BRIEF COMMUNICATIONS

A Single Administration of Testosterone Induces Cardiac Accelerative Responses to Angry Faces in Healthy Young Women

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Recently, it was demonstrated how individuals with high levels of testosterone selectively attend toward angry faces. It was argued that this suggests that high levels of testosterone are associated with an aggressive, dominating personality style. In this study, the authors used a double-blind, placebo-controlled design to examine whether exogenous testosterone would induce cardiac acceleration in response to angry faces. Participants (healthy young women) were exposed to neutral, happy, or angry faces. Administration of a single dosage of testosterone (0.5 mg) induced an accelerative cardiac response to angry faces. It is argued that this effect is due to the encouragement of dominance behavior and the inclination toward aggression. Possible mechanisms behind testosterone-driven changes in behavior are discussed with relevance to steroid-responsive networks in the limbic system that drive and control motivational and physiological aspects of social behavior.

To ensure access to key resources, such as food, shelter and sexual partners, individual animals strive for dominance (Buss, 1999). Lower mammals, such as rodents, typically dominate by means of aggression, but in higher primates evolution seems to have transformed aggressive dominance interactions into ritualized challenges based on gestures and displays (Mazur, 1973). In particular, the angry facial expression seems to have evolved to function as a threatening signal in dominance encounters (Öhman, Dimberg, & Öst, 1985). In primates, status can be established and maintained through a series of short, face-to-face competitions between group members (Mazur, 1985). Striding with a threatening gait while keeping direct eye contact is a sign of dominance, whereas averting the eyes or gaze from threatening individuals indicates submission and prevents aggression (Mazur & Booth, 1998).

In a variety of species, including humans, high basal levels of testosterone are related to aggressive and interpersonal dominance (e.g., Gray, Jackson, & McKinlay, 1991; Rubinow & Schmidt, 1996), whereas high basal levels of cortisol are related to socially avoidant, submissive behavior (e.g., Sapolsky, 1992; Schulkin, Gold, & McEwen, 1998). Recently, using a pictorial emotional Stroop task, researchers demonstrated that women and men with high basal levels of testosterone selectively attend toward angry faces (van Honk et al., 1999). Furthermore, by using a subliminal version of the Stroop task, it was shown that individuals with high basal levels of cortisol avoid the processing of angry faces (van Honk et al., 1998; van Honk, Tuiten, et al., 2000). It was argued that these findings support Mazur’s (1985) observation of the relation between elevated basal testosterone and face-to-face dominance, and Sapolsky’s (1992) suggestions concerning the relation between elevated basal cortisol and socially avoidant behavior (see van Honk et al., 1998, 1999; van Honk, Putman, et al., 2000; van Honk, Tuiten, et al., 2000).

The above findings are correlational in nature, making it difficult to draw firm conclusions concerning a causal role for the steroid hormones. More definite evidence for activational effects of cortisol or testosterone on emotional responding could be obtained by administering exogenous steroids. Furthermore, for a better understanding of the mechanisms involved in selective processing of emotional stimuli, autonomic physiological responses should be assessed as a dependent measure. Such a potentially useful dependent measure is the cardiac response, with an acceleration of the heart beat within 5 s after the presentation of a pictorial stimulus indicating anticipatory defense, suggestive of a preparatory flight–fight stage (Lang, Bradley, & Cuthbert, 1997; Öhman, 1997).

In the present study, we used a double-blind, placebo-controlled design to investigate the effects of a single dose of testosterone on
cardiac responses to neutral, happy, and angry faces in healthy young women. In addition, questionnaires were administered to investigate effects of exogenous testosterone on self-reported mood. Given the more vigilant response to angry faces we earlier observed in women with high basal levels of testosterone (van Honk et al., 1999), it was hypothesized that testosterone administration would induce cardiac acceleration in response to the angry faces exclusively.

Method

Participants

Participants were 16 female volunteers, ages 19 to 25. They were medication free, did not use oral contraceptives, and had regular menstrual cycles. Furthermore, participants had no indications of pituitary or endocrine diseases and no history of psychiatric illness. Informed consent was given, and the protocol was approved by the Medical Ethics Committee of the University Hospital of Utrecht. Only women participated because the time course and the dosage of testosterone necessary to establish physiological and psychological effects after a single administration have not yet been established in men (see Tuiten et al., 2000). Additional pharmacological studies are necessary to establish these parameters in men.

All participants were tested twice in a double-blind, placebo-controlled testosterone design, and care was taken to ensure that both measurements took place within 10 days after their last menstruation. Menstrual cycle and stimulus habituation were controlled for by running the second (placebo or testosterone) session 4 weeks after the first session, providing menstruation had occurred. This phase in the menstrual cycle (i.e., the follicular phase) was chosen because on average, endogenous hormone levels are relatively low and most stable during this period (see Roland, 1982). Data from 2 participants were lost because of apparatus failure and absence on the second test session.

Stimulus Materials and Questionnaires

Ekman and Friesen's (1976) Pictures of Facial Affect and other specially prepared slides were used as stimuli (see van Honk et al., 1999). Ten different female faces were used in the experiment, with each displaying a neutral, happy, or angry expression, resulting in a total of 30 stimuli. The happy face was used in this design to control for an effect of emotionality per se (Öhman, 1986). Two Kodak Carousels with Comput high speed shutter (Electronic Developments, North London, UK) and a projection tachistoscope (Electronic Developments) were used for presentation. Timing of stimulus presentation was regulated by Test Point software (1996) on a Laser Pentium 133 personal computer. The stimuli were back-projected through a milk-colored glass screen into the experimental room. The screen was located 110 cm from the participant at eye level. The resulting slide image was 20 × 30 cm (visual angles approximately 10.3°× 15.3°). A trial began with the presentation of a fixation cross for 5 s, immediately followed by the target slide that was shown for 6 s. The intertrial interval varied between 25 and 35 s.

Mood was established with the state version of the State–Trait Anxiety Inventory (STAI; Spielberger, 1988) and a shortened, 32-item version of the Profile of Mood States (POMS; see Shacham, 1983). The subscales of this version of the POMS provide measures of tension, depression, anger, fatigue, and vigor. To enhance sensitivity, we used visual analog scales that allowed responses between 0 and 100 (see van Honk et al., 1999).

Testosterone and Placebo Administration

Participants received sublingually 0.5 mg testosterone (or placebo) with cyclodextrines as carrier (Stuenkel, Dudley, & Yen, 1991). Experimental studies in our laboratory have established the time course of 0.5 mg sublingual testosterone administration on blood levels and physiological responsivity. These studies showed, without exception, an at least tenfold increase in the levels of total testosterone in plasma (with no changes in binding globulin) 15 min after intake, with a return to baseline within 90 min (e.g., Tuiten & van Honk, 1999; Tuiten, van Honk et al., 2000). Furthermore, it was repeatedly demonstrated that this single dose of testosterone (0.5 mg) significantly elevated physiological responsiveness (vaginal pulse amplitude) in healthy women about 4 hr after administration (e.g., Tuiten & van Honk, 1999; Tuiten et al., 2000; see also van Honk et al., 1999, for the use of this time course in salivary testosterone sampling).

Thus, physiological effects peaked 2.5 hr after the testosterone level in the blood had already returned to baseline. The use of vaginal pulse amplitude enables researchers to establish the exact time course of testosterone administration on physiological responding. Its nonhabitual nature allows multiple measurements throughout the day (see Tuiten et al., 2000). The cardiac response, however, habituates. In the present experiment, we took into account the time course of effects of testosterone administration we found on vaginal pulse amplitude in women and measured cardiac responses 4 hr after drug (testosterone or placebo) administration (see Zillmann, 1998, for strong connections between sexuality and aggression on the endocrinological level).

Apparatus and Physiological Response Measurement

Heart rate was measured with a Contact Precision Instruments photoplethysmograph finger pulse amplifier (London, UK); interbeat intervals were measured to the nearest millisecond. Gain control was set at 80 ms, and a high pass filter was set at 1 Hz. Interbeat intervals were converted off-line to heart rate in beats per minute (bpm). Base period heart rate (1 s prestimulus) was subtracted from the average heart rate for every second after stimulus presentation for a period of 6 s (cf. Greenwald, Cook, & Lang, 1989). When displayed, this often gives a triphasic waveform, consisting of an initial deceleration, followed by a small acceleration, and ending with another deceleration. The mean of this waveform usually lies below baseline. The first 2 s of deceleration indicate orienting behavior, whereas the defense response constitutes the accelerative phase and lies between 3 and 5 s after stimulus presentation. Absence of a true accelerative phase is seen as the absence of the defense response, and more extreme deceleration (bradycardia) during the second phase indicates freezing. Only for strongly aversive stimuli does cardiac acceleration surpass the baseline, indicating a preparatory (Sokolovian) defense response (Lang et al., 1997; Öhman, 1997).

Procedure

On arrival at our laboratory (between 0900 and 1000 for both sessions), participants completed the first version of the POMS and received testosterone or placebo. The completion of the second (randomized parallel) version of the POMS, immediately followed by cardiac-response measuring took place 4 hr after drug (testosterone or placebo) administration. Before cardiac-response measuring, a 5-min resting period allowed participants to adapt to the laboratory setting. Next, presentation of 2 neutral pictures served to accustomed the participants with the procedure before the presentation of the 30 target pictures. During both sessions, varied orders of presentation were used across participants, counterbalanced over the conditions.

1 As a limitation to the present study, it must be noted that for ethical and practical reasons it was decided not to establish the time course of testosterone on blood levels again. A salivary sampling procedure was not an option because earlier studies have shown that sublingual administration of testosterone confounds salivary measures of testosterone for a considerable time.
**Statistical Analyses**

Mood measures were calculated by adding up the scores on the relevant items of the separate mood measures. To investigate the effects of testosterone on mood, we computed six separate univariate repeated measures analyses of variance (ANOVAs) with STAI scores and POMS scores on the subscales Anger, Tension, Fatigue, Vigor, and Depression as dependent variables. Drug (testosterone vs. placebo) and time (time of questionnaire administration: before drug administration or 4 hr after drug administration) were used as within-subjects factors, and order (placebo first vs. testosterone first) was a between-subjects factor. All reported tests were performed two-tailed, with alpha set at .05.

To investigate the effects of testosterone on cardiac responses, we computed a multivariate ANOVA (MANOVA), with drug (placebo vs. testosterone), stimulus valence (neutral, happy, or angry facial expressions), and seconds (one measure at each of the 6 s of slide exposure) as within-subjects factors, and order (placebo first vs. testosterone first) as a between-subjects factor. Both for moods and for cardiac responses, there were no significant effects or interactions containing order (all Fs < 1). Order was therefore excluded, and all ANOVAs were rerun without order as a factor. Results of these analyses are reported.

**Results**

**Mood**

The results of the univariate ANOVAs showed no significant main effects or interactions concerning drug and time (all Fs < 1 and 2, respectively) for any of the moods (i.e., STAI and POMS subscales).

**Cardiac Responses**

There were no significant differences in baseline heart rate between the placebo ($M = 72.8, SEM = 1.5$) and the testosterone condition ($M = 72.6, SEM = 1.2$), as indicated by a two-tailed, paired $t$ test, $t(13) = 0.3, ns$.

A MANOVA revealed a significant Drug x Valence x Seconds interaction, $F(10, 4) = 16.6, p < .01$. To further investigate this significant three-way interaction, we performed separate analyses for the different faces. For neutral and happy faces, the Drug x Seconds interactions were not significant, $F(5, 9) = 2.3, ns$, and $F(5, 9) = 2.0, ns$, respectively. For angry faces there was, however, a significant Drug x Seconds interaction, $F(5, 9) = 9.7, p < .005$. See Figure 1 for a graphical representation of the change in heart rate over time, relative to baseline.

As can be seen in Figure 1, cardiac acceleration was found only in response to the angry faces. Furthermore, in the placebo and in the testosterone conditions, the peaks of these cardiac responses to angry faces were reached 4 s after stimulus onset. A post hoc paired $t$ test (two-tailed) was performed for these values (i.e., the placebo vs. testosterone cardiac reflexes for angry faces after 4 s). This test showed that testosterone administration relative to pla-

![Figure 1](image-url)  
Figure 1. Mean heart rate changes in beats per minute (bpm) from baseline (1 s prestimulus) during 6 s poststimulus for neutral, happy, and angry faces.
cebo resulted in significantly accelerated cardiac responses to angry faces, $t(13) = 2.8$, $p = .015$ (see Figure 2).

Discussion

The results of this study clearly demonstrate that exogenous testosterone induces cardiac accelerative responses to angry faces in young women. This effect was content specific, and the physiological measure per se was not influenced, as indicated by the absence of an effect for neutral and happy faces and the absence of any changes in baseline heart rate. A cardiac accelerative response may indicate aggression proneness and preparation for fight, or fearfulness and preparation for flight. However, testosterone is strongly associated with interpersonal dominance and aggression and seems to reduce social fear. In a variety of animals, it has been demonstrated that testosterone treatment enhances dominance in social confrontations by reducing fearfulness toward threatening conspecifics (for a review, see Boissy & Bouissou, 1994). In humans, testosterone levels have been associated with anger, interpersonal (face-to-face) dominance, and aggressive and antisocial tendencies (e.g., Dabbs & Hargrove, 1997; Mazur & Booth, 1998), and increases in testosterone induced by administration of luteinizing hormone-releasing hormone reduce fear (Hubert, 1990; McAdoo et al., 1979). An example of the aggression-potentiating effects of testosterone directly related to our hypothesis is the finding that adolescent boys with high levels of testosterone respond vigorously to interpersonal provocation and threat (Olweus, Mattson, Schalling, & Löw, 1988). In concordance with this reasoning and the present findings, salivary testosterone levels, self-reported anger, and self-reported antisocial tendencies are associated with vigilant attentional responses to angry faces (van Honk et al., 1999; van Honk, Putman, Hermans, & Tuiten, 2000). On the other hand, socially fearful, submissive individuals show inhibited physiological and behavioral responses to threatening conspecifics to save resources and forestall attack (Flinn, Baerwald, Decker, & England, 1998; Nesse, 2000). In agreement with this finding, salivary cortisol levels and self-reported social anxiety have been shown to be associated with avoidant attentional responses to angry faces (van Honk et al., 1998; van Honk, Putman, et al., 2000). Finally, enhanced vigilant attentional responding to angry faces after slow repetitive transcranial magnetic stimulation at the right prefrontal cortex (d’Alfonso, van Honk, Hermans, Postma, & De Haan, 2000) is most likely due to reductions in anxiety (van Honk, Schutter, d’Alfonso, Postma, & De Haan, 2000). It may therefore be concluded that the more vigilant heart-rate response to the angry face, induced by a single dose of testosterone, does not indicate fearfulness but fearlessness, and enhanced willingness to fight or defend status in face-to-face challenges (cf. Mazur & Booth, 1998).

An additional objective of our study was to measure mood relations. Whereas earlier we found significant relations between endogenous testosterone and the POMS subscales anger and tension (van Honk et al., 1999), in the present study no effects on mood were found. Most studies in eugonadal subjects have shown little or no effects of testosterone treatment on self-reported mood (see Alexander et al., 1997). Changes in self-reported mood are perhaps even more likely to be absent when one evaluates the effects of a single dose of testosterone, as was done here. Nevertheless, in the Tuiten et al. (2000) study, a significant effect on self-reported sexual arousal accompanied the physiological effect. However, this self-reported effect seemed due to repeated measuring during the day, priming subjects on sexual functioning. That is, in a follow-up study, only one measure was taken after 4 hr, and the physiological effect remained significant, but the self-reported effect was lost (Tuiten & van Honk, 1999).

The exact mechanism by which testosterone might affect the physiological response to angry faces is not yet fully understood. However, according to Wood (1996), gonadal steroids act by binding to specific steroid-responsive neurons that occupy a wide, but selective, range of the nuclei in the limbic system. Acting in concert with sensory cues from the external environment, testosterone or its metabolites, estradiol and dihydrotestosterone, can facilitate sexual, aggressive, and dominant behavior by affecting networks of these steroid-receptor-containing neurons, which in turn interact with other integrative neural circuits that influence motivation (Meisel & Sachs, 1994). In fact, anatomical evidence in rodents indicates that steroid-responsive neuron networks can filter and channel sensory information, leading to selection of specific stimuli, and the subsequent initiation of a cascade of events leading to the physiological response (Cottamging & Phaff, 1986). A key neuroanatomical component of these neuron networks is the amygdala (Wood, 1996). The central nucleus of the amygdala innervates brainstem centers that control heart rate (Kling & Brothers, 1992), and recent evidence from neuroimaging studies (positron-emission tomography and functional magnetic resonance imaging) indicates a crucial role for the human amygdala in autonomic responses to threatening facial expressions (for a review, see Whalen, 1998).

In conclusion, the present study demonstrates that testosterone induces a cardiac accelerative response to angry faces in healthy young women. It is argued that this effect is due to an increased readiness to dominate or aggress in social challenges. The mechanisms responsible for these cardiac accelerative responses to angry faces after testosterone administration might be traced to limbic steroid-responsive networks driving motivational and physiological aspects of social behavior.

References


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