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Basal testosterone moderates responses to anger faces in humans

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Abstract

Prior research [van Honk J, Tuiten A, Verbaten R, van den Hout M, Koppeschaar H, Thijssen J, de Haan E. Correlations among salivary testosterone, mood, and selective attention to threat in humans. Horm Behav 1999;36(1):17–24; van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, Verbaten R, van Doornen L. A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. Behav Neurosci 2001;115(1):238–42.] showed relationships in humans between testosterone (T) and vigilance to facial expressions of anger, which are considered signals of an impending dominance challenge. In Study 1, we used a differential implicit learning task (DILT) (see [Schultheiss OC, Pang JS, Torges CM, Wirth MM, Treynor W. Perceived facial expressions of emotion as motivational incentives: evidence from a differential implicit learning paradigm. Emotion 2005;5(1):41–54.]) to investigate the degree to which subjects find anger faces reinforcing. In the DILT, separate sequences of actions were paired with presentations of anger faces, neutral faces or a blank screen. After training, performance on the three sequences was measured in the absence of face stimuli. Saliva was collected for T measurement. Higher T predicted better learning on sequences paired with sub-threshold (i.e., presented too fast for conscious awareness) anger faces, suggesting that T is related to reinforcing qualities of these faces. In Study 2, we examined whether morning or afternoon T better predicted attention and vigilance to anger faces. Participants were tested at 9:00 and 15:00. At each session, saliva was collected for T measurement, and participants completed a Stroop task and a dot-probe task [Mogg K, Bradley BP, Hallowell N. Attentional bias to threat: roles of trait anxiety, stressful events, and awareness. Q J Exp Psychol A 1994;47(4):841–64.] with facial expression stimuli. Morning (peak) T was a better predictor of responses to anger faces than afternoon T. Morning T predicted greater Stroop-like interference to sub-threshold anger faces, as well as attentional orienting away from sub-threshold anger faces. These effects were not present for joy faces or for supraliminal anger faces. T may generally decrease aversion to threatening stimuli, and/or may specifically facilitate approach towards signals of dominance challenge.

Keywords: Testosterone; Human; Dominance signals; Emotion; Facial expressions of emotion; Anger; Instrumental learning; Dot-probe; Stroop; Circadian; Subliminal; Reward; Anxiety

1. Introduction

The steroid hormone testosterone (T) is produced by the gonads and the adrenals of both sexes. T is well known to be involved in aggression and dominance in mammals, including humans. In rodents, the winner of a dominance challenge has an increase in T; the loser often shows a decrease, e.g., [1]. Studies in primates often find higher T in dominant individuals [2,3]. In humans, T is sometimes found to relate positively to status in both men and women [4–6]. The association between T and status probably is due to a bi-directional causal relationship [7]; as in other species, manipulations in status lead to changes in T in humans. For example, T rises after winning contests such as tennis matches or chess games; recent medical school graduates had higher T than other medical students [4,8]. In turn, higher T may cause individuals to be less averse to dominance challenges from others: animals with higher T are less likely to submit and more likely to win in future challenges [1,9,10], and in humans, T influences the decision to re-enter competition [11]. These findings are consistent with recent applications of the "challenge hypothesis" [12] to primates. T is hypothesized to rise during contexts associated with mating-related competition in order to motivate competitive and aggressive behavior; in turn, success in such competitions and the associated increase in

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status lead to further increases in T [13,14]. This framework is supported by various lines of evidence in humans [13]. Most relevant to the present research is the finding, in humans, of a reciprocal relationship between T and responses to faces displaying anger [15–17], which will be discussed further below. These findings provide suggestive evidence for relationships between T and approach towards dominance challenge-related cues in this species.

Perceived facial expressions of emotion (FEEs) are important social stimuli that can serve as signals of emotional state and thus future behavior of the displayer [18]. As such, even non-conscious viewing of FEEs can impact the viewer's behavior [19,20]. FEEs of anger are particularly salient, assumedly since they signal imminent verbal or physical attack. Individuals selectively attend to anger faces [21,22]; aversive stimuli condition particularly well to anger faces [23,24]; and anger faces activate brain regions involved in emotional salience and emotional learning, such as the amygdala [25,26].

Van Honk et al. [16] demonstrated that selective attention to anger faces in a face version of a Stroop task depended on basal T levels in men and women. T appears to have a causal effect on response to anger faces: T administration increased heart rate responses to anger faces but not to joy faces [17]. These findings suggest that T levels may impact the salience of anger faces, which are important dominance and/or dominance-related cues for humans. However, the valence, if any, of that salience remains to be determined; i.e., whether anger faces are perceived as signals to approach or to avoid to high T individuals. In general, subjects could preferentially attend to anger faces because they signify threat, and are therefore aversive [27]. However, it is also conceivable that a signal of an impending dominance challenge could be rewarding to individuals that have a history of success in such encounters, or, alternatively, that an anger face signifies the sender's frustration and thus represents a positive outcome of such a challenge for the perceiver. If basal T is a marker of dominance status, it is conceivable that anger faces have approach-related properties for individuals high in T relative to their sex.

In Study 1, we aimed to evaluate whether the incentive value (rewarding/aversive properties) of anger faces differs according to basal T. To best investigate the rewarding or punishing effect of a stimulus, subjects should be trained on an arbitrary behavior that is then associated with the stimulus; subjects' subsequent performance of the behavior will reflect the degree to which they are willing to work for the stimulus [28,29]. We used a differential implicit learning task (DILT) in Study 1 to investigate the degree to which subjects find anger faces reinforcing. In the DILT, separate sequences of actions are paired with presentations of anger faces, neutral faces or a blank screen. After training, performance on the three sequences is measured in the absence of face stimuli. Prior to the DILT, saliva was collected for T measurement.

Importantly, the relationship between T and selective attention to anger faces in van Honk et al. [16] was revealed for a T measurement taken 6 h prior to the Stroop task. The authors refer to the classical, relatively slow, genomic pathway of steroid hormone action to explain this effect. However, steroid hormones can also affect neuronal transmission via fast-acting, membrane-bound receptors, e.g., [30]. Note also that T, like many other steroid hormones, displays a robust diurnal rhythm with a morning peak [31–33]. Since T measurements in van Honk et al. [16] began in the morning and Stroop testing took place in the afternoon, an alternate explanation for the six-hour delay is that peak T is a better predictor of T-related behavior than levels of T as they decrease throughout the day. As a parallel, evidence exists for greater stability in waking (peak) cortisol, and relationships between waking cortisol and behavioral measures that do not appear for cortisol measured later in the day [34].

To test whether peak T better predicts attention to anger faces, in Study 2 we collected saliva and measured attention to anger faces at 9:00 and 15:00 in a within-subjects design. If T only predicts attention to anger faces after a six-hour delay, we would expect T at 9:00 to predict attention at 15:00 but not at 9:00. If peak T is the crucial measure, on the other hand, T at 9:00 should predict attention both at 9:00 and 15:00. In order to measure attention directed at anger faces, we utilized two tasks: a Stroop task similar to that used by van Honk and colleagues [15,16], and a dot-probe task [35], both using face stimuli. We expected that relationships between T and attention directed at anger faces could differ between these two tasks, as they appear to capture distinct mechanisms of attention [22,36]. In addition to anger faces, joy faces were included for comparison in both tasks in Study 2.

Finally, since conscious awareness of faces can change effects on brain activity, hormones, and behavior [15,22,37–39], we used two presentation times for face stimuli in both Studies 1 and 2, one allowing for conscious awareness of the face and one not (as tested repeatedly in our laboratory [40,41]).

2. Study 1: methods

Participants were 41 women and 29 men, mean age 21.5± 3.7 years, recruited by advertisement and paid $25 for approximately 2.5 h of participation. The study had received prior approval by the University of Michigan Institutional Review Board, and all participants provided informed consent. Sessions took place between 9:00 and 18:00. These participants are a subset of a larger sample recruited for a study on the incentive values of FEEs; effects of implicit motives on the incentive values of these stimuli have been reported elsewhere [41]. During the session, subjects first provided a saliva sample, which was later analyzed for T. Then, subjects worked on the DILT task on a computer, as described below. The task was administered on a computer, and was programmed using Experimental Run Time System software (ERTS; Berisoft Corp., Frankfurt, Germany).

For the DILT, subjects were required to press a key corresponding to one of four screen locations at which an asterisk could appear. They were instructed to do so as quickly and accurately as possible. During the DILT "learning" phase, asterisks appeared in three distinct repeating sequences of 12 keypresses each. Sequences were differentiated by color of asterisks; thus, sequence color served as a discriminatory stimulus. Subjects were simultaneously engaged in a distractor

task (counting randomly presented tones) which prevented conscious awareness of the sequences. One sequence was always followed by presentation of an anger face on the screen, another sequence was followed by the same face with a neutral expression, and the third sequence had no face associated with it (mask only.) Sequence-face pairings were held constant within subjects and counterbalanced across subjects. Stimulus faces, one of each gender showing an angry and one a neutral expression, all Caucasian, were taken from Matsumoto and Ekman's slides [42], and were cropped cheekbone to cheekbone and hairline to chin. Sequences were presented in 18 blocks, with each sequence presented 3 times in random order in each block. Face presentation time was varied between subjects (36 subjects in each condition), and was either 12 ms or 209 ms. In both cases, the face was followed by a mask (scrambled face), presented for 80 ms. These presentation times were chosen based on pilot trials that indicated that no conscious recognition of faces occurred when presented at 12 ms and masked; faces were easily recognized at 209 ms. Awareness tests confirmed that participants could recognize faces and emotion of faces presented at 209 and not at 12 ms; see Schultheiss et al. [41]. Face gender was also varied between subjects.

During the DILT "extinction" phase, subjects engaged in the task with no faces presented. They performed on the same three sequences on which they had previously worked ("fixed" sequences) as well as random presentations of the asterisk ("random" sequences) using the same three colors of asterisks. Response times were recorded by the software. Following the "extinction" phase, subjects underwent awareness tests to confirm that they had not become consciously aware of the three sequences, and that face stimuli presented at 12 ms were not consciously recognized. Subjects performed at chance levels at identifying faces presented at 12 ms; they showed close to 100% accuracy at identifying faces presented at 209 ms. A more detailed description of the DILT and the awareness tests appears in Schultheiss et al. [41].

For saliva collection, participants used sugar-free chewing gum to collect 7.5 ml saliva in a sterile polypropylene vial. After three freeze–thaw cycles with subsequent centrifugation to free samples from mucopolysaccarides and other residuals, T was assayed in saliva samples via radioimmunoassay, using a DPC Coat-A-Count testosterone RIA kit (Diagnostic Products Corporation, Los Angeles, CA) with a modified protocol for saliva [43]. T was measured using 400 μl saliva samples in combination with water-diluted standards (analytical range: 5 to 400 pg/ml). Free (unbound) steroid hormones enter saliva by migrating through cell membranes. T measured in saliva, then, is a measure of bioactive T. Saliva measurements correlate well with plasma measurements of free T [44,45]. In this assay, lower limit of detection (B0−3∗SD) was 2.5 pg/ml. Mean intra-assay coefficient of variation was 9.8% for female and 14.9% for male samples.

Statistical analyses for both Studies 1 and 2 were conducted with SYSTAT 10 and involved repeated-measures regression and ANOVA analysis, correlation analysis, Fisher's post-hoc comparison and *t*-tests. Descriptive statistics are given as mean ± SD. An alpha level of 0.05 (two-tailed) was employed in all analyses.

3. Study 1: results

Implicit learning of the sequences was demonstrated by significantly faster response times on "fixed" sequences (358 ± 9 ms) than on "random" sequences (387 ± 8 ms) in the extinction phase. Repeated-measures ANOVA on response times, with fixed vs. random and sequence type (emotional face, neutral face, no face) as within-subjects factors, revealed a main effect of fixed vs. random, $F(1, 71) = 76.23$; $P < 0.000001$. Overall, the interaction between fixed vs. random and sequence type was not significant; in other words, across all subjects, learning did not differ as a function of what kind of face stimulus was associated with a given sequence.

Mean salivary T levels were 93.4 ± 24.8 pg/ml in men and 27.8 ± 18.3 pg/ml in women. T levels were converted to *z*-scores within each sex to allow comparison across sexes. T levels in women were not normally distributed, and so were log-transformed prior to calculating *z*-scores. All analyses using T were performed using these T *z*-scores.

Experimental sessions took place at various times of day. In both men and women, there appeared to be a negative relationship between time of day and T; however, it failed to reach significance (men: $R = -0.194$; $P = 0.314$; women: $R = -0.170$; $P = 0.321$). It appears that in this sample, individual differences in T overwhelmed differences due to the daily T rhythm. Time of day was therefore excluded from further analyses.

To test for effects of experimental conditions and T levels on participants' learning gains, we calculated learning scores for each sequence type (i.e. anger, neutral, no face) by subtracting response times on fixed from response times on random sequences, such that a higher difference score (in milliseconds, ms) indicates better learning on that sequence.

Repeated-measures regression analysis revealed a nearly significant testosterone × face gender × sequence type interaction on learning, $F(2, 132) = 2.89$; $P = 0.050$. Restricting the analysis to subjects assigned to the 12 ms presentation condition, the T × face gender × sequence type interaction became significant, $F(2, 60) = 3.98$; $P = 0.024$. This interaction was not significant in the 209 ms presentation condition ($F(2, 64) = 0.91$; $P = 0.4$). The moderating role of stimulus presentation condition is also reflected in a trend towards a T × face gender × condition × sequence type interaction in the whole sample ($F(2, 124) = 2.51$; $P = 0.08$). These interactions were not moderated by participant sex.

Correlations between T and learning scores, divided according to face gender and stimulus presentation condition, are shown in Table 1. In the 12 ms male face condition, T shows

Table 1
Correlations between testosterone *z*-scores and learning advantage scores

Face presentation time:		Sequence type		
		Anger face	Neutral face	No face
12 ms	Female faces	−0.298	0.273	0.078
	Male faces	0.455*	−0.221	0.133
209 ms	Female faces	0.189	−0.053	−0.076
	Male faces	0.026	−0.339	−0.352

* $P < 0.07$.

a marginal positive correlation with learning on anger face sequences, but is uncorrelated with learning on neutral face sequences and no-face sequences (see Table 1). Correlation coefficients for T and learning on anger vs. neutral face sequences were found to significantly differ by Fisher's post-hoc comparison, $Z=2.66$, $P<0.004$. A trend existed towards the difference of correlation coefficients for T and learning on anger vs. no-face sequences, $Z=1.33$, $P<0.1$. No significant correlations were found between T and learning with 12 ms female faces, or with either face gender in the 209 ms condition.

4. Study 1: discussion

In Study 1, the significant T × face gender × sequence type interaction, as well as the significantly different correlation coefficients, indicate that endogenous T levels affected the degree of instrumental learning of sequences associated with sub-threshold anger faces. Specifically, higher T predicted better learning of sequences associated with sub-threshold male anger faces, in contrast to neutral faces, female faces or supra-threshold faces, in which T did not relate to sequence learning.

Participants' improved or impaired learning on sequences paired with anger faces compared to sequences paired with neutral or no faces indicates a rewarding or aversive quality of the anger face, respectively, compared to the neutral/no face stimulus. Though the DILT does not reflect pure instrumental conditioning, because face presentation during learning did not depend on participants' performance on the sequences, association of a rewarding or aversive stimulus with an instrumental task can affect learning of the task via mixed Pavlovian/instrumental learning [28,41]. Results of Study 1 therefore suggest that sub-threshold male anger faces have positive reinforcing qualities (or at least greater salience) for high-T, and negative reinforcing qualities for low-T participants. However, a limitation of Study 1 is that time of day was not controlled; stronger results may have been obtained with a more reliable measurement of T. Study 2 was therefore designed specifically to address whether T relates differently to attention to anger faces at different times of day. Nonetheless, results of Study 1 indicate that, despite circadian fluctuation, T measured at the time of the task can be predictive of behavioral responses to dominance/threat stimuli.

5. Study 2: methods

Participants were 26 men and 26 women, mean age 20.8 ± 2.5 years. Participants were recruited through advertisements announcing a paid research study, as well as through introductory psychology subject pool. The former received $20 and the latter received 2 h of experiment completion credit for their time. The study had received prior approval by the University of Michigan Institutional Review Board, and all participants provided informed consent. One woman did not complete the morning Stroop task, and was excluded from all Stroop analyses. Due to hormone assay error, T measurement was not available for another woman. Thus, the complete data set comprised data from 26 men and 24 women.

All participants attended an experimental session at 9:00 and another at 15:00 on the same day. Sessions lasted 45–60 min. All tasks were administered on a computer, and were programmed using ERTS software, as in Study 1. In each session, participants provided a saliva sample while completing a selection from the University of Wales Mood Adjective Check List [46]. They then completed a Face Stroop Task and a Face Dot-Probe Task (details below) for assessment of attention directed at FEEs of joy and anger, shown at two presentation times. In the morning session, participants also completed a Picture-Story Exercise for assessment of implicit motives to test other hypotheses. In the afternoon session, participants completed the sub-scales of the Personality Research Form (PRF) [47] assessing dominance, aggression, and affiliation; a forced-choice task to assess awareness of the FEEs at the two presentation times; and a biographical data questionnaire, including questions about time in menstrual cycle and oral contraceptive status.

As in Study 1, face stimuli were taken from Matsumoto and Ekman's slides [42], and were cropped cheekbone to cheekbone and hairline to chin. Eight posers per emotion (joy and anger) were chosen along with their corresponding neutral faces; two of each gender and race (Japanese and Caucasian). Face stimuli for the Face Stroop Task were modified using Adobe Photoshop to be predominantly bright yellow, blue, red, or green in color. In both tasks, face stimuli were presented in some trials for 22 and in some trials for 245 ms. Again, extensive testing in our laboratory has confirmed that faces shown at 200 ms or longer are easily recognized, whereas participants perform at chance levels at recognition of faces shown for 22 ms [40,41].

In the Face Stroop Task, participants were provided with microphones and were instructed to keep their attention on the fixation cross and then name the color of face/mask stimuli that appeared on the computer screen as quickly and accurately as possible. In a trial, after a fixation cross was presented, a yellow, blue, red or green face was presented mid-screen on a black background at either 22 or 245 ms, followed by a mask (scrambled face) of a color matching the face. Presentation of face or mask terminated upon initiation of participant's vocal response, and response time was recorded by the software as time between onset of face presentation and onset of vocal response. Participants completed practice trials to ensure task comprehension and that the microphone was working prior to the actual task. In the task itself, all 32 faces were shown in all 4 colors at both presentation times, in a randomized fashion, for a total of 256 trials (2 [face gender] × 2 [face race] × 2 [poser] × 2 [joy/anger] × 2 [emotional/neutral] × 4 [color] × 2 [stimulus presentation time, SPT] within-subjects design). The entire task lasted approximately 8 min. Slower response times in such color-naming tasks are considered indicative of interference due to emotional salience of the stimulus; i.e., an "emotional Stroop effect", e.g., [48].

The Face Dot-Probe Task was modified from the task developed by Mogg and Bradley [35,49]. In this task, participants are instructed to fixate on the cross and then press a key (left or right CTRL) to indicate whether a dot appeared on the left or right side of the screen after presentation of stimuli. They are instructed to respond as quickly and accurately as

possible. No specific instructions are given whether to attend to stimuli that appear prior to the dot. In a trial, following fixation cross presentation, two faces are presented, an emotional (joy or anger) and neutral face of the same poser, on the left and right sides of the screen, for 22 or 245 ms, followed by masks shown for 69 ms. Following the masks, a dot appears on the left or right side of the screen. Stimuli were displayed on a black background; dots were white. Each of the 16 pairs of faces was displayed in 8 types of trials: with the emotional face on the right and the left, the dot probe appearing on the right and the left, and for both presentation times (22 and 245 ms), for a total of 128 types of trials (2 [face gender]×2 [face race]×2 [poser]×2 [joy/anger]×2 [emotion location]×2 [probe location]×2 [SPT] within-subjects design). Participants performed 2 blocks each containing all 128 types of trials, presented in a randomized fashion, for a total of 256 trials. Response times were recorded by the experimental software. Prior to the task, participants completed 24 practice trials with feedback if they pressed the wrong key or did not respond before 1 s. The task itself took approximately 5 min. Participants are faster at responding to the dot if their attention is already directed at the side of the screen where the dot appears. Therefore, dot-probe tasks assess attention directed toward a stimulus, in this case an emotional face, by comparing reaction time when the dot and the stimulus appear on the same side of the screen vs. when the dot and stimulus appear on opposite sides of the screen [35,40,49].

Saliva samples were collected, processed and assayed as described in Study 1. Lower limit of detection (B0–3*SD) was 1.2 pg/ml. Mean intra-assay coefficient of variation was 11.7%.

6. Study 2: results

Mean T in women was 12.3±6.8 pg/ml in the morning (T1) and 8.4±5.5 pg/ml in the afternoon (T2). Mean T in men was 72.1±20.3 pg/ml in the morning and 52.5±18.1 pg/ml in the afternoon. Paired *t*-tests revealed a significant drop in T between the two measurements in both sexes (women, T(25)=3.09, $P=0.005$; men, T(25)=5.61, $P<0.0001$).

Ten of the 24 women reported using hormonal contraceptives. Morning T was significantly lower in women using hormonal contraceptives (8.9±3.8 pg/ml) compared to women who were not (14.6±7.4 pg/ml), T(23)=2.23, $P=0.035$. Afternoon T did not significantly differ between the two groups (7.1±5.2, women using vs. 9.3±5.8 pg/ml, women not using hormonal contraceptives). Because of this difference, a factor coding for hormonal contraceptive status (PILL) was added as a covariate in all analyses, but it exerted no moderating effects on the findings reported below.

6.1. Face Stroop Task

Response times on Stroop faces were averaged across face color, face race and poser (4 faces each in 4 colors=16 response time variables), as these factors were not hypothesized to influence emotional Stroop effects. This left 5 within-subjects factors, for a 2 [morning/afternoon, 'AM/PM']×2 [stimulus presentation time, 'SPT']×2 [face gender]×2 ['JOY/ANGER']×2 [emotional/neutral, 'EMO/NEUT'] within-subjects design.

Table 2
Reaction times to name the color in the emotional Face Stroop Task, in ms: *M* (SD)

Time of day and SPT[a]		Face emotion			
		Anger	Neutral[b]	Joy	Neutral[c]
Morning (9:00)					
22 ms	Female faces	636 (125)[d]	614 (132)	622 (142)	610 (133)
	Male faces	620 (145)	615 (133)	619 (135)	618 (128)
245 ms	Female faces	636 (143)	642 (138)	652 (149)	653 (146)
	Male faces	640 (139)	618 (129)	631 (139)	638 (156)
Afternoon (15:00)					
22 ms	Female faces	573 (76)	570 (76)	584 (88)	587 (96)
	Male faces	579 (91)	562 (80)	575 (75)	575 (89)
245 ms	Female faces	591 (90)	577 (81)	600 (92)	593 (91)
	Male faces	579 (91)	577 (87)	576 (81)	584 (94)

[a] SPT: stimulus presentation time.
[b] Neutral faces of the same posers as the anger faces.
[c] Neutral faces of the same posers as the joy faces.
[d] Note that these reaction times are averaged across picture set and face race (four faces per data point.)

Within-subjects regression analysis with factors AM/PM, SPT, face gender, JOY/ANGER and EMO/NEUT demonstrated that participants had longer response latencies in the morning, to long SPT (i.e., 245 ms) faces, and to female faces (main effects of AM/PM, SPT, and face gender; Table 2). Participants also tended to display longer response latencies to anger faces compared to the corresponding neutral faces, suggesting an overall Stroop interference effect for anger faces. In comparison, there was no difference in response times between joy faces and their corresponding neutral faces. This is reflected in a trend for a JOY/ANGER×EMO/NEUT interaction, $F(1, 50)=3.60$, $P=0.06$. Follow-up *t*-tests comparing response times on emotional faces vs. their corresponding neutral faces identified longer response latencies for 245 ms male anger faces in the morning (T(50)=2.77, $P=0.008$), for 22 ms male anger faces in the afternoon (T(50)=2.15, $P=0.037$), and for 245 ms female anger faces in the afternoon (T(50)=1.95, $P=0.046$). No significant effects were found for joy faces.

Regression analysis on all 5 within-subjects factors with morning T (T1) as a between-subjects factor and participant sex as a covariate revealed a significant interaction of SPT×face gender×JOY/ANGER×EMO/NEUT×T1, $F(1, 47)=4.64$, $P=0.036$. This effect was not moderated by AM/PM, participant sex or PILL. Follow-up analyses traced this effect to a positive relationship between T1 and the Stroop interference effect for 22 ms female anger faces, reflected in a positive correlation between T1 residuals after the effect of participant sex was regressed out (TR1) and morning response time residuals for 22 ms female anger faces after regressing out 22 ms female neutral faces ($R=0.35$, $P=0.01$; Fig. 1). Thus, there is a positive relationship across both sexes between morning T and Stroop interference (at both times) for female anger faces presented too quickly for conscious awareness.

However, a similar regression analysis on afternoon T (T2) including participant sex as a factor revealed a significant

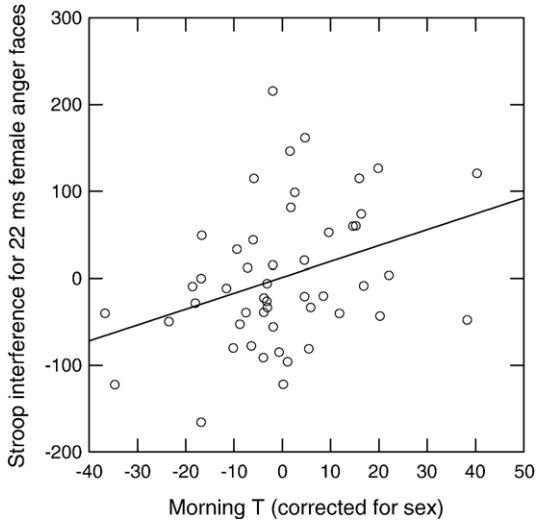

Fig. 1. Stroop interference on short-SPT (22 ms) female anger faces in the morning, as a function of morning salivary T. T values shown are residuals after regressing out effects of participant sex. *Y*-axis shows reaction time residuals for color-naming latency on short-SPT female anger faces at 9:00 after regressing out reaction times on short-SPT female neutral faces at 9:00.

interaction of AM/PM × face gender × JOY/ANGER × EMO/NEUT × participant sex × T2, $F(1, 46)=11.87$, $P=0.001$. This effect was not moderated by SPT or by PILL. Follow-up analyses traced the effect to a negative correlation in women between T2 and Stroop interference effect for female anger faces in the morning. The important variable appeared to be drop in T: change in T (ΔT), calculated by subtracting T2 from T1, positively predicted Stroop effects for female anger faces (collapsed over SPT) in the morning in women, $R=0.56$, $P=0.004$ (Fig. 2). Exclusion of women using hormonal contraceptives left the correlation intact, $R=0.77$, $P<0.001$. This effect was not present in men, for joy faces, or for anger faces in the afternoon in women.

6.2. Face Dot-Probe Task

Evidence for an overall attentional orienting effect appeared in the first set of Caucasian faces (the set among the Matsumoto and Ekman faces rated as expressing emotion most reliably and powerfully, [42]). Regression analysis for response times on trials using these faces revealed a significant interaction of SPT × emotional face location (EMOLOC) × dot probe location (PROLOC), $F(1, 51)=5.07$, $P=0.029$. This effect was not moderated by face gender or by emotion (joy vs. anger). Follow-up analyses localized the effect to 245 ms face presentation trials, in which participants were slower to respond when the emotional face and the dot appeared in the same location, a possible attentional avoidance effect.

Attentional bias scores were created separately for the within-subjects factors AM/PM, SPT, face gender, face race, and emotion by subtracting the average of response times on trials in which the emotional face and dot probe appeared on the same side of the screen from the average of response times on trials in which the emotional face and probe appeared on opposite sides of the screen (i.e., avg(LR, RL) − avg(RR, LL)). Positive attentional bias scores, then, reflect faster response times when the emotional face and the dot appeared on the same side of the screen, which indicates that participants' attention was drawn to the emotional face.

Regression analysis on morning attentional bias scores with SPT, face gender, face race, and emotion as within-subjects factors and TR1 (morning T with effects of participant sex regressed out) as a between-subjects factor revealed a significant interaction of SPT × face race × emotion × TR1, $F(1, 49)=4.77$, $P=0.034$. This interaction was also significant for TR2 (afternoon T with effects of participant sex regressed out), $F(1, 49)=5.81$, $P=0.020$. Neither participant sex nor hormonal contraceptive status (PILL) moderated the interaction. The effect was traced to a relationship between T and morning

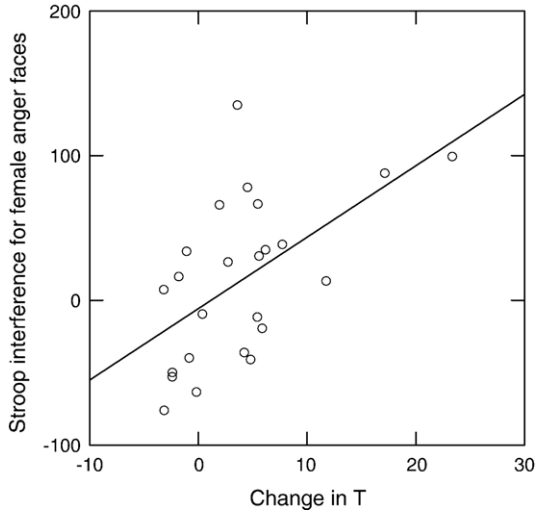

Fig. 2. Stroop interference on female anger faces in the morning as a function of drop in T (morning T- afternoon T) in women. *Y*-axis shows reaction time for female neutral faces (averaged over SPT) subtracted from reaction time for female anger faces.

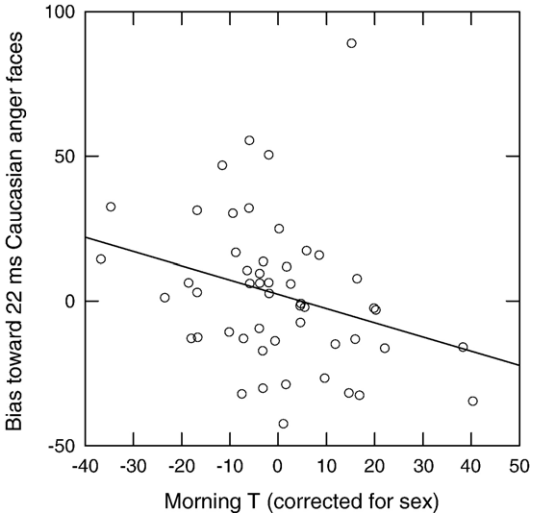

Fig. 3. Attentional bias toward short-SPT Caucasian anger faces in the morning as a function of morning T (residuals after regressing out effects of participant sex). Positive attentional bias scores indicate attention directed toward and negative scores indicate attention directed away from anger faces in the dot-probe task.

attentional bias to Caucasian anger faces shown for 22 ms: There was a negative correlation between TR1 and attentional bias toward short-SPT Caucasian anger faces (averaged over face gender and poser), $R=-0.29$, $P=0.038$ (Fig. 3). In addition, a trend for a negative correlation existed between afternoon T (TR2) and averaged attentional bias toward short-SPT Caucasian anger faces, $R=-0.26$, $P=0.064$. No relationships existed between TR1 or TR2 and attentional bias toward long-duration anger faces, or afternoon bias toward anger faces. There were no consistent relationships between TR1 or TR2 and attentional bias toward joy faces or toward Japanese faces of either emotion.

7. Study 2: discussion

Results of Study 2 show relationships between T, particularly measured in the morning, and attention directed, in the morning and afternoon, at sub-threshold (22 ms) anger faces in both a Stroop and a dot-probe task. In the Stroop task, participants' morning T related to longer latencies to name the color of sub-threshold anger faces, and in a dot-probe task, morning T predicted attentional bias away from these stimuli.

The different pattern of effects on the dot-probe and Stroop tasks is not surprising given literature demonstrating very different mechanisms of attention captured by lexical versions of these two tasks [36]. This makes conceptual sense considering the different nature of the two tasks: in the Stroop task, subjects must attend to the color of the stimulus in front of them while ignoring its content, whereas in the dot-probe task, subjects have a choice of two stimuli to attend to and their responses are not tied to the stimuli per se. Also, increased anxiety increases attentional orienting towards anger faces in the dot-probe task [35,50], whereas anxiety was found to have no relation to Stroop interference to anger faces [22]; rather, trait anger and a measure of the behavioral activation system (BAS) were related to Stroop interference, which the authors describe as greater vigilance for the anger faces. Putman and colleagues [22] suggest that BAS promotes an approach-orientation towards signals of dominance challenge, as reflected in greater interference to anger face stimuli in a Stroop task, whereas the behavioral inhibition system (BIS), which is related to anxiety (e.g., [51]), promotes vigilance towards the threatening nature of dominance challenges, and is reflected in greater attentional orienting towards anger faces in the dot-probe task as found by Mogg and Bradley (e.g., [35]). As discussed further below, T is related to greater sensitivity to reward (i.e., BAS) and decreased anxiety (related to BIS). T could decrease vigilance towards anger faces in the dot-probe task by reducing BIS/anxiety, while simultaneously increasing attention to anger faces in the Stroop task by increasing anger and/or dominance. In this vein, the present data underscore the fact that the Stroop task is probably better suited to capture dominance and anger-related attention (which are associated with BAS or approach motivation) than is the dot-probe task, which seems better suited to measure anxiety-related vigilance.

Notably, in Study 2, morning T was the most important variable in predicting morning and, to some extent, afternoon Stroop interference and attentional bias to anger faces. Our findings suggest that, unlike what was reported in van Honk et al. [16], a six-hour delay is not necessary to predict responses to anger faces with T; rather, it is possible that peak (i.e., morning) T is what drives some of the behavioral effects of T. In the present study, T levels predicted behavior on tasks performed soon after saliva sampling; this could be explained either by fast actions of T or its metabolites at membrane-bound receptors, or a longer-term (over days or weeks) adaptation of the brain to typical peak T levels. It also should be noted that though the adrenal is a significant source of T in women, the effects of T on responses to anger faces do not seem to be due to a general effect of circadian effects on adrenal hormones; we also assayed cortisol in samples in Study 2 (unpublished data) and found no relationships between cortisol and attentional orienting or Stroop interference to anger faces.

8. General discussion

The present results provide further evidence that responses to facial expressions of anger depend on basal levels of circulating T. In Study 1, we showed evidence that the incentive value of anger faces positively depends on T: study participants' later performance on sequences that had been associated with sub-threshold (i.e., too fast for conscious awareness) presentations of male anger faces depended on T levels relative to their sex. Results from Study 2 corroborate others' demonstrations [16,17] that basal T levels relate to attention directed at anger faces: in a face version of the emotional Stroop task, participants' morning T related to greater interference for sub-threshold anger faces, suggesting that endogenous T increases vigilance toward signals of dominance challenge. In a dot-probe task, on the other hand, morning T predicted attentional bias away from anger faces, which is consistent with effects of T to decrease anxiety.

Notably, relationships between T and responses to anger faces in Study 1 and in the Stroop and dot-probe tasks in Study 2 were only found for faces presented too quickly for conscious awareness. Face stimuli have been shown to have effects on the brain and behavior in the absence of awareness, e.g., [26]. Also, in many studies it is found that only faces presented outside of awareness interact with hormone levels [15,22], anxiety [52], and activation of brain regions involved in automatic emotional responses [37]. It is thought that higher-level processing of emotional stimuli may allow for inhibition of more automatic responses to these stimuli (see, e.g., [53]). For example, more sophisticated perceptual processing possible at longer exposure times could reveal that the faces are only photographs shown on a computer screen and thus could allow for down-regulation of the responses to them generated by the brain's emotional centers.

It is also of interest that in the present studies, the pattern of relationships between T and responses to anger faces was the same in both sexes. It appears that the crucial variable in these measures is relative T levels compared to one's own sex. This finding is in line with research supporting a connection between T and aggression/dominance in both sexes [5,54], despite far greater T levels in men. Similarly, T affects responses to anger faces in both sexes in a similar manner [16,17]. A related issue is effects of hormonal contraceptives and menstrual cycle status

on T-behavior relationships in women. Despite lower T levels in women using hormonal contraceptives, this variable did not moderate effects of T on responses to anger faces. Menstrual cycle effects on T are small in comparison to circadian fluctuations [31], and our samples are too small to reliably assess whether cycle phase moderates the results. However, given evidence that cycle phase influences anger reactivity [55], larger-scale studies with multiple sessions in different cycle phases would be helpful to address whether women display more approach toward and less avoidance of anger faces at times in the cycle when T is highest.

As discussed in the Introduction, T is well-known to be associated with dominance and aggression in humans and other species. The relationship between T and dominance appears to be reciprocal: winners of dominance challenges show increases in T, and in turn, higher T leads to a greater likelihood to aggress and/or to pursue further dominance challenges, in nonhuman animals and potentially also in humans [1,7,11,13]. To a human viewer, facial expressions of anger are important social signals that can signal impending social threat and/or dominance challenge. T could affect the salience and/or incentive value of such a signal to the viewer: high-T individuals may be more willing to engage in dominance challenges and so may have more of an "approach" orientation towards a signal of such a challenge, whereas low-T individuals may avoid signals of impending dominance challenge [56]. The present results are also consistent with Archer's elaboration of the challenge hypothesis [13], in that higher T may reflect recent dominance successes or contexts relevant to mating competition, and these higher T levels in turn alter subjects' responsiveness to signals of a challenge to dominance/status.

In addition to its specific effects on dominance and aggression, a considerable literature links T to reward processes and inhibition of stress processes. T can induce conditioned place preference in animals, a measure of positive reinforcing qualities of a treatment, in part via its conversion to GABA-active neurosteroids [57–59]. T also tends to be inhibitory to anxiety and stress systems: it down-regulates the HPA axis [60,61] and anxiety-related behavior [62–64]. In humans, T is also related to reward and approach-motivation and reduces some aspects of anxiety. Van Honk et al. demonstrated that exogenous T administration disrupted performance on the IOWA gambling task [65], a task which requires adequate punishment-sensitivity (BIS) compared to reward-sensitivity (BAS) [66]. Specifically, T appeared to increase reward-sensitivity and/or decrease sensitivity to punishment. The same group has also demonstrated that T administration reduces fear-potentiated startle [67] and non-conscious responses to anxiety-related stimuli [68].

Given this pattern of effects of T, it is conceivable that higher basal T levels result in multiple, overlapping changes in response to threat signals such as anger faces. T could decrease anxiety and punishment sensitivity, and thus decrease aversion to threat signals in general; T could specifically reduce avoidance of dominance challenges; T also may be associated with increased anger [16], and thus could increase willingness to engage in dominance challenges, leading to greater vigilance to and positive reinforcing effects of anger faces in high-T individuals. These effects of T may be mediated in part through the amygdala, which is highly responsive to threat signals [25,26] and contains androgen receptors [69,70]. In fact, preliminary evidence from fMRI studies conducted in our laboratory (unpublished data) and by others [71] suggest a relationship between T and amygdala responsiveness to anger faces.

In conclusion, in Study 1 we have shown, in humans, evidence for a relationship between basal T levels and reinforcing properties of anger faces. In Study 2 we have shown that morning T predicts morning (i.e., without a delay) and afternoon attention directed at or vigilance to anger faces, and that there is an opposite relationship between T and attention to anger faces on a task that taps into BAS and vigilance (Stroop) and a task that taps into BIS and avoidance of threat (dot-probe). Future research should elucidate the brain mechanisms behind these relationships, as well as whether T influences behavioral responses to dominance-related stimuli in real-life situations.

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