Testosterone Rapidly Increases Neural Reactivity to Threat in Healthy Men: A Novel Two-Step Pharmacological Challenge Paradigm

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Background: Previous research suggests that testosterone (T) plays a key role in shaping competitive and aggressive behavior in humans, possibly by modulating threat-related neural circuitry. However, this research has been limited by the use of T augmentation that fails to account for baseline differences and has been conducted exclusively in women. Thus, the extent to which normal physiologic concentrations of T affect threat-related brain function in men remains unknown.

Methods: In the current study, we use a novel two-step pharmacologic challenge protocol to overcome these limitations and to evaluate causal modulation of threat- and aggression-related neural circuits by T in healthy young men ($n = 16$). First, we controlled for baseline differences in T through administration of a gonadotropin releasing hormone antagonist. Once a common baseline was established across participants, we then administered T to within the normal physiologic range. During this second step of the protocol we acquired functional neuroimaging data to examine the impact of T augmentation on neural circuitry supporting threat and aggression.

Results: Gonadotropin releasing hormone antagonism successfully reduced circulating concentrations of T and brought subjects to a common baseline. Administration of T rapidly increased circulating T concentrations and was associated with heightened reactivity of the amygdala, hypothalamus, and periaqueductal grey to angry facial expressions.

Conclusions: These findings provide novel causal evidence that T rapidly potentiates the response of neural circuits mediating threat processing and aggressive behavior in men.

Key Words: Aggression, amygdala, androgens, anger, emotion, fMRI, testosterone

Testosterone (T) has been clearly associated with aggression across numerous species (1). However, evidence in humans has revealed a relatively weak and inconsistent association between baseline T concentrations and aggression (2). These inconsistencies might arise from an exclusive focus on baseline T rather than experimentally evoked T responses, which are recognized as highly variable and rapidly fluctuating (3,4). Indeed, current theory holds that changes in T during competitive interactions are key for modulating ongoing and/or future aggression and dominance-related behaviors (5,6). In support of this model, studies have found that changes in T during competition are positively correlated with subsequent competitive motivation (7,8) and reactive aggression (9–11). Although acute changes in T have proven useful in predicting aggression (12), the extent to which T plays a causal role in shaping variability in human aggression is not clear. Explication of possible causal pathways between T and aggression is particularly timely and relevant, because T augmentation is increasingly being promoted as a pharmacologic approach to recovering and maintaining physical and reproductive vitality in aging men with "low T" (13).

Of particular value in this context is identifying the effects of T augmentation on neural circuitry—including the amygdala, hypothalamus, and periaqueductal gray (PAG)—that mediate threat processing and aggressive behavior (14–16). Notably, individual differences in T concentrations are associated with variability in the function of these neural structures in humans. Specifically, functional neuroimaging studies in men and women indicate that endogenous T concentrations are positively correlated with the reactivity of the amygdala (17–20) and hypothalamus (18) to facial threat displays (e.g., angry and fearful faces). Going beyond correlational work, evidence indicates that a single administration of T increases amygdala, hypothalamic, and midbrain reactivity to facial signals of threat (18,20–22). Although these studies provide support for a causal role of T in potentiating threat-related neural function, they are limited in certain ways. Most notably, these pharmacologic challenge studies have been performed exclusively in women, for whom there is lacking evidence for a relationship between acute endogenous changes in T and aggressive behavior (9,11). Also, the standard sublingual T protocols employed in women fail to account for baseline differences and increase T concentrations above the normal physiologic range (mean = 18.41 nmol/L vs. .6–7.2 nmol/L) (23,24). Finally, most previous work in women has used a significant time lag (4–4.5 hours) between drug administration and assessment of physiological and behavioral processes, and

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there is some evidence that T might have much more rapid, perhaps non-genomic effects on neural responses to social threat (18). Thus, the extent to which raising T concentration to within the normal physiologic range in healthy young men would rapidly potentiate threat-related neural function remains unclear.

Understanding the modulatory role of T on heightened threat-related amygdala reactivity in men is particularly important, because aggression is generally more common in men than women (25), and pathological extremes of aggression are also more often exhibited in men than women (26). Psychopathology characterized by heightened reactive aggression (e.g., intermittent explosive disorder, borderline personality disorder) is associated with heightened amygdala reactivity to the presentation of angry facial expressions (27,28). Also, functional genetic polymorphisms in pathways linked to aggression, such as the androgen receptor (29) and monoamine oxidase A (30), are associated with increased amygdala reactivity to fearful (19) and angry (31) facial expressions. On the basis of this evidence and work in animal models (1), we have proposed that acute changes in T within the context of competitive interactions might modulate aggressive behavior through its effects on amygdala, hypothalamic, and PAG function (12).

To begin to address this hypothesis, the current study developed a novel human pharmacologic challenge protocol similar to that used in California mice (32). In this animal model, male mice are physically castrated before engaging in a resident-intruder paradigm, a manipulation that serves to clamp the hypothalamic-pituitary-gonadal (HPG) axis, thus ensuring that endogenous changes in T do not occur in response to competitive interactions. After winning a competitive interaction, mice are given an acute dose of T or placebo. This manipulation reveals that mice are more aggressive in subsequent competitive interactions but only if they received T after winning an initial interaction (32). A remarkably similar effect has been observed in healthy young men: winning is associated with increased aggressive behavior, an effect mediated by T reactivity (11).

We adapted the T suppression/replacement animal model to experimentally modulate T in healthy young men with a dual-stage, placebo-controlled, double-blind, within-subject design. Specifically, we first used a gonadotropin releasing hormone (GnRH) antagonist to acutely suppress the HPG axis. By antagonizing GnRH receptors on the anterior pituitary, this drug effectively inhibits the release of luteinizing hormone, ultimately suppressing endogenous T concentrations to within the hypogonadal range (33). Critically, this first challenge simultaneously reduces variability in baseline T concentrations. After clamping the HPG axis and achieving T suppression, participants then received T or placebo and performed a challenge paradigm involving perceptual processing of emotional facial expressions during blood oxygen level–dependent (BOLD) functional magnetic resonance imaging (fMRI).

The primary goal of this study was to examine whether acute manipulation of T affects threat-related neural function in men. On the basis of recent pharmacologic challenge work in women (18,20) and neurobiological models of aggression in animal models (1), we hypothesized that T administration would increase the reactivity of the amygdala, hypothalamus, and periaqueductal gray (PAG) to angry facial expressions. Testosterone administration has also been shown to decrease fear-potentiated startle (34), suggesting that T administration might decrease amygdala reactivity to fearful facial expressions. However, recent evidence indicates that T administration increases amygdala reactivity to fearful expressions in women (22); and correlational studies, which have combined angry and fearful facial expressions in their analyses, have found positive correlations between T and amygdala reactivity to both types of threat-related expressions (17,19). Given inconclusive evidence with regard to T and neural responses to fearful expressions, we made no directional hypotheses concerning the role of T in modulating neural reactivity to these stimuli. Finally, we also examined the effect of T administration on neural responses to surprise facial expressions.

Methods and Materials

Participants

Participants were 16 healthy adult male volunteers (18–44 years of age; mean age 26.81) who self-identified as Caucasian (75%), Hispanic (12.5%), African-American (6.25%), and Asian (6.25%). Exclusion criteria were history of endocrine or psychiatric disorder, current prescription medication use, left-hand dominance, history of closed-head injury, and presence of ferromagnetic foreign bodies or medical devices. Participants were given an honorarium of $125 per visit. The protocol was approved by the Wayne State University Institutional Review Board.

Procedure

The current study employed a placebo-controlled, double-blinded, within-subject, crossover design. The procedure entailed 2 testing days spaced at least 3 weeks apart. On both visits, participants came to the clinic between 8:00 AM and 9:00 AM, completed a Profile of Mood States questionnaire (POMS) (35) and provided demographic information. Blood (10 mL) was then collected from the antecubital vein. Next, participants received a subcutaneous injection of a GnRH antagonist (cetrorelix acetate, 3 mg). After receiving the GnRH antagonist, participants were free to go about their normal daily activities before returning between 5:00 PM and 6:00 PM for the fMRI portion of the study. Upon arrival, participants had an indwelling catheter inserted into their antecubital vein, and blood (10 mL) was drawn every 15 min for the 2-hour visit. We were unable to obtain blood samples from two participants. After their first blood draw, participants were randomly assigned to receive either 100 mg T gel (Androgel; Abbvie, North Chicago, Illinois) or placebo gel (order of gel administration was fully counterbalanced) and then completed a second POMS questionnaire. Approximately 50 min after gel application, participants completed a third POMS questionnaire before entering the scanning suite. The MRI scan lasted approximately 60 min. After the scan, participants completed their fourth and final POMS questionnaire. See Figure 1 for experimental protocol. After the final visit, participants were paid, debriefed, and asked whether they believed they received the T gel on the first or second visit. A binomial test indicated that participants were no better than chance at guessing when they received T ($p = .607$). None of the research staff conducting the questionnaire or fMRI portion of the study had knowledge of assignment to Androgel (Abbvie) or placebo until completion of the entire study.

Hormone Assessment

To assess the efficacy of our T manipulation protocol, total T concentrations were assayed with commercially available enzyme linked immunoassay kits (DRG International, Springfield, New Jersey). Moreover, we also assayed for estradiol and serum hormone binding globulin (SHBG) to assess the

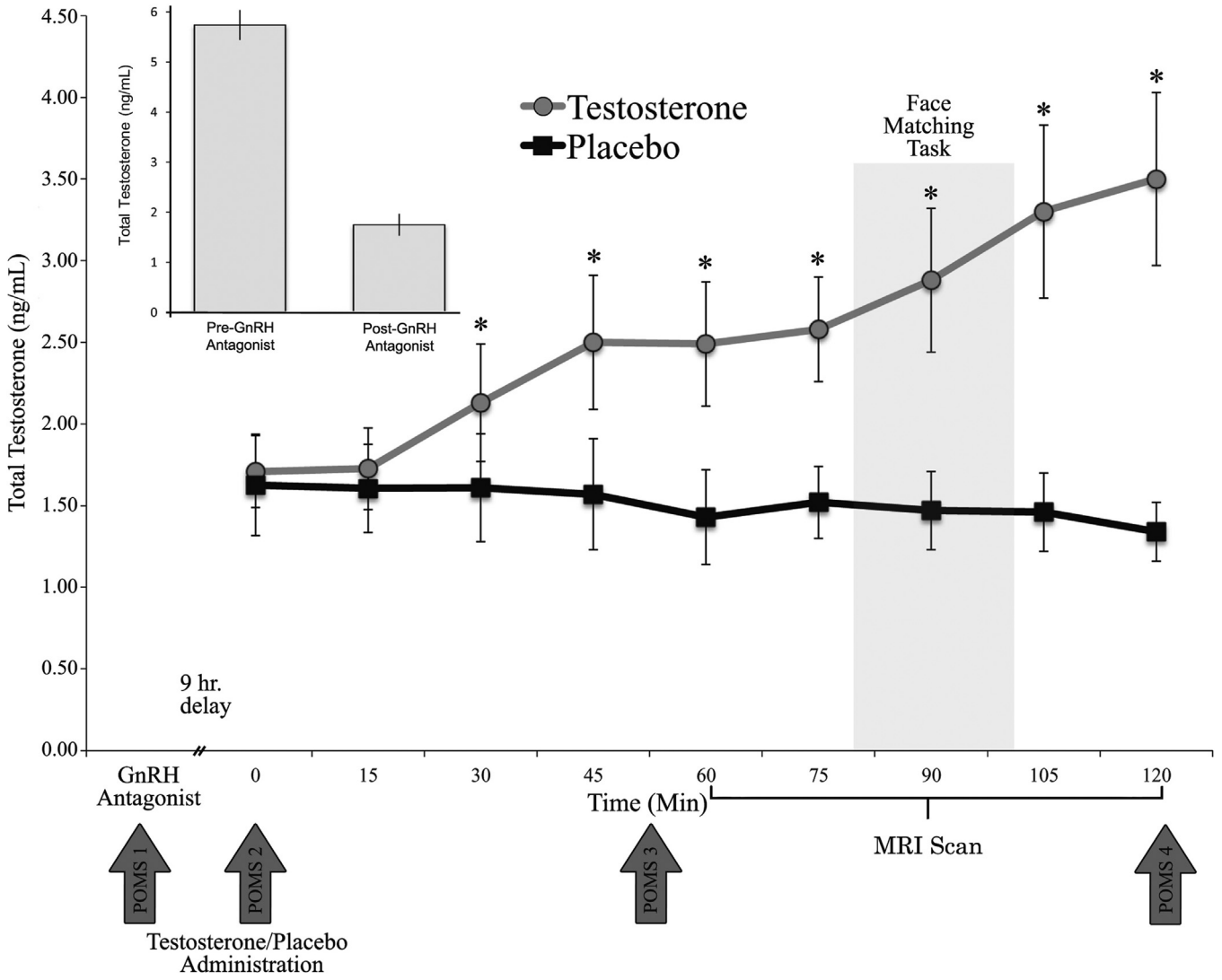

Figure 1. Experimental design and serum testosterone concentrations. Androgel (Abbvie, North Chicago, Illinois) administration increased serum testosterone concentrations above the placebo condition within 30 min of drug application. Error bars depict SEM. *$p < .05$. GnRH, gonadotropin releasing hormone; MRI, magnetic resonance imaging; POMS, Profile of Mood States.

specificity of the T suppression/replacement manipulation. All samples were spun in a refrigerated centrifuge (4°C) for 15 min at 3000 rpm. The plasma supernatant was aliquoted and stored at −80°C. For T, the intra- and inter-assay coefficients (CVs) of variation were 6.72% and 8.29%, respectively. For SHBG, the intra- and inter-assay CVs were 7.20% and 6.57%, respectively. For estradiol, the intra- and inter-assay CVs were 11.05% and 7.50%, respectively.

fMRI Task

The fMRI challenge paradigm used in the current study has been used extensively to elicit a robust and replicable amygdala response across an array of experimental protocols and sample populations (36–40). In the paradigm, there are four blocks of a perceptual face-matching task interleaved with five blocks of a sensorimotor control. During task blocks, participants view a trio of faces and select one of two faces (on the bottom) identical to a target face (on the top). Each task block consists of six different trios, balanced for gender, all of which were derived from a standard set of pictures of facial affect (41). Thus, in each block, participants see 18 faces (6 trials × 3 faces of the same expression). We used the Duke Neurogenetics Study version of the task (42–44) consisting of one block each of fearful, angry, surprised, and neutral facial expressions presented in a pseudorandom order across participants. These four task blocks are interleaved with five control blocks, in which subjects match simple geometric shapes (circles and vertical and horizontal ellipses). Each control block consists of six different shape trios. All blocks are preceded by a brief instruction ("Match Faces" or "Match Shapes") that lasts 2 sec. In the task blocks, each of the six face trios is presented for 4 sec with a variable interstimulus interval of 2–6 sec (mean = 4 sec), for a total block length of 48 sec. A variable interstimulus interval is used to minimize expectancy effects and resulting habituation and maximize amygdala reactivity throughout the paradigm. In the control blocks, each of the six shape trios is presented for 4 sec with a fixed interstimulus interval of 2 sec, for a total block length of 36 sec. Total task time is 390 sec. The stimuli were presented with E-prime software (version 2.0; Psychology Software Tools, Pittsburgh, Pennsylvania).

Neural Regions of Interest

Because of our strong a priori hypotheses, we focused our analyses primarily on the amygdala, hypothalamus, and PAG. Functional reactivity of the amygdala was assessed within anatomical regions of interest (ROIs) on the basis of cytoarchitectonic probability maps as implemented in the anatomy toolbox for SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom) (45,46). In keeping with recent imaging work (47–49) and in recognition of the functional and anatomical heterogeneity of the amygdala (50), we specifically considered the corticomedial (centromedial and superficial) and basolateral subregions in our analyses. The PAG ROI was a 6-mm sphere centered on Montreal Neurological Institute (MNI) coordinates x = 1, y = −29, z = −11 (51). The hypothalamic ROI was constructed on the basis of the PickAtlas toolbox of SPM8 (http://fmri.wfubmc.edu/software/PickAtlas) and was dilated (×1) to accommodate between-subject variability in this small structure.

To correct for multiple comparisons within our ROIs and to guard against Type I error, we used the Alphasim function in AFNI. Monte Carlo simulations (10,000 iterations) were performed with the smoothness values estimated from the residuals obtained from the GLM. At a per-voxel *p* value of .05 (two-tailed), the following cluster sizes provided for a corrected family-wise error rate of $\alpha < .05$: 38 voxels in corticomedial amygdala (CMA), 43 voxels basolateral amygdala (BLA), 14 voxels in the hypothalamus, and 28 voxels in the PAG. Unless stated otherwise, all results reported in this article survive correction for multiple comparisons.

Behavioral Data Analysis

The response time (RT) and accuracy data for each participant during the imaging task were obtained. A total of 17 of 1728 trials (16 trials of shapes, 1 trial of neutral faces) with RTs more than 3 SDs from the mean were excluded. The RT and accuracy data were averaged for participants by different stimulus type and drug condition and reconstructed for paired comparisons. As expected, participant accuracy was very high ($M = 97.7\%$, SD = 5.9). There were no effects of drug or drug × expression interaction on either accuracy (*p* values > .71) or RT (*p* values > .69).

POMS Questionnaires

The POMS questionnaire consisted of 37 items. Six subscales (tension, depression, anger, vigor, fatigue, and confusion) were aggregated from individual items. To account for missing items, the average score was used instead of the sum. A Total Mood Disturbance score was calculated as the average of the six subscales. The six subscales of POMS (tension, depression, anger, vigor, fatigue, and confusion) achieved high reliability (average Cronbach's $\alpha = .81$). There were no effects of drug condition on any of the subscales (*p* values > .05), and thus we do not report further on this measure.

BOLD fMRI Data Acquisition

Each participant was scanned with a research-dedicated Siemens Vario 3T scanner at Wayne State University. T2*-weighted BOLD images were acquired with echo-planar imaging (EPI) (repetition time/echo time/flip angle = 2000 msec/25 msec/90; field-of-view = 220; voxel size = 3.44 × 3.44 × 4 mm; interslice skip = 0). Siemens MRI motion correction software was used to retroactively reduce the relative motion across the dataset by applying post-processing interpolation of frame-to-frame movement. After this, mean movement for each of six translational (x, y, z) and rotational (pitch, roll, yaw) movement directions were calculated. Average participant movement was <.5 mm across three translational directions and <.3° across three rotational directions. No significant differences in movement were found between the two drug conditions for any of the six movement parameters (*p* values > .05).

BOLD fMRI Data Preprocessing

Preprocessing steps were performed with SPM8 software (Wellcome Department of Imaging Neuroscience). The first six EPI volumes were discarded to allow for signal stabilization. Images were then realigned and spatially normalized to the MNI template with the participant-specific transformation parameters created by fitting mean functional images to the single reference EPI standard SPM template (final resolution of functional images = 2 mm isotropic voxels). After normalization, images were spatially smoothed with a relatively small Gaussian kernel of 4 mm full width at half maximum as used in recent work (52–54). Finally, low-frequency BOLD signal drift was removed by applying a standard high-pass filter (128-sec cutoff).

After preprocessing, linear contrasts with canonical hemodynamic response functions convolved with the block duration were used to estimate expression-specific (anger > neutral; fear > neutral; surprise > neutral) BOLD responses for each individual. These individual contrast images (i.e., weighted sum of the beta images) were then used in second-level random-effects models to determine mean expression-specific neural reactivity with one-sample *t* tests and simple main effects of drug challenge (i.e., T > placebo and placebo > T) with paired *t* tests. To examine the specificity of the effects of drug on threat-related neural activation within our a priori ROIs, we performed a 2 × 3 flexible factorial analysis with drug condition (T vs. placebo) and expression (anger > neutral vs. fear > neutral vs. surprise > neutral) as within-subject factors. Statistical parametric maps for the effect of drug were superimposed onto a high-resolution T1-weighted image of a single individual transformed into MNI space.

Results

Hormone Responses to Drug Challenge

Total T. A repeated-measure analysis of variance (ANOVA) revealed main effects of drug and time on total T concentrations (*p* values < .001). Testosterone concentrations were reduced to within the hypogonadal range after GnRH antagonist administration ($M_{preGnRHantagonist} = 5.73$ ng/mL vs. $M_{postGnRHantagonist} = 1.74$ ng/mL; $t_{13} = 12.185$, $p < .001$). The main effects of drug and time were qualified by a significant drug × time interaction ($F_{9,99} = 10.075$, $p < .001$). Post hoc analyses indicated that total T concentrations were significantly higher in the T condition compared with the placebo condition within 30 min of gel application ($M = 2.13$ ng/mL vs. $M = 1.64$ ng/mL, respectively; $t_{13} = 5.819$, $p < .001$) (Figure 1) and continued to increase throughout the session (maximum at 2 hours, T: $M = 3.48$ ng/mL; placebo: $M = 1.34$ ng/mL).

SHBG. Repeated measures ANOVA revealed no main effects or drug × time interaction (all *p* values > .128).

T Effects on Neural Responses to Angry vs. Neutral Expressions

Results revealed significant amygdala (CMA and BLA) reactivity to angry expressions in comparison with neutral expressions

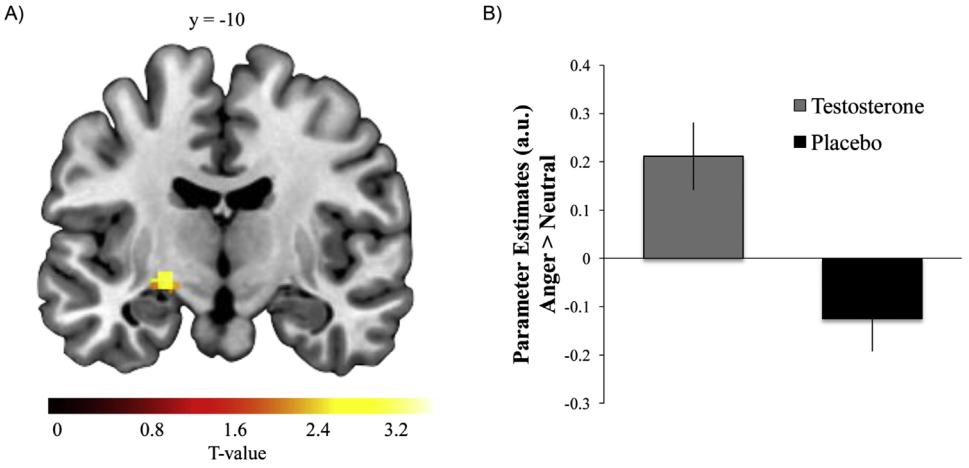

Figure 2. Testosterone (T) administration increased corticomedial amygdala (CMA) reactivity to angry compared with neutral faces. **(A)** Statistical parametric map illustrating relatively increased left CMA reactivity to angry compared with neutral expressions ($p < .05$, corrected for multiple comparisons within the CMA) after T administration. **(B)** Parameter estimates obtained from peak voxel in CMA demonstrating effect of drug (T > placebo) for the contrast of angry versus neutral faces. Error bars depict SEM. a.u., arbitrary units.

across placebo and T conditions (Figure S1A in Supplement 1). Direct comparisons between T and placebo conditions showed the T condition was associated with increased left CMA reactivity (Figure 2) and heightened reactivity within the PAG and hypothalamus (Table 1).

T Effects on Neural Responses to Fearful vs. Neutral Expressions

There was significant amygdala (CMA and BLA) reactivity to fearful expressions in comparison with neutral expressions across placebo and T conditions (Figure S1B in Supplement 1). Also, we observed significant PAG reactivity across placebo and T conditions ($Z = 2.26$, x = 0, y = −24, z = −14, cluster size = 42 voxels, $p < .05$, corrected). Direct comparisons between T and placebo conditions showed no significant effects of drug condition (T > placebo or placebo > T) on amygdala, hypothalamus, or PAG reactivity.

T Effects on Neural Responses to Surprise vs. Neutral Expressions

Results revealed significant amygdala (CMA and BLA) reactivity to surprise expressions in comparison with neutral expressions across placebo and T conditions (Figure S1C in Supplement 1). Also, we observed significant PAG reactivity across placebo and T conditions ($Z = 2.82$, x = 0, y = −24, z = −14, cluster size = 104 voxels, $p < .05$, corrected). Direct comparisons between T and placebo conditions showed no significant effects of drug condition (T > placebo or placebo > T) on amygdala, hypothalamus, or PAG reactivity (Table 1).

Flexible Factorial Analysis

To formally investigate the extent to which the effect of T on threat-related neural function was specific to the processing of angry or fearful expressions (i.e., drug × emotional expression interaction), a 2 × 3 flexible factorial analysis was performed with drug condition (T vs. placebo) and expression (angry, fearful, and surprise vs. neutral) as within-subject factors. Results revealed a significant drug × emotional expression interaction in the hypothalamus ($F = 7.13$, x = −6, y = −2, z = −4, cluster size = 16 voxels, $p < .05$, corrected) but not the amygdala or PAG (Figure 3). Also, there was a significant main effect of drug condition (T > placebo) within the PAG ($F = 7.41$, x = 4, y = −32, z = −6, cluster size = 46 voxels, $p < .05$, corrected).

Table 1. Effects of Testosterone on Amygdala, Hypothalamus, and Periaqueductal Gray Function

Contrast	ROI	X	Y	Z	Peak *Z* Value	Cluster Size
Angry > Neutral						
Testosterone	Left CMA	−26	−12	−6	2.78	71
	Right CMA	28	−10	−10	2.31	89
	Hypothalamus	−6	−6	−8	3.12	20
	PAG	2	−22	−12	2.22	48
Placebo	Right BLA	34	−6	−16	2.24	43
Testosterone > placebo	Left CMA	−24	−12	−6	2.71	44
	Hypothalamus	−8	−6	−8	2.67	17
	PAG	0	−32	−6	2.85	110
Fearful > Neutral						
Testosterone	Left CMA	−16	−6	−20	2.13	105
	Left BLA	−20	−6	−20	2.03	68
Placebo	Left CMA	−16	2	−20	3.22	71
	Left BLA	−20	2	−24	3.09	119
	Right BLA	32	−4	−16	2.88	76
	PAG	2	−28	−18	2.28	28
Surprise > Neutral						
Testosterone	Left CMA	−26	−2	−10	2.09	169
	Right CMA	28	−6	−10	2.30	198
	Left BLA	−28	−12	−12	2.43	120
	Right BLA	26	−6	−18	2.45	197
	PAG	0	−32	−14	2.19	113
Placebo	Left CMA	−28	−2	−16	2.02	44
	Right CMA	32	−2	−16	2.07	47
	Right BLA	34	−6	−16	2.21	70
	PAG	0	−24	−14	2.27	29

Activations are reported at $p < .05$, corrected for multiple comparisons within the regions of interest (ROIs). Peak coordinates of each cluster are reported in Montreal Neurological Institute space. There were no effects of testosterone > placebo or placebo > testosterone for the fearful > neutral or surprise > neutral contrasts.

BLA, basolateral amygdala; CMA, corticomedial amygdala; PAG, periaqueductal gray.

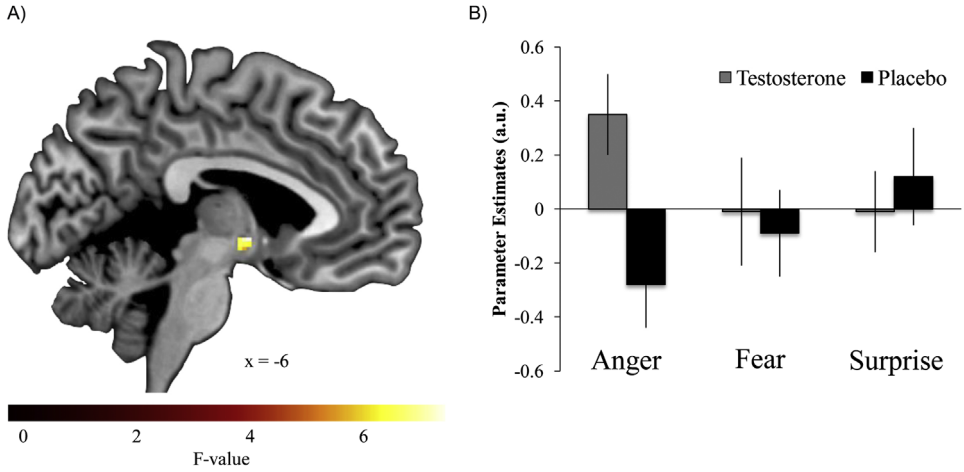

Figure 3. Testosterone administration increased hypothalamus reactivity to angry but not fearful or surprise compared with neutral expressions. **(A)** Statistical parametric map illustrating a significant drug × emotion interaction in the hypothalamus ($p < .05$, corrected for multiple comparisons within the hypothalamus). **(B)** Parameter estimates obtained from peak voxel in the hypothalamus demonstrating drug × emotion interaction. Error bars depict SEM. a.u., arbitrary units.

Discussion

With a novel pharmacologic challenge protocol that effectively controls for variability in baseline concentrations of T and uniformly raises these concentrations to a normal physiologic range, we provide causal evidence that T rapidly increases threat-related reactivity of core neural structures mediating aggression. Importantly, the current study is the first to examine the causal role of T in mediating neural responses to ecologically valid facial threat cues in healthy young men and thus represents a significant extension of the existing literature on the neuroendocrine modulation of threat- and aggression-related neural function.

The effects of T on amygdala reactivity were found within the centromedial subregion, which encompasses the central and medial nuclei. Critically, the central nucleus of the amygdala can mediate physiologic arousal and threat vigilance through projections to the hypothalamus, brain stem, and basal forebrain cholinergic cell populations (55). Terburg and van Honk (56) have argued that T increases social aggression by modulating neural function within the medial amygdala. Consistent with this idea, stimulation of the medial amygdala increases rage behavior in cats (57,58), mainly through its downstream effects on the hypothalamus and PAG. Notably, we also observed increased hypothalamic and PAG reactivity to angry facial expressions after T administration. Collectively, these neural structures are rich in both androgen and estrogen receptors (59–63) and form part of the neural circuitry underlying reactive aggression (1).

Most previous pharmacologic challenge work in humans has assessed the effects of T on brain and behavior 4–4.5 hours after T administration (21). This is a legacy effect from the initial landmark study demonstrating that effects of sublingual T administration on vaginal pulse amplitude in response to sexual stimuli emerged only 4 hours after drug administration (64). This relatively long temporal interval between drug administration and behavioral testing is consistent with a genomic mechanism wherein binding of T to androgen receptors (or estrogen receptors after aromatization) in the cytosol initiates their translocation to the nucleus where they act as transcription factors directly regulating gene expression (65).

In contrast, the effects observed in our study were prominent within 90 min after T administration, which is consistent with a rapid non-genomic mechanism (66). Moreover, work by van Wingen *et al.* (20) indicates that T administration in women increases threat-related amygdala function within 45 min. Research in animal models has identified extranuclear androgen and estrogen receptors in the hippocampus, amygdala, hypothalamus, and cortex (67–70). Such extranuclear sex steroid receptors are positioned to regulate rapid membrane and cytoplasmic signaling in axons and dendrites (71), thus facilitating the modulation of brain function and social behavior through non-genomic mechanisms (72–74). These findings converge to suggest that T can have both rapid and sustained effects on threat-related neural processes.

Although the present findings provide novel causal evidence for the importance of T in modulating threat-related neural processing, some study limitations should be noted. First, our T administration protocol only raised T concentrations to within the low normal range (75). Other pharmacologic challenge work conducted in healthy young women increased T concentrations to a much higher degree (64). Thus, perhaps the effects observed in the current study would have been more robust (similar to that observed in young women) had we used a larger dose of Androgel (Abbvie) (76). Despite differences in absolute T concentrations achieved after drug administration, both our study and the aforementioned work in young women converge on the finding that T modulates the neural circuitry underpinning threat processing and aggressive behavior in a similar fashion. Nevertheless, we believe that it will be critical to determine the extent to which T has dose-dependent effects on threat-related neural function in both men and women.

Another limitation of our work is that, although the effect of T on threat-related neural processing seemed to be specific to angry facial expressions, the factorial analysis failed to show significant drug × expression interactions for the amygdala and PAG. Thus, we cannot make strong claims concerning the specificity of the effect of T on the processing of angry facial expressions within these two regions. In contrast, the factorial analysis revealed that the effect of T on hypothalamic reactivity was emotion-specific. T was associated with increased hypothalamic reactivity to angry but not for fearful or surprise expressions, consistent with our hypothesis and with previous work (18,20).

Currently it is unclear whether the few behavioral studies in humans reporting associations between changes in T and subsequent aggressive behavior are caused by these neuroendocrine responses or are related only indirectly through a third variable (8,9,11). Our findings indicate that acutely raising T concentrations (similar to the changes in T observed during competitive interactions) can rapidly increase threat-related neural processing. A next critical step will be to implement our dual-stage pharmacologic challenge during protocols assessing aggressive behavior explicitly (e.g., Point Subtraction Aggression Paradigm, Taylor Aggression Paradigm). This will extend our current work to testing the role of increased amygdala, hypothalamus, and PAG reactivity in mediating the effects of T on aggressive behavior. Furthermore, such application of our challenge design could be used more broadly to examine the effects of T on other behavioral processes previously linked to T (e.g., risk-taking, cooperation).

In summary, we provide novel, causal evidence that exogenously administered T potentiates threat-related neural function in healthy young men. These effects were observed shortly after T administration, which is consistent with a rapid non-genomic mechanism of action. Adopting the current approach in behavioral studies will be an important next step in establishing the role that hormone dynamics, particularly those of T, play in modulating competitive and aggressive behavior.

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