

# Endogenous Testosterone Modulates Prefrontal–Amygdala Connectivity during Social Emotional Behavior

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**It is clear that the steroid hormone testosterone plays an important role in the regulation of social emotional behavior, but it remains unknown which neural circuits mediate these hormonal influences in humans. We investigated the modulatory effects of endogenous testosterone on the control of social emotional behavior by applying functional magnetic resonance imaging while healthy male participants performed a social approach–avoidance task. This task operationalized social emotional behavior by having participants approach and avoid emotional faces by pulling and pushing a joystick, respectively. Affect-congruent trials mapped the automatic tendency to approach happy faces and avoid angry faces. Affect-incongruent trials required participants to override those automatic action tendencies and select the opposite response (approach-angry, avoid-happy). The social emotional control required by affect-incongruent responses resulted in longer reaction times (RTs) and increased activity at the border of the ventrolateral prefrontal cortex and frontal pole (VLPFC/FP). We show that endogenous testosterone modulates these cerebral congruency effects through 2 mechanisms. First, participants with lower testosterone levels generate larger VLPFC/FP responses during affect-incongruent trials. Second, during the same trials, endogenous testosterone modulates the effective connectivity between the VLPFC/FP and the amygdala. These results indicate that endogenous testosterone influences local prefrontal activity and interregional connectivity supporting the control of social emotional behavior.**

**Keywords:** androgen, control, emotion, fMRI, VLPFC

## Introduction

It is well known that the steroid hormone testosterone plays an important role in the regulation of social emotional behavior (Van Honk et al. 1999; Viau 2002; Archer 2006; Eisenegger et al. 2010), but it remains unknown how testosterone modulates the neural systems involved during that behavior. An example of social emotional behavior are the approach and avoidance responses evoked by positive and threatening faces (Lang et al. 1990), more specifically the action tendencies automatically primed by those stimuli (Chen and Bargh 1999; Roelofs et al. 2010; Seidel et al. 2010). In this context, regulation of social emotional behavior can be operationalized with tasks requiring subjects to override such action tendencies (Rotteveel and Phaf 2004; Roelofs, Minelli, et al. 2009, 2010). We have recently shown that the left ventrolateral prefrontal cortex (VLPFC) is involved in supporting the voluntary control of these action tendencies (Roelofs, Minelli, et al. 2009). In the present study,

we have used functional magnetic resonance imaging (fMRI) to study the modulatory effects of endogenous physiologic levels of testosterone on activity and connectivity of cerebral circuits involved during the voluntary control of social approach–avoidance (AA) behavior.

Testosterone is the end product of the hypothalamic–pituitary–gonadal axis, activation of which is associated with reactive aggression and social approach motivation (Van Honk et al. 1999; Archer 2006). For example, participants with higher testosterone levels show more approach-related behavior during short social encounters (Dabbs et al. 2001). Furthermore, testosterone increases when obtaining victory during a contest, particularly in persons with an uninhibited and assertive personality (Schultheiss et al. 1999; Mehta and Josephs 2006). Recent findings have shown that this hormone influences neural activity of specific portions of the brain, in particular the amygdala and ventral prefrontal areas (Manuck et al. 2010; Mehta and Beer 2010; Root et al. 2010; Stanton et al. 2009; Van Wingen et al. 2009). For instance, endogenous testosterone levels showed a negative relation with activity in the orbital frontal cortex (OFC) when participants received an unfair monetary offer (Mehta and Beer 2010). Also, testosterone administration increased amygdala responses to negative face stimuli in middle-aged women (Van Wingen et al. 2009). Those findings support the notion that testosterone plays a significant role in the perception of emotions and aggressive behavior. However, it remains unknown how testosterone influences the cerebral mechanisms involved in the regulation of social emotional behavior.

We have evoked social motivational actions using an AA task. During this task, participants approach or avoid photographs of happy and angry faces by pulling or pushing a joystick toward or away from their bodies, respectively. This task exploits the automatic tendency that people have to approach positive stimuli and to avoid negative stimuli (Chen and Bargh 1999; Roelofs et al. 2005; Rinck and Becker 2007). For instance, participants are faster to provide affect-congruent responses (approach-happy; avoid-angry) than affect-incongruent responses (approach-angry; avoid-happy; the congruency effect). The VLPFC is involved in supporting the control of these responses, being particularly active during affect-incongruent responses (Roelofs, Minelli, et al. 2009). Both behavioral and cerebral effects are specific to the voluntary control of social motivational behavior, being present when participants are asked to explicitly evaluate the emotional valence of the faces (AA task) and disappearing when participants evaluated an emotionally irrelevant feature of the visual stimuli (gender

evaluation task, GE task) (Roelofs, Minelli, et al. 2009). Here, we build on that experimental paradigm, using fMRI during performance of the AA and GE tasks to test for a modulatory role of endogenous testosterone during the voluntary control of social motivational behavior. Given the strong interactions between testosterone and cortisol (Viau 2002), and the relevance of the latter in modulating social motivational behavior (Van Peer et al. 2007; Roelofs, Van Peer, et al. 2009), we also consider endogenous cortisol levels. We focus our analyses on the frontal cortex and the amygdala, a focus justified by the available evidence on cerebral correlates that support emotional control and that are sensitive to testosterone, as reviewed above. More precisely, given the negative relation between testosterone levels and OFC activity during complex social behavior (Mehta and Beer 2010), we predict that the VLPFC involvement in controlling affect-incongruent responses is particularly prominent in subjects with low testosterone levels. Furthermore, given the suggestion that emotional control can operate through VLPFC downregulating amygdala responses to emotional stimuli (Ochsner and Gross 2005, 2008), we predict that low testosterone levels lead to a negative amygdala-VLPFC coupling during affect-incongruent trials.

## Materials and Methods

### Participants

Twenty-four right-handed males (Edinburgh Handedness Inventory; Oldfield 1971; all above 75, age: 19–28 years) participated in the study after giving written consent according to the guidelines of the local ethics committee (Commissie Mensgebonden Onderzoek Region Arnhem-Nijmegen). All participants had normal or corrected to normal vision and no history of psychiatric disorders, as indicated by the participants. They received payment or course credits for their contribution. Four participants were excluded due to technical problems (joystick malfunction, MR-scanner artifacts), resulting in 20 participants for the final analyses.

### Procedure

Upon arrival in the laboratory between 12:00 PM and 3:00 PM, the participants were reminded of the experimental schedule. They completed several questionnaires dealing with personality, mood and life events, and saliva for the testosterone and cortisol measurement was collected. After providing the saliva samples, the participants were positioned in the MR scanner and familiarized with the task setup by means of a short training. Immediately after this familiarization period, the first fMRI session started (duration: 30 min). After a short break outside the scanner (5 min), the participants were positioned again in the MR scanner and the second fMRI session started (duration: 30 min), followed by a resting state scan (6 min—not included in this report) and an anatomical scan (9 min). At the end of both fMRI sessions, saliva for additional cortisol measurements was collected. The participants completed 2 additional tasks after the fMRI measurement.

### Experimental Tasks

The AA task and the GE task were administered in 2 separate fMRI sessions, with order counterbalanced across participants. During both tasks, the participants were asked to respond to visually presented emotional faces by means of a joystick. The participants either pulled the joystick toward themselves (approach) or pushed the joystick away from themselves (avoid). During the AA task, the participants were asked to categorize the faces as happy, angry, and neutral, based on their affective expressions. During the GE task, the participants were asked to categorize an affectively irrelevant feature (gender) of the same faces. Joystick displacements of 65% or more along the sagittal plane, and delivered within 3 s from stimulus presentation, were marked as valid responses. Invalid responses were signaled for 1 s with

visual feedback indicating “you did not move your joystick far enough.” After moving the joystick, the participants had to return to the starting position (defined as the central area covering 15% along the sagittal plane) before the end of the intertrial interval (ITI; 1–3 s). Otherwise, visual feedback indicated “return the joystick to the starting position” and the ITI was repeated after the participant returned the joystick.

Each task consisted of 24 blocks (with 12 trials per block); each block was followed by a baseline period (21–24 s). During each block, 2 of the 3 affective expressions were presented as stimuli because only 2 responses could be given to categorize the stimulus. This resulted in 6 different block types used 4 times in both tasks, representing the affect (happy-angry, happy-neutral, angry-neutral)  $\times$  movement (approach-avoid) combinations. At the start of each block the participant received written instructions regarding their required response mapping (see Fig. 1A,B). The affect  $\times$  movement combination of the first block was counterbalanced across participants, and the other affect  $\times$  movement combinations were pseudorandomly and evenly distributed within each task (with no affect combination repetition). Within each block, affective expressions and gender types were pseudorandomly presented, avoiding 3 or more sequential presentations of the same expression/gender, and 2 presentations of the same facial model. See Figure 1C for the trial sequence. The training at the beginning of the task consisted of 6 blocks; 1 block of 8 trials for each of the 6 affect  $\times$  movement combinations. Different visual stimuli were used during the training and scanning blocks.

### Materials and Apparatus

The fMRI images were acquired on a 1.5 T MRI scanner (Avanto, Siemens Medical Systems) equipped with an 8-channel head coil using a multiecho GRAPPA sequence (Poser et al. 2006) (repetition time [TR]: 2.14 ms, echo times [TEs, 5]: 9.4/21/33/44/56 ms, 34 transversal slices, ascending acquisition, distance factor: 17%, effective voxel size 3.3  $\times$  3.3  $\times$  3.5 mm, field of view [FoV]: 212 mm). At the end of the second experimental session, high-resolution anatomical images were acquired using an magnetization prepared rapid gradient echo sequence (TR: 2250 ms, TE: 2.95 ms, 176 sagittal slices, voxel size 1.0  $\times$  1.0  $\times$  1.0 mm, FoV: 256 mm).

The MR-compatible joystick (Fiber Optic Joystick, Current Designs), with a sampling rate around 550 Hz, was placed on the abdomen of the participants to ensure comfortable push and pull movements (see Fig. 1D). The participants wore MR-compatible headphones to reduce the scanner noise (Commander XG MRI Audio System, Resonance Technologies Inc).

The visual stimuli consisted of faces from 36 models (18 male) taken from several databases (Ekman and Friesen 1976; Matsumoto and Ekman 1988; Martinez and Benavente 1998; Lundqvist et al. 1998). Each model showed 3 affective expressions (happy, neutral, angry). The pictures were in grayscale, matched for brightness and contrast values, displayed against a black background. To exclude influence from hair and nonfacial contours, the faces were trimmed (Van Peer et al. 2007; Roelofs, Minelli, et al. 2009). The stimuli were projected at the center of a screen, viewed via a mirror above the subject's head, with a visual angle of 4°  $\times$  6° (width  $\times$  height). Stimuli presentation and acquisition of joystick positions were controlled by a PC running Presentation software version 10.2 (<http://www.neurobs.com>).

### Salivary Measurements

Saliva was collected using commercially available devices. To obtain saliva for the testosterone measurement, the participants were instructed to fill a container with 15 mL saliva (PLADI504, 60 mL professional urine container from Blockland). For the cortisol measurement, the saliva was collected using Salivette. The participants had to gently chew on a cotton swab for about 1 min and then put it back in the device without touching it with their hands. These devices were stored at –25 °C for later analyses. Testosterone concentration was measured using a competitive chemiluminescence immunoassay (LIA) with a sensitivity of 0.0025 ng/mL (IBL). Cortisol concentration was measured using a commercially available chemiluminescence immunoassay (CLIA) with high sensitivity of 0.16 ng/mL (IBL). For

### A. Block of AA-task

Instruction 1

If you see an ANGRY face,  
Move the joystick TOWARDS yourself as fast as possible.

If you see a HAPPY face,  
Move the joystick AWAY from yourself as fast as possible.

Instruction 2

Move the joystick to start.

### B. Block of GE-task

Instruction 1

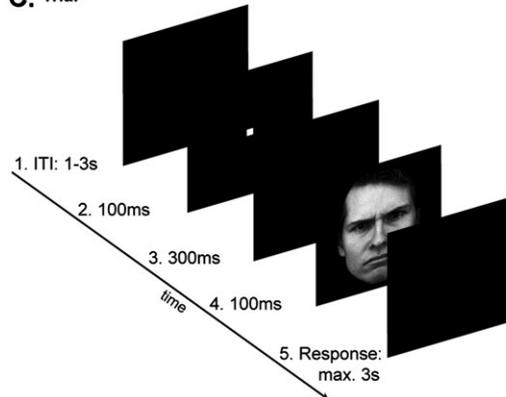
If you see an FEMALE face,  
Move the joystick TOWARDS yourself as fast as possible.

If you see a MALE face,  
Move the joystick AWAY from yourself as fast as possible.

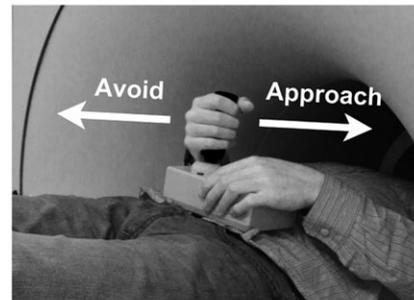
Instruction 2

Move the joystick to start.

### C. Trial



### D. Experimental setup



**Figure 1.** Block sequence for the AA task and the GE task, trial sequence, and experimental setup. (A and B, instruction 1) Each block starts with an interblock interval of 21–24 s, followed by the block-specific instruction. (A and B, instruction 2) After 3 s, an extra instruction is presented indicating that the participant can start the trials by moving the joystick. After moving the joystick, the instructions disappear. (C) Each trial starts with an ITI. Then a fixation screen is presented to make sure the participant fixates in the center of the screen. After this a blank screen is presented, followed by the stimulus. During the response period, another blank screen is presented. (D) The experimental setup with the participant lying in the MRI scanner and the approach and avoidance movements.

the CLIA assay, the intraassay and interassay coefficients are less than 8% and for the LIA assay these are between 10% and 12%.

To control variables influencing testosterone and cortisol levels, the participants did not use any caffeine-containing drinks or food, smoked no more than 5 cigarettes, and minimized physical exercise on the day of the appointment, and refrained from any food, cigarettes, and drinks (except water) from 1 h before the experiment.

#### Behavioral Analysis

Trials with incorrect responses or a RT shorter than 100 ms or longer than 1500 ms were excluded. We also excluded trials in which the joystick peak velocity or the joystick path length differed more than 3 standard deviations (SDs) from the subject-specific data distribution. Median RTs were calculated for each level of the 3 experimental factors (Task, Movement, and Valence) and then entered in a 3-way repeated measures analysis of variance (ANOVA), with factors Task (AA, GE), Movement (approach, avoid), and Valence (happy, neutral, angry). The standardized testosterone and cortisol from the first saliva measurement were included as subject-specific covariates. The  $\alpha$ -level was set at  $P < 0.05$ .

#### Functional MRI Data—Single Subject Analysis

The imaging data were preprocessed and analyzed with SPM5 (Statistical Parametric Mapping; <http://www.fil.ion.ucl.ac.uk/spm>). The first 4 volumes of each participant's data set were discarded to allow for  $T_1$  equilibration. Given the multiecho GRAPPA MR sequence (Poser et al. 2006), the head motion parameters were estimated on the MR images with the shortest TE (9.4 ms), since these images are the least affected by possible artifacts. These motion-correction parameters, estimated using a least-squares approach with 6 rigid body transformation parameters (translations, rotations), were then applied to the 5 echo images collected for each excitation. After spatial realignment, the 5 echo images were combined into a single MR volume using an optimized echo weighting method (Poser et al. 2006).

The time series for each voxel were temporally realigned to the first slice in time to correct for differences in slice time acquisition. The  $T_1$ -weighted image was spatially coregistered to the mean of the functional images. The fMRI time series were transformed and resampled at an isotropic voxel size of 2 mm into the standard Montreal Neurological Institute (MNI) space using both linear and nonlinear transformation parameters as determined in a probabilistic generative model that combines image registration, tissue classification, and bias correction (i.e., unified segmentation and normalization) of the coregistered  $T_1$ -weighted image (Ashburner and Friston 2005). The normalized functional images were spatially smoothed using an isotropic 8 mm full-width at half-maximum Gaussian kernel.

The fMRI time series of each subject were analyzed using an event-related approach in the context of the general linear model. We considered the following effects for each scanning session (AA, GE) separately: approach-happy, approach-neutral, approach-angry, avoid-happy, avoid-neutral, and avoid-angry. Trials excluded from the behavioral analyses were modeled with a separate regressor (Misses), as were trials during which instructions or feedback information was provided (Info). The vectors describing the onset and duration of each of these events were convolved with the canonical hemodynamic response function, yielding 8 task-related regressors.

The potential confounding effects of residual head movement-related effects were modeled using the original, squared, cubic, first-order, and second-order derivatives of the movement parameters as estimated by the spatial realignment procedure (Lund et al. 2005). Three further regressors, describing the time course of signal intensities averaged over different image compartments (i.e., white matter, cerebrospinal fluid, and the portion of the MR image outside the skull) were also added. This procedure accounts for image intensity shifts due to movement of the hand within or near the magnetic field of the scanner (Culham et al. 2003; Verhagen et al. 2006). Finally, the fMRI time series were high-pass filtered (cutoff 120 s). Temporal autocorrelation was modeled as a first-order autoregressive process.

## Functional MRI Data—Group Analysis

### Multiple Regression Analyses

Consistent effects across subjects were tested using a random effects multiple regression analysis that considered, for each subject, 8 contrast images. These images represented the estimated cerebral effects from 8 conditions of the experimental design (Task [AA, GE] × Movement [approach, avoid] × Valence [happy, angry]). Testosterone and cortisol levels were included in the multiple regression analysis as subject- and condition-specific covariates, generating another 16 regressors.

We considered 2 effects. First, we tested for a significant Movement × Valence interaction on the AA task, that is, task-related differences during affect-incongruent conditions (avoid-happy; approach-angry) and affect-congruent conditions (approach-happy; avoid-angry). Furthermore, we tested whether these effects were specific to the voluntary control of social motivational behavior. In other words, we tested whether the regions showing an increased differential congruency effect during the (explicit) AA task had significantly weaker congruency effects during the (implicit) GE task. This second test was implemented by masking the statistical map describing the relevant Movement × Valence interaction of the AA task (affect-incongruent > affect-congruent) with the statistical map describing the 3-way interaction (AA task [affect-incongruent > affect-congruent] > GE task [affect-incongruent > affect-congruent]). In addition, we also tested for the presence of the congruency effect (either with or without the 3-way interaction mask) in a region of the VLPFC previously shown to be sensitive to this effect (Roelofs, Minelli, et al. 2009) by using a volume of interest (VOI) centered on  $-48, 30, 8$  (MNI coordinates) with the same spatial distribution as Roelofs, Minelli, et al. (2009).

Second, we tested whether the congruency effect isolated in the first analysis was significantly modulated by the endogenous concentration of testosterone (and cortisol). This was done by assessing the same contrast described in the first group-level analysis (both without and with the 3-way interaction mask [Task × Movement × Valence]) on the regressors parametrizing the interindividual differences in testosterone or cortisol levels on the task-related conditions. We tested for these hormonal modulations on the congruency effect by using a VOI focusing on those areas showing significant effects in the first group-level analysis.

The reported activations are corrected for multiple comparisons using family-wise error (FWE) correction. For the whole-brain analyses, we made inferences at the cluster-level (Friston et al. 1996; FWE:  $P < 0.05$ , on the basis of an intensity threshold of  $t > 4$ ) and for the VOI analysis, a small volume correction was performed (Worsley et al. 1996; Friston 1997; FWE:  $P < 0.05$ ). Anatomical inference is drawn by superimposing the SPMs showing significant signal changes on the structural images of the subjects. Anatomical landmarks were identified using the atlas of Duvernoy et al. (1991). The Brodmann areas (BAs) were assigned by superimposing the significant SPMs on the MRIcron template (<http://www.sph.sc.edu/comd/rorden/micron/>). Further anatomical details on the prefrontal effects were inferred based on the articles of Rajkowska and Goldman-Rakic (1995), Ramnani and Owen (2004), and Chiavaras and Petrides (2000).

### Effective Connectivity Analyses

The aim of the following analysis was to test whether testosterone modulated the interregional coupling between VLPFC/frontal pole (FP) (see Results) and amygdala, as evoked during the congruency effect in the context of the AA task. To test for this effect, we used the psychophysiological interactions (PPIs) method (Friston et al. 1997). More specifically, we tested for significant differences between the regression coefficients of amygdala activity over VLPFC/FP activity during the affect-incongruent versus the affect-congruent conditions of the AA task. To select the voxels to be included in the VOI, we used the following anatomical and functional constraints (Friston et al. 1997; Stephan et al. 2010). For each subject, the anatomical location consisted of voxels falling within a sphere of 8 mm radius around the peak voxel corresponding to the activated cluster of the testosterone modulation on the congruency effect (coordinates:  $-28\ 54\ 8$ ; see Results). Within this location, we included those voxels showing task-related effects ( $P < 0.05$  uncorrected), as assessed by an  $F$ -contrast considering the

“approach-happy,” “approach-angry,” “avoid-happy,” and “avoid-angry” conditions of the AA task. Participants showing no voxels with significant task-related effects within this location were excluded from the PPI analysis ( $N = 2$ ). For the remaining participants, subject-specific contrast images were generated describing the PPI between the time course of the VLPFC/FP VOI and the time course of the affect-incongruent versus affect-congruent conditions within the AA task. The strength of testosterone modulation on the task-related coupling between VLPFC/FP and amygdala was then assessed by using a multiple regression design on these subjects-specific contrast images and adding their corresponding testosterone values as a subject-specific regressor. In addition to a whole-brain analysis, we assessed significant voxel-level effects (FWE corrected for multiple comparisons,  $P < 0.05$ ) within the amygdala, defined on the basis of the aal atlas (Tzourio-Mazoyer et al. 2002) using the WFU PickAtlas tool (Maldjian et al. 2003).

## Results

### Behavioral Data

The participants performed the tasks accurately (error rate: 7.9%; omissions: 1.1%; and undefined responses: 2.7%) and consistently (Table 1). Their endogenous testosterone (mean: 79.3 pg/mL, SD: 27.8) and cortisol (mean: 7.2 nmol/L, SD: 4.1) values were comparable with data reported previously (Dabbs 1990; Van Bokhoven et al. 2006; Mehta and Beer 2010; Roelofs, Minelli, et al. 2009).

The 3-way ANOVA (on Task [AA, GE], Movement [approach, avoid], and Valence [happy, neutral, angry], with testosterone and cortisol levels as covariates) showed a significant Movement × Valence interaction ( $F_{2,16} = 4.4$ ,  $P = 0.020$ ) over both tasks, a significant Task × Valence interaction ( $F_{2,16} = 8.0$ ,  $P = 0.001$ ), and significant main effects of Task ( $F_{1,17} = 10.2$ ,  $P = 0.005$ ) and Valence ( $F_{2,16} = 22.4$ ,  $P < 0.001$ ). When considering each task separately, the Movement × Valence effects did not reach significance ( $F_{2,16} < 3$ ,  $P > 0.05$ ). Table 1 indicates that trials with neutral faces generally evoked RTs intermediate between those of the other 2 emotional expressions. Cortisol levels significantly modulated the Task × Movement × Valence interaction ( $F_{2,16} = 5.8$ ,  $P = 0.007$ ), and the Movement × Valence interaction ( $F_{2,16} = 6.8$ ,  $P = 0.003$  over both tasks). Furthermore, when considering each task separately, cortisol modulated the Movement × Valence interaction in the context of the AA task ( $F_{2,16} = 8.3$ ,  $P = 0.001$ ) but not of the GE task. These results indicate that, during the AA task, there was a positive association between the congruency effect (slower affect-incongruent than affect-congruent responses) and cortisol ( $r = 0.653$ ,  $P = 0.002$ ). This relationship was mainly caused by the happy faces ( $F_{1,17} = 7.4$ ,  $P = 0.014$ ) and not the angry faces. No other significant effects, such as a main effect of movement, were found. Adding the gender of the model as an additional factor to the analyses did not affect the results and revealed no significant interactions

**Table 1**

RTs in millisecond during the AA and GE tasks for the approach and avoidance movements to happy, neutral, and angry faces

	AA task		GE task	
	Approach	Avoid	Approach	Avoid
Happy	523 (21)	541 (21)	511 (17)	519 (14)
Neutral	575 (24)	587 (22)	516 (17)	525 (16)
Angry	592 (25)	577 (17)	537 (18)	531 (16)

Note: Values are presented as mean (SE).

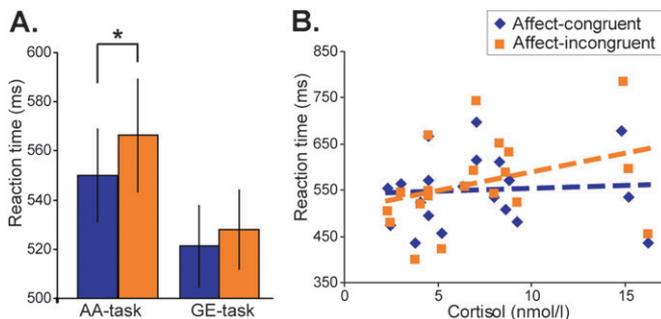
between Gender and Movement. Testosterone levels did not significantly modulate the participants' RTs.

In previous studies using the AA task, only emotional faces (happy and angry) and no neutral faces were presented (Roelofs, Minelli, et al. 2009). When excluding the neutral faces from our analyses, we found results consistent with those reported above but also a significant congruency effect for the AA task (Movement  $\times$  Valence [happy, angry]:  $F_{1,17} = 4.6$ ,  $P = 0.047$ ) and not for the GE task ( $F_{1,17} < 2$ ) (see Fig. 2). These findings are consistent with congruency effects reported previously (Roelofs, Minelli, et al. 2009), indicating that, during the AA task, processing affect-incongruent conditions evoked significantly longer RTs than during affect-congruent conditions.

### Functional MRI Data

#### Multiple Regression Analyses

Areas with stronger responses during the affect-incongruent than the affect-congruent conditions in the context of the AA task are reported in Table 2. These areas include the left and right VLPFC/FP, the fusiform gyrus, the left supramarginal, and inferior parietal gyrus. The VLPFC/FP responses are located on the border of BA 10 and BA 47/12, which corresponds to the VLPFC but also include parts of the frontal pole (Ramnani and Owen 2004). Using orbital frontal sulci probability maps (Chiavaras and Petrides 2000) and a cytoarchitectonic map of human area 46 (Rajkowska and Goldman-Rakic 1995), we could infer that the VLPFC/FP responses were located dorsal to the OFC and ventral and anterior to BA 46. The VLPFC area found by Roelofs, Minelli, et al. (2009) was also significantly more active during affect-incongruent relative to affect-congruent trials (extent: 3 voxels; coordinates of local maxima:  $-48, 32, 12$ ). The VLPFC/FP responses were stronger when the participants had to approach an angry face or avoid a happy face compared with the more automatic responses of approaching a happy face and avoiding an angry face. When comparing the effects on the AA task with the implicit (GE) task (by masking the contrast with the 3-way interaction [Task  $\times$  Movement  $\times$  Valence]), the VLPFC/FP responses were significant during the AA task but not during the GE task. This indicates that the results are specific to the (explicit) AA task.



**Figure 2.** Behavioral results. (A) RTs for the affect-congruent and affect-incongruent conditions of the AA task and the GE task (mean  $\pm$  standard error [SE] of the mean, Valence: happy and angry). Subjects were significantly slower to provide affect-incongruent (approach-angry; avoid-happy) than affect-congruent responses (approach-happy; avoid-angry). (B) Scatter plot visualizing the relation between cortisol and the RTs during affect-congruent (in blue) and affect-incongruent (in orange) conditions of the AA task. There was a positive correlation between the affect-incongruent responses and cortisol.

The left VLPFC/FP was significantly modulated by testosterone during the affect-incongruent conditions compared with the affect-congruent conditions (see Fig. 3A, extent: 5 voxels; coordinates of local maxima:  $-28, 54, 8$ ;  $t = 3.89$ ; masking with the 3-way interaction [Task  $\times$  Movement  $\times$  Valence] gave the same result). As illustrated in Figure 3B, the congruency effect in the left VLPFC/FP is stronger for the participants with low endogenous testosterone than for the participants with high endogenous testosterone. Post hoc analyses revealed that these effects were evoked by both happy and angry face trials (conjunction analysis; Nichols et al. 2005,  $P = 0.027$ ). Cortisol did not modulate the cerebral congruency effect. An extra analysis including the gender of the stimuli as added factor revealed no additional testosterone modulation on the neural processing of the congruency effect or of the individual emotions with respect to gender.

#### Effective Connectivity Analyses

Having shown that during performance of the AA task, left VLPFC/FP activity is significantly modulated by testosterone, we explored whether testosterone also modulates the interregional connectivity of this area. We performed PPI analyses with the left VLPFC/FP as seed region during the affect-incongruent versus the affect-congruent conditions of the AA task, using testosterone as a covariate. There were no significant whole-brain effects, but the VOI analysis showed that the connectivity strength between the left VLPFC/FP and the right amygdala (see Fig. 4A, extent: 3 voxels,  $t = 4.55$ ; coordinates of local maxima:  $32, -2, -20$ ) was significantly modulated by testosterone. Figure 4B shows that the connectivity changes are positively correlated with testosterone level. Participants with low endogenous testosterone values show a negative or more decreased coupling between the VLPFC/FP and the amygdala during the affect-incongruent conditions, whereas participants with high testosterone values demonstrate the opposite pattern.

### Discussion

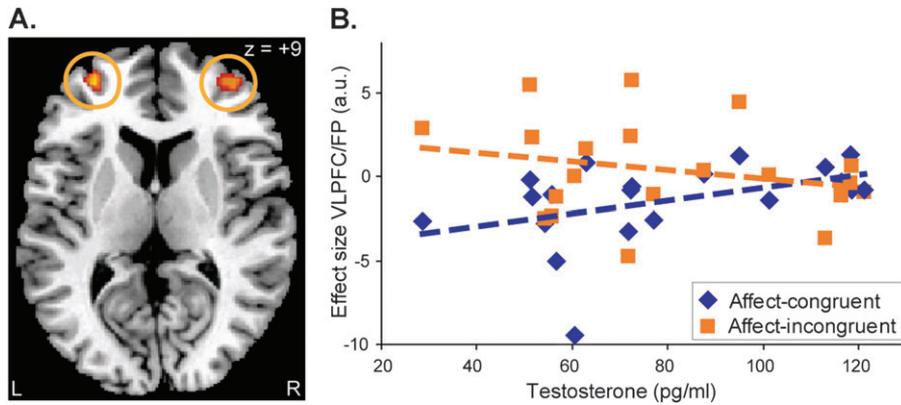
The purpose of this study was to investigate the influence of endogenous testosterone on the cerebral substrate underlying the control of social emotional behavior. We studied the control of emotional behavior, rather than focusing on perceptual aspects of emotion processing as previously done (Hermans et al. 2008; Derntl et al. 2009; Manuck et al. 2010; Root et al. 2009; Stanton et al. 2009; Van Wingen et al. 2009). Two major findings emerged. First, testosterone levels predicted the involvement of the VLPFC/FP when participants had to voluntarily override their automatic AA tendencies. Second, in the same condition, testosterone modulated the connectivity

**Table 2**

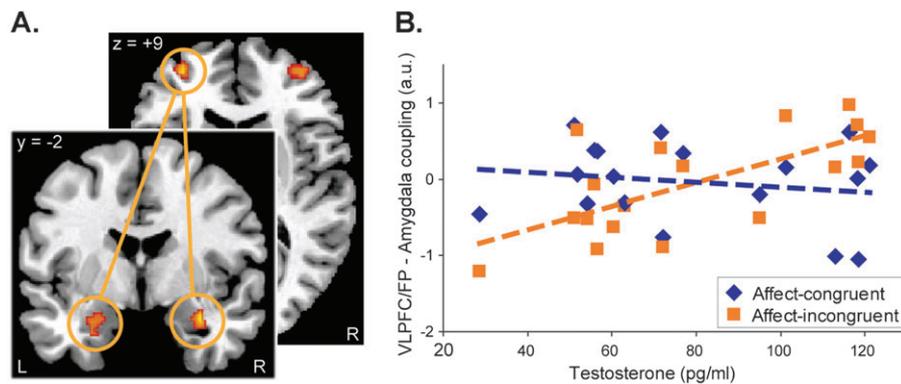
The suprathreshold activation clusters showing larger activity for the affect-incongruent versus the affect-congruent conditions on the AA task

Anatomical region	Putative BA	Side	X	Y	Z	No. of voxels	P value	t Value
Fusiform gyrus	BA 37	L	-52	-56	-14	56	0.010	5.30
		R	58	-54	-6	85	0.002	5.14
VLPFC/FP	BA 10	R	32	54	8	95	0.001	5.00
Supramarginal gyrus	BA 40, 48	L	-62	-48	30	92	0.001	4.96
VLPFC/FP	BA 10, 47/12	L	-30	58	2	117	<0.001	4.79
Inferior parietal gyrus	BA 40	L	-38	-52	54	44	0.021	4.53

Note: Coordinates are defined in MNI space. The P values represent the FWE cluster-level corrected values.



**Figure 3.** The left VLPFC/FP (local maxima:  $-28\ 54\ 8$ ) was significantly (FWE,  $P < 0.05$ ) modulated by testosterone during the affect-incongruent trials compared with the affect-congruent trials. (A) Cluster showing the activation at an uncorrected threshold of  $P < 0.05$ . The visualization was optimized for the local maxima, but note that a cluster of activation was also present on the right side (uncorrected threshold of  $P < 0.05$ , coordinates of local maxima:  $38\ 52\ 8$ ). (B) Scatter plot visualizing the relation between the left VLPFC/FP activity and testosterone during affect-congruent trials (in blue) and affect-incongruent trials (in orange) of the AA task. There was a negative relation between testosterone and activity in the left VLPFC/FP for the affect-incongruent versus the affect-congruent trials, indicating that the cerebral congruency effect was particularly pronounced in participants with low endogenous testosterone levels.



**Figure 4.** The coupling between left VLPFC/FP and right amygdala was significantly (FWE,  $P < 0.05$ ) modulated by testosterone during the affect-incongruent trials compared with the affect-congruent trials. (A) Cluster showing the task-dependent modulation of the coupling between the VLPFC/FP and the right amygdala (coordinates of local maxima:  $32\ -2\ -20$ ) at an uncorrected threshold of  $P < 0.05$ . The visualization was optimized for the local maxima, but note that a cluster of activation was also present on the left side (uncorrected threshold of  $P < 0.05$ , coordinates of local maxima:  $-28\ 0\ -22$ ). (B) Scatter plot visualizing the positive correlation between testosterone and the VLPFC/FP-amygdala connectivity for the affect-incongruent versus the affect-congruent trials.

strength between the VLPFC/FP and the amygdala. There was no difference in VLPFC/FP activity during a control task, in which participants responded to an affectively irrelevant feature of the faces (gender) that evoked more automatic aspects of AA behavior. Taken together, these findings indicate that endogenous testosterone influences the cerebral circuits involved during the control of voluntary social emotional behavior. Cortisol enhanced the behavioral congruency effect on the AA task, but these behavioral effects did not translate into detectable cerebral effects, confirming the results of a previous study (Roelofs, Minelli, et al. 2009). In the following sections, we discuss the relevance of our findings for understanding how steroid hormones influence the regulation of social emotional behavior in humans.

#### Testosterone Effects on Emotional Brain Circuits

Testosterone plays a crucial role in the regulation of aggressive and approach-related behavior (Albert et al. 1986; Harris et al. 1996; Van Honk et al. 1999; Archer 2006; Eisenegger et al. 2010). The present study showed that lower endogenous testosterone levels were associated with a greater activity in the VLPFC/FP during voluntary control

of automatic social AA tendencies. We also found that testosterone modulated changes in effective connectivity between the VLPFC/FP and the amygdala. Male subjects with low testosterone levels showed a negative coupling for the affect-incongruent trials, whereas the opposite pattern was seen in males with high testosterone levels. Although our data cannot resolve the anatomical direction of these connectivity changes, human and animal literature strongly suggest that prefrontal areas can have an inhibitory influence on the amygdala during emotion regulation (Rosenkranz and Grace 2002; Rosenkranz et al. 2003; Quirk et al. 2003; Etkin et al. 2006; Passamonti et al. 2008). For instance, a study investigating interindividual differences in behavioral approach motivation (Passamonti et al. 2008) reported top-down effects from the ventral anterior cingulate cortex to the amygdala during passive viewing of angry and neutral faces. Accordingly, we suggest that in subjects with lower endogenous testosterone levels, voluntary control of social motivational behavior relies on stronger inhibition from the VLPFC/FP on amygdala responses.

Previous studies have shown modulations of amygdala and OFC activity as a function of endogenous testosterone during

emotional processing (Derntl et al. 2009; Manuck et al. 2010; Mehta and Beer 2010; Stanton et al. 2009; Van Wingen et al. 2010). For instance, OFC activity is negatively correlated with endogenous testosterone in subjects receiving an unfair monetary offer (Mehta and Beer 2010). It has also been shown that, in midaged women with lower androgen levels than young women, administration of testosterone diminished OFC activity and its effective connectivity with the amygdala during a matching task using angry and fearful faces (Van Wingen et al. 2009, 2010). This finding fits with the known involvement of the OFC in emotional processing and stimulus-outcome predictions that do not require an explicit behavioral adaptation (Ostlund and Balleine 2007; Watanabe and Sakagami 2007). In contrast to those studies, here we focus on the control of emotional processing. For instance, correct performance of the incongruent trials in the AA task involves applying an episodic rule to emotional features of the visual stimulus, while suppressing a prepotent automatic reaction to those emotional features. Accordingly, this study highlighted the relevance of the VLPFC/FP in implementing such emotional control. The VLPFC/FP has been suggested to facilitate context-based evaluation of stimuli's emotional values and the related, appropriate action selections, in the context of emotion regulation (Ochsner and Gross 2005). Furthermore, anterior portions of the VLPFC have been implicated in branching control, defined as the relational integration of top-down information from episodic events and goals with the ongoing behavioral situation (Ramnani and Owen 2004; Koechlin and Summerfield 2007; Badre and D'Esposito 2009). In this context, the VLPFC/FP is important when the implementation of a particular rule requires one to override more automatic stimulus-response mappings (Rushworth et al. 2005; Thompson-Schill et al. 2005; Diekhof and Gruber 2010). Here, we show that testosterone influences the cerebral correlates involved in the control of social emotional behavior by modulating anterior portions of the VLPFC bordering the FP, rather than those portions of the orbitofrontal cortex previously associated with emotional processing (Derntl et al. 2009; Manuck et al. 2010; Mehta and Beer 2010; Van Wingen et al. 2010).

These findings appear relevant for understanding cerebral alterations in patients with antisocial disorders, who often show increased testosterone levels (Harris et al. 1996; Van Honk et al. 1999; Archer 2006) and reduced structural VLPFC-amygdala connectivity (Elovathingal et al. 2006; Grant et al. 2007; Craig et al. 2009). These patients have reduced control of their social approach behavior, and it has been suggested that this impairment emerges from alterations in the prefrontal modulation of subcortical areas, such as the amygdala (Davidson et al. 2000; Blair 2004; Lewis et al. 2006; Sterzer and Stadler 2009). In healthy male subjects, increased testosterone has been associated with uninhibited behavior and an extraverted, dominant personality style (Archer 2006). The present findings extend this scenario, showing that healthy males with increased testosterone levels recruit the VLPFC/FP less to control their social emotional behavior and show a different pattern of effective connectivity between prefrontal and amygdala regions than healthy males with lower testosterone levels. These results indicate that there may be a relevant relation between previous findings of altered testosterone levels and reduced frontal-amygdala connectivity in patients with psychopathy (Archer 2006; Blair 2008). The present results also illustrate the

importance of taking endogenous hormonal influences into account when studying emotional or social behavior in healthy individuals as well as patient samples. Several studies in humans have already shown that differences in endogenous testosterone can have profound effects on neural activity (Derntl et al. 2009; Manuck et al. 2010; Stanton et al. 2009; Van Wingen et al. 2009). This is the first study showing that endogenous testosterone also modulates the connectivity strength between frontal and limbic areas in humans.

### ***Interpretational Issues***

Only males participated in this study, therefore it remains to be seen whether the findings also apply to females. It is interesting to compare males and females, but it will be difficult given the large gender differences in steroid hormone activity (Dabbs 1990; Casanueva and Dieguez 1999; Kirschbaum et al. 1999; Wood 2008), as well as in emotion processing (Rotter and Rotter 1988).

The modulatory effect of testosterone on cerebral activity was not accompanied by a behavioral effect. In addition, the modulatory role of cortisol on the participants' RTs had no task-specific cerebral counterpart similar to what we found in a previous study (Roelofs, Minelli, et al. 2009). These negative findings are important insofar they indicate that, despite the known interactions between testosterone and cortisol (Viau 2002), the testosterone modulation reported here cannot be explained by salivary cortisol levels. Baseline cortisol might have more generic effects on cerebral activity than those specifically assessed in this task and/or influence emotional control through different cerebral systems than testosterone. For instance, it has been recently suggested that cortisol increases serotonergic function (Summers and Winberg 2006; Fiocco et al. 2007). In turn, the serotonergic system affects the association between testosterone and motivational behavior (Birger et al. 2003). There are several neurochemical mechanisms by which testosterone could influence frontal-amygdala functioning (Bodo and Rissman 2006; Wood 2008). For example, testosterone binds to androgen receptors or, when aromatized to estradiol, it binds to estrogen receptors (Balthazart and Ball 2006; Wood 2008). For an extended review, see Bialek et al. (2004). Further research is needed to gain more insight in how testosterone interacts with other hormones, neurotransmitters, and receptors during the regulation of social emotional behavior.

### **Conclusion**

The voluntary regulation of AA responses provides a privileged viewpoint for investigating the interface between hormonal and neural involvement during social emotional behavior. We have shown that, in healthy males, testosterone modulates the cerebral balance between changes in local activity and interregional connectivity in circuits involved during the control over automatic approach and avoidance tendencies. The results emphasize the importance of considering the actual behavioral response tendencies evoked by the perception of emotional material and the underlying psychoneuroendocrinologic interactions. The significant portion of cerebral intersubject variability accounted for by endogenous testosterone using this paradigm suggests that testosterone might provide a relevant physiological measure for studying alterations in social emotional behavior, such as antisocial and anxiety disorders.

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