrf) as implemented in the SPM5 software. A separate GLM was run on the trust assessment runs in both sessions for the effect of the trustworthiness judgment (untrustworthy vs. trustworthy) and the interaction with drug. In this GLM the control runs were left out and two regressors were entered into the analysis, one for the stimuli judged untrustworthy, and one for the stimuli judged as trustworthy. In both models the realignment parameters and a discrete cosine transform high pass filter with a cut-off of 128 s were entered into the analyses to reduce unexplained variance.

For a first group analysis on the neural correlates of trustworthiness judgments, contrast maps of stimulus vs. baseline from all four runs were entered in a factorial 2×2 ANOVA with drug administration (testosterone or placebo) and task (trust or control) as separate factors. For the analysis on the effect of testosterone on trust assessment, the control task was omitted from the analysis, and only the trust-runs were entered in a factorial 2×2 ANOVA with drug administration and trust judgment (trustworthy or untrustworthy) as separate factors. To increase sensitivity, but also regarding our specific hypothesis for the amygdala, for analysis of drug effects on this region, percent signal changes within both amygdalae were extracted from the data based on the automated anatomical labeling (AAL) template (Tzourio-Mazoyer et al., 2002) using MarsBaR (Brett et al., 2002). The output values from MarsBaR of both left and right amygdala were entered in a multivariate factorial 2×2 ANOVA with drug administration and trust judgment as separate factors. Finally, a connectivity analysis was performed using an anatomically defined bilateral amygdala as a seed region (Fig. 4B). The time course of activation in the bilateral amygdala, which was adjusted for the general effects of the task, was extracted from the data, and an interaction term for this time course was calculated with the presentation of a face. Both time course and psychophysiological interaction (PPI) term were entered in the regression model. Subsequently, contrast

maps of the testosterone and placebo sessions were entered in an ANOVA with drug as a within-subject factor.

The statistical threshold for all the calculated linear contrasts was set at $P<0.05$ Family Wise Error (FWE) corrected. Also, based on previous imaging studies investigating the effect of testosterone on emotion processing (Bos et al., 2010a; Hermans et al., 2008; van Wingen et al., 2010), some anatomical regions of interest were defined, including the inferior OFC, the anterior cingulate cortex, the insula, and the thalamus. For correct statistical thresholding, small volume corrections were applied to bilateral anatomically defined regions based on the AAL template (Tzourio-Mazoyer et al., 2002). Additionally, due to the large extent to which the OFC refers, small volume corrections within the OFC were based on the location of peak *t*-values for neural responses towards faces as reported in a previous study investigating the effects of testosterone by Hermans et al. (2008).

Results

Imaging data

The first analysis, which addressed the neural correlates of trustworthiness judgments in general, showed an effect for the contrast of trustworthiness judgment versus age judgment in the bilateral inferior OFC (whole brain FWE corrected $P<0.05$) and the dorsal anterior cingulate cortex (ACC) (SVC at $P<0.05$) (Table 1). The pattern of activation in the inferior OFC encompassed a large part of Brodmann area 47 (Fig. 1) and extended to the left superior temporal pole and anterior insula. No effect of drug administration was found for any contrast in this analysis. Next we focused on the differences in brain activation between faces judged as trustworthy versus untrustworthy, rated on an individual basis by the participants during the trust tasks in both sessions, omitting the control task. Faces rated as untrustworthy, as compared to those rated as trustworthy, showed stronger activation in the left inferior OFC, anterior insula, temporal pole, and in the dorsal part of the left ACC (all ROI small volume corrected at $P<0.05$; Fig. 1). Whole brain analyses revealed no regions that showed an interaction between drug and trustworthiness. Because of our a priori prediction about the amygdala, we performed a more sensitive test for this region by extracting and averaging all data from the bilateral amygdala based on an anatomical mask. The crucial analysis on the effects of testosterone administration on the amygdala showed a significant interaction between testosterone administration and trustworthiness judgment in the left and right amygdala ($F(2,14)=4.15$, $P<0.05$; Fig. 2). This effect was driven by an interaction between testosterone administration and trustworthiness judgment in the right amygdala ($F(1,15)=7.25$, $P<0.05$), but not the left amygdala ($F(1,15)=1.11$, $P=0.31$). A direct *T*-test within the right amygdala showed enhanced responsiveness in the testosterone condition, specifically for faces that were judged untrustworthy compared to trustworthy ($t(15)=3.24$, $P<0.01$; Fig. 2), while no such difference was observed in the placebo condition ($t(15)=0.27$, $P=0.79$). Subsequently, we tested the hypotheses that testosterone would reduce functional connectivity between the amygdala and the OFC, and increase connectivity with the brainstem. To this end, we examined correlations of activity between the anatomical bilateral amygdala and the OFC and brainstem during the trust task. There was no effect of testosterone on the time course of activation during the whole fMRI trust task, but we did find a psychophysiological interaction with the presentation of the faces. There was a significant reduction of functional connectivity of the bilateral amygdala with the left OFC (peak voxel: −24, 58, −2; $t=3.49$) in the testosterone condition compared to placebo (Fig. 3B). We did however not observe any change in connectivity with the brainstem. Importantly, this amygdala-OFC decoupling by testosterone occurred in response to all unfamiliar faces, irrespective of whether the face was seen as trustworthy or not.

Table 1
Z values for significantly activated voxels, corresponding MNI coordinates, and cluster sizes.

Experimental effect	Peak voxel location			CS	Local maximum (Z)
Region	*x*	*y*	*z*		
Main effect: Trust > age					
L frontoinsular cortex	−40	22	−18	64	5.04[a]
R orbital part inferior frontal gyrus	50	26	−10	8	4.89[a]
L anterior cingulate cortex	−2	−18	34	2	3.86[b]
Main effect: Untrustw. > trustw.					
L anterior cingulate cortex	−6	−12	34	3	3.85[b]
L anterior insula	−34	22	−6	13	3.83[b]
L orbital part inferior frontal gyrus	−36	22	−6	14	3.76[b]
	−28	22	−26	3	3.61[b]
L superior temporal pole	−40	18	−16	7	3.59[b]

CS, cluster size in voxels; R, right, L, left.
[a] Whole brain FWE corrected at $p<0.05$.
[b] Small volume corrected at $p<0.05$.

Behavioral data

As expected, we found no effect of testosterone administration on any of the behavioral measures in the current task. The comparison of the number of faces judged trustworthy in the placebo compared to the testosterone condition showed not significant ($t(1,15)=0.41$, $P=0.68$). In our previous experiment (Bos et al., 2010b), which was specifically designed to detect subtle hormone-induced effects on social cognition, trustworthiness was assessed on a scale ranging from −100 (very trustworthy) to +100 (very untrustworthy). In the current experiment, participants could only choose between the judgment 'trustworthy' or 'untrustworthy' (see supplemental material for the trustworthiness scores for all participants in both conditions). Our trustworthiness fMRI task, as anticipated, is much less sensitive in measuring behavioral changes in social cognition, but is properly adapted for detecting neural modulations during these trust judgments (Winston et al., 2002). With regard to the control task, testosterone did not have any effect on age ratings, neither were there effects on reaction times in any task.

Finally, testosterone did not affect participants' mood, as there was no effect on any of the POMS scales (see Materials and methods). Also, participants' guesses on which day they had testosterone did not deviate from chance regarding the true day of testosterone administration (binominal = 0.18 NS, two-tailed).

Testosterone baseline data/2D:4D ratio

The raw testosterone baseline ratings were positively skewed (skewness = 2.24; $P<0.05$), and therefore log-transformed. The participants' baseline testosterone levels in the placebo condition were unrelated to the trust judgments or reaction times during the placebo scan session (both $P>0.38$). This concurs with our previous finding, where we did not show this same relation using a much more sensitive measure for trustworthiness (Bos et al., 2010b). However, baseline testosterone did predict the response towards untrustworthy faces in the right amygdala in the placebo condition, indicated by a strong positive correlation ($r=0.66$, $P<0.01$; Fig. 4). In the left amygdala no such correlation was found ($r=0.22$, $P=0.41$), and the effect was thus confined to the amygdala, which also shows the strongest effect of testosterone administration. Furthermore, baseline testosterone did not predict the effect of testosterone administration on the amygdala, or interact with the drug administration (all *P*-values > 0.5). Lastly, testosterone-baseline did not change the effect of testosterone on amygdala connectivity when entered as a covariate in the ANOVA. We also investigated whether the 2D:4D ratio predicted amygdala activation, or the effect of testosterone on the

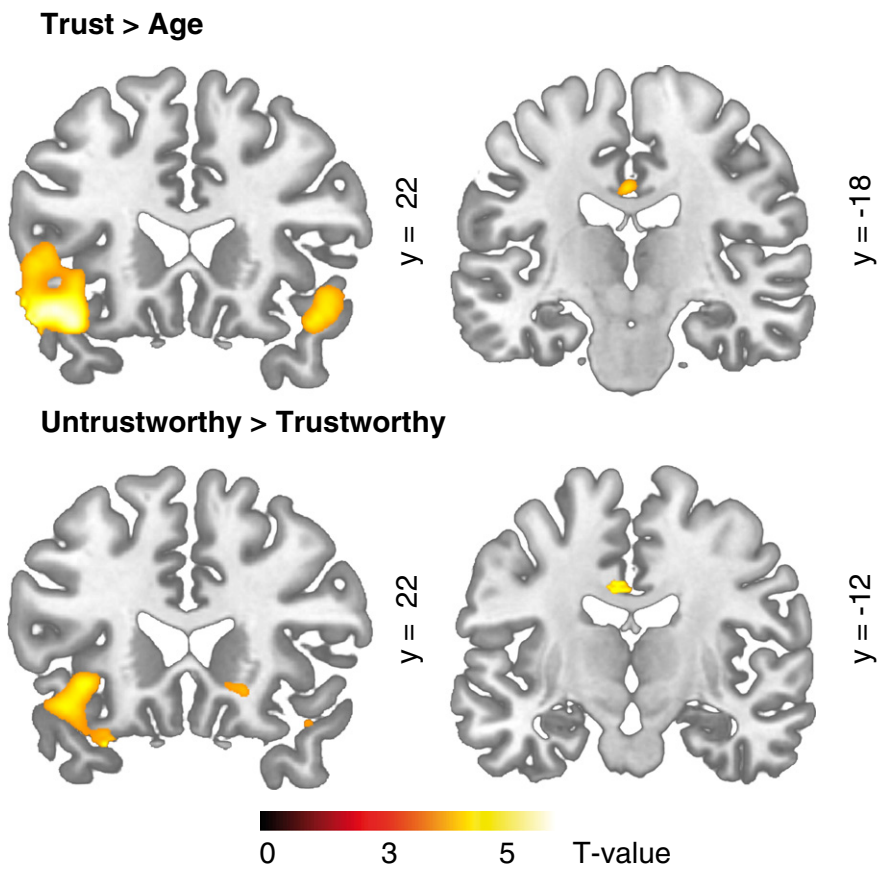

Fig. 1. Coronal slices with accompanying MNI coordinates of the Y-axis of the *t*-maps for the main effects of trust vs. control task and untrustworthy vs. trustworthy faces. On the left side the activation is shown in the inferior OFC, extending to the anterior insula and temporal pole. On the right activation in the dorsal ACC is shown for both contrasts. The *t*-maps are thresholded at $P<0.001$ uncorrected for illustration purposes and overlaid onto a T1-weighted canonical image.

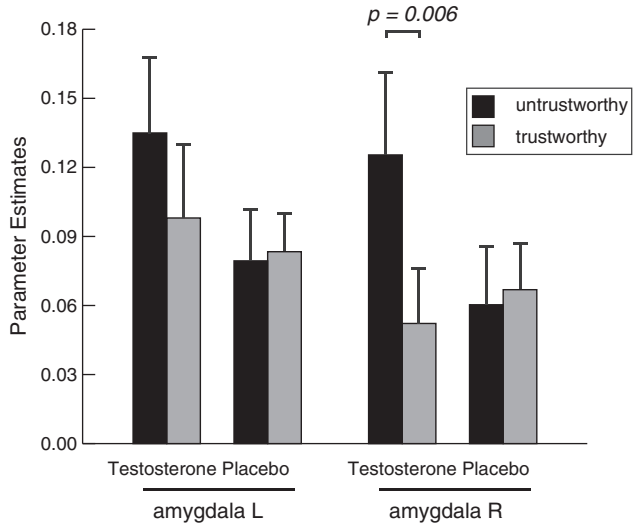

Fig. 2. Extracted parameter estimates are shown for both amygdalae in the placebo and testosterone condition for faces judged trustworthy and untrustworthy. There is a significant interaction in both amygdalae between testosterone administration and trustworthiness judgment ($F(2,14)=4.15$, $P<0.05$), which is most pronounced in the right amygdala ($F(1,15)=7.25$, $P<0.05$). A direct comparative *T*-test within the right amygdala showed enhanced responsiveness in the testosterone condition, specifically for faces that were judged untrustworthy compared to trustworthy ($t(15)=3.24$, $P<0.01$).

amygdala, but no such effect was found. Neither did the 2D:4D ratio predict the effect of testosterone on amygdala connectivity, nor the behavioral scores.

Discussion

Our data demonstrate that a network of brain regions including the bilateral OFC, temporal pole, anterior insula, and the left dorsal ACC, is implicated in the assessment of the trustworthiness of unfamiliar faces, and that this network is more strongly activated in response to untrustworthy faces. Importantly, as hypothesized, testosterone leads to increased amygdala responses during trustworthiness evaluations. Baseline testosterone levels correlate with amygdala responses towards untrustworthy faces, and administration of testosterone heightens responsiveness of the amygdala to untrustworthy faces, effects defensibly pointing at heightened social vigilance. Furthermore, expected reductions in functional connectivity between the amygdala with the OFC during trust judgments were also observed, but we did not see any changes in the connectivity between the amygdala and the brainstem (Bos et al., 2010b; Johnson and Breedlove, 2010)

Testosterone reduced amygdala connectivity with the OFC during all facial (social) evaluations, but activated the amygdala specifically to the faces that were evaluated as untrustworthy. These findings strongly suggest that the more general amygdala-OFC decoupling was primary, and underlying to the heightened amygdala activation in response to untrustworthy faces. That is, by reducing access of, and therefore modulation by the OFC (Barbas et al., 2003; Davidson, 2002; Wager et al., 2008), testosterone prevents inhibitory action of the OFC on social vigilance, which subsequently results in increased

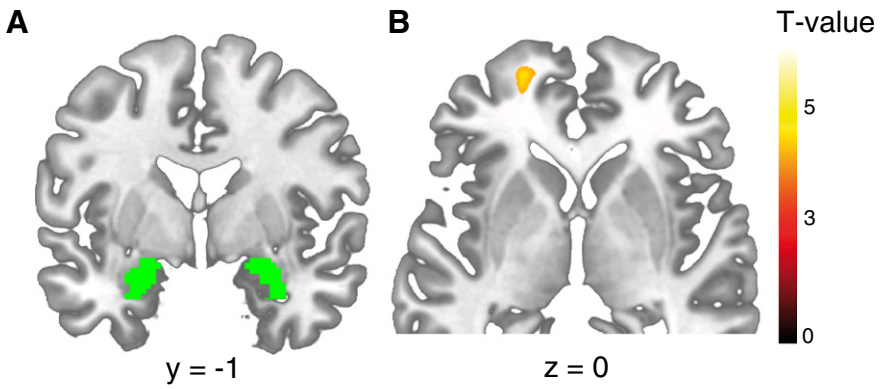

Fig. 3. (A) Coronal image of the mask for the bilateral amygdala which was chosen as a seed region in the connectivity analysis. (B) Frontal part of the axial slice of the prefrontal cortex which shows the region of the OFC in which connectivity to the bilateral amygdala is significantly reduced after testosterone compared to placebo. The *t*-map which overlays a T1-weighted canonical image is thresholded at $P<0.001$ uncorrected for illustration purposes.

amygdala responsiveness to untrustworthy faces (Fig. 5). This functional mechanism concurs with findings from previous studies investigating the effect of testosterone administration on amygdala function, which show increased amygdala responses to angry facial expressions (Hermans et al., 2008; van Wingen et al., 2009), and reduced functional connectivity between the amygdala and the OFC in response to angry and fearful faces (van Wingen et al., 2010). These effects of testosterone on the amygdala further show that the extent of this modulation is not limited to threat detection. Testosterone is also involved in aggressive and sexual behavior, behavioral repertoires which are both importantly subserved by the amygdala (Hamann et al., 2004; Hermans et al., 2008). It remains to be investigated if a common neural circuitry underlies these behaviors, or whether different sub-nuclei of the amygdala are perhaps involved (Davis and Whalen, 2001).

An important question is by what neurobiological mechanisms our effects on OFC-amygdala connectivity and amygdala activation might be established. A parsimonious explanation of these effects is that in uncertain social conditions, when meeting strangers (unfamiliar faces) testosterone decreases the functional connectivity between OFC and amygdala, which results in increased amygdala activation to the strangers looking most untrustworthy, because of reduced inhibitory control by the OFC on the amygdala (Barbas et al., 2003; Davidson, 2002; Mobbs et al., 2007). Although the currently applied methods only allow speculation on the neurobiological mechanisms, it could be that testosterone affected connectivity between the OFC and the amygdala by increasing dopamine action in the OFC (Aubele and Kritzer, 2011; Blasi et al., 2009; Kritzer and Creutz, 2008). Testosterone also acts on vasopressinergic mechanisms in the amygdala, but until now, there is no direct evidence that vasopressin affects amygdala-OFC coupling; two recent neuroimaging studies investigating the effects of vasopressin administration on emotion processing did not find connectivity-changes between the frontal cortex and the amygdala (Rilling et al., 2011; Zink et al., 2010). Based on these findings, the amygdalar neuropeptide mechanism is perhaps not the most plausible neurobiological mechanisms underlying our effects of testosterone on interpersonal trust (Bos et al., 2010b; Johnson and Breedlove, 2010). However, there are several other pathways by which testosterone could also exert effects on the brain. The hormone acts on androgen receptors, which are densely distributed in the amygdala, but also located in the prefrontal cortex (Clark et al., 1988), and testosterone, more rapidly can induce a diverse array of non-genomic effects even within seconds (Michels and Hoppe, 2008). Furthermore, by fast local metabolism through aromatase, administration of testosterone may increase levels of estradiol in the brain (Biegon et al., 2010; Cornil et al., 2006). Estradiol can induce a host of neural and behavioral effects by acting on estrogen receptors, and also via fast non-genomic mechanisms (Cornil et al., 2006; McEwen and Alves, 1999). It must be noted that

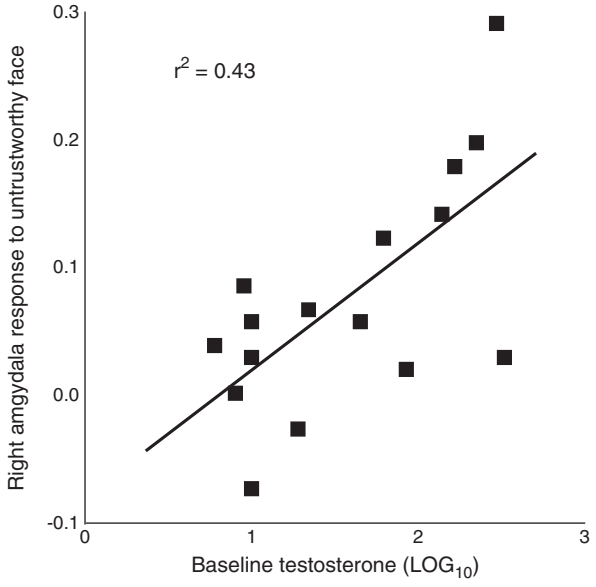

Fig. 4. Correlation between baseline testosterone levels and averaged values in the anatomically defined right amygdala towards untrustworthy faces, both in the placebo condition, showing that higher levels of testosterone lead to more amygdala reactivity towards untrustworthy faces ($r = 0.66$, $P<0.01$).

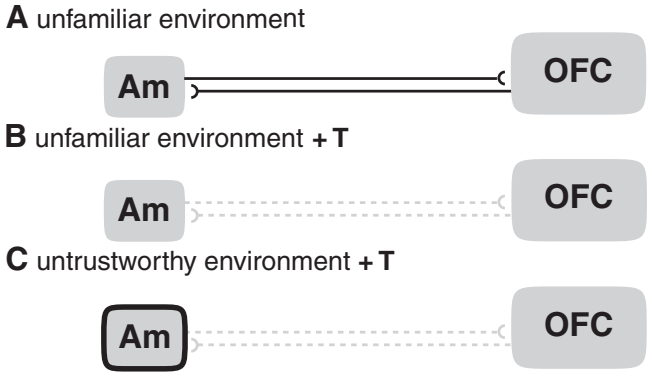

Fig. 5. Neural model for the effect of testosterone on interpersonal trust. In an unfamiliar environment (A), administration of testosterone reduces amygdala-OFC coupling, possibly by increasing dopamine release (B). Less coupling removes inhibitory control of the OFC over the amygdala subsequently leading to increased amygdala responses in a untrustworthy environment (C). (Am = amygdala; OFC = orbitofrontal cortex; T = testosterone).

fMRI data cannot uncover such neurobiological mechanisms, hopefully in future research other methods can and will be employed to investigate these issues.

Notably, we did not find a main effect on the amygdala of untrustworthy versus trustworthy faces, while this contrast in a previous study did yield activation of the amygdala (Winston et al., 2002). However, in subsequent investigations, researchers have shown that robust amygdala activation towards untrustworthy faces is difficult to obtain, and have therefore developed more sensitive designs to elucidate amygdala activation during trustworthiness judgments (Engell et al., 2007; Said et al., 2009; Todorov et al., 2008a). Furthermore, testosterone at present did not increase connectivity between the amygdala and the brainstem. Although testosterone's action, that is, the decoupling of the amygdala–OFC circuit, forecasts vigilance on the brainstem level (Barbas et al., 2003), the specific stimuli used in the current study, might also not be threatening enough to elicit robust brainstem responses (Mobbs et al., 2007; Van Honk et al., 2011).

Overall, the network of regions that was activated during trust judgments as compared to the control task, consists of regions that all have an important role in evaluating emotional information, and shows strong overlap with the neural network important for affective evaluation found in large meta-analyses of imaging data (Kober et al., 2008). The same regions are also part of the network importantly involved in detection of salient information, which is composed of the orbital frontoinsular cortex, and the dorsal ACC (Seeley et al., 2007). More specifically, the strong activation of the lateral OFC fits with the proposed role for this region in evaluating the punishment or reward value of stimuli (Kringelbach and Rolls, 2004). The ACC is also importantly involved in evaluation of social information, and based on its connections with motor-related regions, the ACC has been proposed to be more involved in adjusting behavior in response to this information (Rushworth et al., 2007). While the OFC and ACC are thus important for selection of appropriate responses towards socially relevant stimuli, together with the anterior insula, these regions form a network more generally involved in detection of emotionally relevant information (Seeley et al., 2007). Within this network, the anterior insula might play a more affective role, as this region has been proposed to integrate internal bodily states and motivational components with information from the environment (Craig, 2009), thereby reflecting subjective 'gut' feeling, based on which social and emotional decisions can be made. The pattern of activation during the trust judgments and during untrustworthy faces also encompassed the temporal pole. Although this region has not gained much attention in emotion research, it is suggested to be an important part of emotion-processing networks, since it has tight anatomical connection with the OFC and the amygdala, and is involved in face recognition, and complex emotional processing such as mind-reading (Olson et al., 2007). Together, the activation of these regions in the current task, confirms their important role in the evaluation of, and acting upon, relevant social–emotional information.

In conclusion, we show that testosterone administration reduces the functional coupling between the OFC and the amygdala during evaluation of unfamiliar faces. Furthermore, testosterone administration also resulted in an increased amygdala response selectively during evaluation of untrustworthy faces, and concurring correlational data in the latter respect were obtained with endogenous testosterone levels. We proposed that in social conditions, testosterone initially induces sustained amygdala–OFC decoupling, provisionally via prefrontal-dopaminergic mechanisms, which subsequently, due to breakdown of OFC-inhibitory mechanisms, produces heighted responses of the very amygdala to signals of untrustworthiness (Fig. 5) (Barbas et al., 2003; Davidson, 2002; Wager et al., 2008). The findings presented here identify a potential neural mechanism by which testosterone may reduce interpersonal trust (Bos et al., 2010b).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.neuroimage.2012.04.002.

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