Psychoneuroendocrinology 115 (2020) 104612

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Endogenous testosterone levels are predictive of symptom reduction with exposure therapy in social anxiety disorder

M.H.M. Hutschemaekers[a,b,*], R.A. de Kleine[c], M.L. Davis[d], M. Kampman[a,b], J.A.J. Smits[d], K. Roelofs[b,e]

[a] Overwaal Centre of Expertise for Anxiety Disorders, OCD and PTSD, Institution for Integrated Mental Health Care Pro Persona, Nijmegen, The Netherlands, Tarweweg 2, 6534 AM Nijmegen, the Netherlands
[b] Behavioural Science Institute, Radboud University Nijmegen, Montessorilaan 3, 6525 HR Nijmegen, the Netherlands
[c] Leiden University, Institute of Psychology, Wassenaarsweg 52, 2333 AK Leiden, the Netherlands
[d] Department of Psychology and Institute for Mental Health Research, The University of Texas at Austin, United States
[e] Donders Institute for Brain, Cognition and Behaviour, Center for Neuroimaging, Radboud University, the Netherlands

ARTICLE INFO

Keywords:
Endogenous testosterone
HPG-axis
Challenge hypothesis
Social anxiety disorder
Exposure therapy
Avoidance behavior

ABSTRACT

The Hypothalamus-Pituitary-Gonadal (HPG)-axis, and testosterone in particular, play an important role in social motivational behavior. Socially avoidant behavior, characteristic of social anxiety disorder (SAD), has been linked to low endogenous testosterone levels, and can be alleviated by testosterone administration in SAD. Although these beneficial effects of testosterone may translate to exposure therapy, it remains unknown whether testosterone increases prior to exposure improve therapy outcomes. In this proof-of-principle study, we tested whether pre-exposure (reactive and baseline) endogenous testosterone levels were predictive of exposure outcome in SAD. Seventy-three participants (52 females) with a principal SAD diagnosis performed four speech exposures: three during one standardized exposure therapy session and one at post-assessment one week later. Subjective fear levels were assessed before and after each speech exposure and social anxiety symptoms were assessed at pre- and post-treatment. Pre-treatment testosterone levels were assessed before (baseline) and in response to a pre-exposure instruction session (reactive). Pre-treatment testosterone levels were not related to fear levels *during* exposure therapy, but predicted pre- to post-treatment reductions in social anxiety symptom severity. Specifically, low baseline and high reactive pre-treatment testosterone levels were associated with larger reductions in social anxiety symptom severity. These findings support the role of HPG-axis in social fear reduction. Specifically, our finding that high reactive testosterone as well as low baseline testosterone predicted exposure outcome in SAD, suggests that good reactivity of the HPG-axis is a promising marker for the symptom-reducing effects of exposure therapy.

1. Introduction

Social anxiety disorder (SAD) is one of the most common anxiety disorders, with a lifetime prevalence rate of 13 % (Bandelow and Michaelis, 2015). Persistent avoidance behavior in SAD is a major factor that hinders extinction of fear during social situations (Arnaudova et al., 2017; Clark and Wells, 1995). Avoidance behavior is the target of exposure therapy, which, although it is a first-line treatment for the disorder, leaves ample room for improvement (response rates vary between 45–55 % and effect sizes are small to moderate, Hedges'g 0.48-0.62 - Carpenter et al., 2018; Hofmann and Smits, 2008; Loerinc et al., 2015). Accordingly, studying social avoidance and its biomarkers has the potential to improve outcomes for individuals with SAD and related disorders.

Produced by the Hypothalamus-Pituitary-Gonadal(HPG)-axis, testosterone constitutes an important regulator of social motivational behavior in general, including avoidance behavior (Hermans and Van Honk, 2006). The social challenge hypothesis (Wingfield et al., 1990), originally based on testosterone and aggression associations in monogamous birds (Wingfield et al., 2001) and later also established in primates (Muller and Wrangham, 2004) and humans (Neave and Wolfson, 2003; Bateup et al., 2002) is the most predominant theory of testosterone reactivity. It states that testosterone levels rise in preparation to a challenging encounter in which social status may be threatened, thereby initiating approach motivation and reducing fear (Archer, 2006; Bos et al., 2012). Consistent with this hypothesis, high

* Corresponding author at: Tarweweg 2, 6534 AM Nijmegen, the Netherlands.
E-mail address: m.hutschemaekers@propersona.nl (M.H.M. Hutschemaekers).

https://doi.org/10.1016/j.psyneuen.2020.104612
Received 27 September 2019; Received in revised form 18 December 2019; Accepted 6 February 2020

endogenous testosterone has been associated with social dominance and approach behavior (Maner et al., 2008; Mazur and Booth, 1998), and low testosterone levels have been linked to socially submissive, anxious and avoidant behavior (Archer, 2006; Josephs et al., 2006; Sapolsky, 1991). Importantly, reduced levels of endogenous testosterone have been found in those suffering from SAD (Giltay et al., 2012) and other social avoidance-related disorders such as depression (Almeida et al., 2008; Giltay et al., 2012).

The anxiolytic properties of testosterone have been linked to its effect on GABAergic transmission in neural fear circuits (Gutierrez-Garcia et al., 2009; McHenry et al., 2014) whereas the threat-approach facilitating properties have been linked to its effects on the amygdala and striatum (i.e., biasing the amygdala towards threat approach and reward anticipation, Radke et al., 2015; Hermans et al., 2010, respectively).

Relevant to the treatment of SAD, causal studies on the relationship between testosterone and fearful avoidance behavior, further confirm the social motivational aspects of testosterone. For example, administering testosterone to healthy participants prior to threat exposure has been shown to reduce fear, enhance reward sensitivity and promote social approach motivation (Bos et al., 2012; Enter et al., 2014; Terburg et al., 2016). When administered specifically in patients with SAD, testosterone alleviates social avoidance and promotes prosocial behavior, including increased eye contact as well as behavioral approach towards angry faces (Enter et al., 2016a). In addition, testosterone administration reduces automatic threat bias to angry faces in SAD patients (Enter et al., 2016b; van Peer et al., 2017). These findings converge to suggest that enhanced testosterone-reactivity prior to exposure therapy may facilitate its outcomes (Enter et al., 2018).

In light of the consistently established anxiolytic and prosocial properties of testosterone in SAD, it is remarkable that the association between pre-treatment testosterone and treatment efficacy has not yet been investigated. The present proof-of-principle study sought to test whether endogenous pre-treatment testosterone increases efficacy of a standardized exposure therapy session for adults with social anxiety disorder, as measured by fear levels during exposure and change in social anxiety symptoms following one standardized exposure session. In line with the challenge hypothesis, proposing that testosterone rises *in preparation* to a challenging encounter, we examined pre-treatment testosterone levels, both before (baseline) and in response to a pre-exposure instruction session (reactive). We hypothesized that participants with higher pre-treatment testosterone reactivity and baseline levels would show more fear decline during the session and greater reductions in self-reported social anxiety symptoms following the session.

2. Materials and methods

2.1. Participants

Seventy-three participants (52 females, M_{age} = 25.66, *SD = 7.48, range* = 18–50) diagnosed with SAD (principal diagnosis; i.e., the most important source of current distress), who endorsed fear of public speaking as their predominant fear were recruited at the University of Texas at Austin and in the Austin community. Exclusion criteria were: A) current use of corticosteroid medicines/testosterone enhancing products, B) a history of bipolar disorder or psychotic disorders, C) alcohol or substance use disorders in the past six months, D) significant suicidal ideation, E) current treatment for SAD and F) prior non-response to exposure therapy. Participants using psychotropic medication were allowed to participate in the study if they were on a stable dose of the medication for three weeks prior to the study. Participants received course credit for their participation.

All participants took part in a study examining the effects of pre-treatment power posing (i.e., holding postures associated with high and low power) for augmenting exposure therapy for SAD (Davis et al., 2017); clinicaltrials.gov/ct2/show/NCT02482805. The experiment was performed in accordance with relevant guidelines and regulations. Participants received one personalized exposure therapy session modeled after the procedures outlined by (see below (Rodebaugh et al., 2013), and were randomly assigned to submissive, dominant, or neutral power pose groups. In line with previous work (Ranehill et al., 2016; Simmons and Simonsohn, 2017), the findings, reported by Davis et al. (2017), revealed that engaging in power versus submissive posing resulted in no single differential effect in terms of symptom reduction, in-session fear responses nor with respect to testosterone responses (Davis et al., 2017). This paper also reported that there was no relation between testosterone reactivity to the *power pose manipulation* (i.e., pre- to post-posing) and the reductions in symptoms following exposure therapy. Therefore, to address the current research question, testing the effects of pre-treatment testosterone levels on therapy outcome, we could collapse the data across the power pose groups. The posing together with the therapy rationale and instructions formed a pre-treatment instruction period during which we measured testosterone reactivity, enabling for the first time testing the predictive effects of pre-treatment testosterone levels on exposure therapy outcome in a well-powered sample of patients with SAD.

2.2. Exposure session

Participants all completed one standardized exposure session, based on the protocol developed by Rodebaugh, Levinson, and Lenze (Rodebaugh et al., 2013). During this session, participants planned a 5-min speech exposure which they expected to elicit considerable fear (i.e. predicting a fear rating of 75 on a scale from 0 (no fear) to 100 (extreme fear)); participants were first familiarized with the rating scale and anchors). Participants completed the same speech-exposure three times during the session (i.e. 3 x 5 min) in front of a small public, including the therapist and 0–3 confederates. This method has been used in previous studies examining exposure effects in SAD (Powers et al., 2004; Ressler et al., 2004; Sloan and Telch, 2002; Smits et al., 2013; Telch et al., 2014; Wolitzky and Telch, 2009).

2.3. Outcome measures

2.3.1. In-session fear

Participants rated their highest fear level during the exposure (i.e., peak fear) using Subjective Units of Distress (SUDs) (Wolpe and Lazarus, 1966) scale (ranging from 0; no fear to 100; extreme fear) immediately after each of the four exposure practice exercises.

2.3.2. Symptom severity

Social anxiety symptom severity was assessed with the Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987), which asks participants to rate how fearful they would feel and how often they would try to avoid 24 different social situations during the past week. Scores range from 0 to 144 and the scale has sound psychometric properties (Heimberg et al., 1999; Safren et al., 1999). The LSAS was completed at pre-and post-treatment (one week after completion of the standardized exposure session).

2.4. Saliva measures

To assess endogenous testosterone, saliva samples were collected from the participants during their first visit to the clinic (i.e. pre-treatment and standardized exposure session) (2 ml passive drool saliva by Salicap; Hamburg, Germany) at different time points illustrated in Fig. 1: T1) 50 min prior to exposure (30 min after arrival), T2) 25 min prior to exposure, see procedure paragraph), T3) directly prior to exposure, T4) after exposure (around 20 min after start exposure) and T5) 50 min after the start of exposure. The samples were stored at −20 °C until radio immune assays were performed by Dr. Clemens Kirschbaum's laboratory in Dresden, Germany, for descriptions of specific

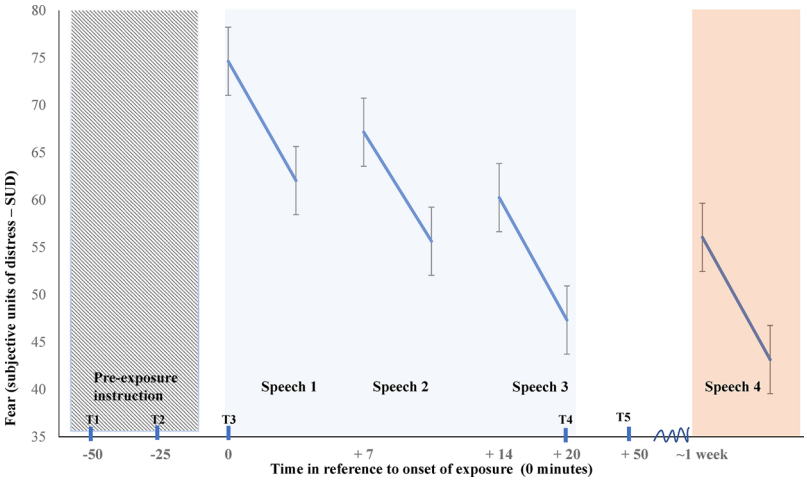

Fig. 1. The mean peak and mean end Subjective Units of Distress (SUDs) per Speech exposure from all 73 participants (speech 4, N = 66). The Figure also depicts the timing of testosterone samples (T1-5) relative to the Speech exposures that took place either during the exposure session (in the middle (blue) area: Speech 1–3) or at post-assessment (in the right (orange) area: Speech 4). The Exposure session was preceded by a pre-exposure instruction phase (black and white striped area) from which the relevant baseline and reactive testosterone samples were taken (T 1-3) to test effects of pre-treatment testosterone reactivity on therapy efficacy and outcome. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

methodology used by this laboratory, see: (Miller et al., 2013; Reardon et al., 2016).

2.5. Procedures

After informed consent, participants were screened for eligibility via questionnaires. All participants were telephoned afterwards for further screening (using the Mini-International Neuropsychiatric Interview (MINI; (Sheehan et al., 1998)) to assess for study inclusion and exclusion criteria), and were invited to participate in the study. After enrollment, participants were randomly assigned to a posture condition (power, submissive or no posture/rest). Saliva was collected at the mentioned time points in Section 2.4 and the posture manipulation protocol was performed. Afterwards, participants participated in the standardized exposure session. One week after the standardized exposure session, participants completed the same 5-min speech as during the exposure session to assess for post-treatment levels of speech fear. Fear levels were assessed at the beginning (initial) and immediately after all the speeches (end and peak SUDs). Participants completed the LSAS prior to the speech exposure session and at post-treatment. See (Davis et al., 2017) for a detailed description of the study procedures.

2.6. Statistical analyses

To test the hypothesis that testosterone reactivity in preparation for a challenging encounter facilitates fear reduction, we focused on pre-exposure testosterone levels: Testosterone reactivity was calculated for each individual, based on the absolute difference in testosterone levels from the start (Sample 1) to the pre-exposure sample (Sample 3). The resulting subtraction-value was divided by the start (sample 1) level to control for initial differences (for a similar method see Jiménez et al., 2012; Zilioli et al., 2014). We used sample 3 versus 1 to capture the full anticipatory period from arriving in the lab until the start of the first speech. In addition, we computed individual baseline testosterone levels by averaging both pre-power posing samples (1 and 2). For all statistical analyses, both reactive and baseline testosterone values were standardized per gender.

To test effects of testosterone responses on exposure outcome (in-session fear and symptom levels), we conducted separate mixed model analyses, using the Lme4 package in R (Bates et al., 2013). P-values were calculated using the Likelihood Ratio Tests using the mixed function in the Afex package (Singmann, 2013). We ran four separate models: namely for baseline and for reactive testosterone levels separately and with fear levels and symptom severity as dependent variables separately. In all models our effect of interest was the testosterone x time interaction. In all analyses the independent continuous predictors were centered and sum to zero contrasts were used. In line with recommendations for mixed models (Pek and Flora, 2018), we report unstandardized effect sizes (i.e. the estimates).

2.6.1. In-session fear analyses

For the analyses regarding fear levels, all peak SUD scores during the speech exposure session were modeled as the dependent variable. Testosterone (baseline or reactive) and Time (speech 1, 2 and 3, in the exposure session) were included as predictors (fixed factors). Participant was included as random slope and intercept and gender, age and initial symptom severity (i.e. baseline LSAS scores) were included as covariates. In addition to the in-session fear analyses, we conducted analyses to see whether testosterone levels were related to fear reduction across sessions (i.e. from speech 1 to speech 4 one week later), therefore the same analysis was repeated for fear levels with Time (Speech 1, Speech 4).

2.6.2. Symptom severity analyses

LSAS scores were the dependent variable; Testosterone (reactive or baseline) and Time (pre/post assessment) were included as predictors, Participant as random intercept, and Gender and Age as covariates.

3. Results

3.1. Sample characteristics

As expected, testosterone levels were higher for males compared to females (all p-values <0.001) and showed a negative (though non-significant) relation with age (correlations for males ranged from $-.17$ to $-.30$ and for females from $-.11$ to $-.22$, all p-values $>.050$). Log-transformations were performed to handle the non-normality of testosterone data. To be able to combine data of females and males, baseline testosterone was standardized per gender (see also (Tyborowska et al., 2016)). Means and standard deviations of the non-transformed data are presented in Table 1. Because we detected one multivariate outlier in the data for baseline as well as reactive testosterone, we repeated the analyses after winsorizing testosterone. For this procedure, extreme values were set to the second and 98th percentile of baseline and reactive testosterone to reduce the effect of spurious outliers. The results remained the same after this procedure (see suppl. page 4 for details).

3.2. In-session fear

The mixed model analysis for in-session fear levels with reactive testosterone as predictor showed that peak SUDs reduced over time, confirming that exposure resulted in the expected within-session reductions in fear levels, *Estimate* $= -7.19(0.97)$, $F(1,70) = 55.46$, $p < .001$. Peak

Table 1
Participants characteristics per gender.

Variable	Females (n = *52*) Mean *(SD)*	Males (n = *21*) Mean *(SD)*	Total sample (N = 73) Mean *(SD)*
Age	25.25 (*6.88*)	26.67 (*8.91*)	25.66 (*7.48*)
LSAS (pre)	80.33 (*22.35*)	61.10 (*17.58*)	74.79 (*22.43*)
LSAS (post)	70.74 (*24.72*)	53.58 (*19.87*)	66.01 (*24.58*)
T-sample 1	21.95 (*17.34*)	169.60 (*94.50*)	
T-sample 2	22.45 (*24.87*)	145.23 (*84.33*)	
T-sample 3	19.93 (*21.99*)	146.10 (*106.26*)	
T-sample 4	19.68 (*20.35*)	147.28 (*95.54*)	
T-sample 5	16.44 (*15.68*)	132.96 (*63.35*)	
Baseline testosterone	22.20 (*19.24*)	157.41 (*58.06*)	
Testosterone reactivity	−.05 (*0.26*)	−.03 (*0.07*)	

T-sample = testosterone sample.
Note: Testosterone levels are in pg/ml. Some of the participants did not fill out the LSAS at post-assessment. Therefore $n = 69$ for post-assessment values.

SUDs diminished over the three speeches ($M_{speech1} = 74.63$, $SD = 16.58$; $M_{speech2} = 67.15$, $SD = 14.01$; $M_{speech3} = 60.25$, $SD = 17.71$). A main effect of Gender, *Estimate* = −4.38(1.87), F(1,67) = 5.33, $p = .024$, further indicated higher SUD scores for females ($M = 70.05$, $SD = 16.19$), as compared to males ($M = 60.65$, $SD = 17.73$). However, contrary to our hypothesis no interaction effect of Time x Testosterone reactivity was found, *Estimate* = −0.08(0.15), F(1,70) = 0.28, $p = .60$. Reductions in fear levels over speeches were not related to testosterone reactivity. Similar findings were observed for Peak SUDs at the post-treatment Speech (Speech 4), Time, *Estimate* = −6.27(0.89), F(1,67) = 49.53, $p < .001$, Gender, *Estimate* = −5.37(1.96), F(1,67) = 7.46, $p = .008$, Time x Testosterone, *Estimate* = −0.16(.14), F(1,64) = 1.33, $p = .25$ (see suppl. for details). Analyses testing the predictive effects of baseline testosterone yielded similar results to those for reactive testosterone (see suppl. for details).

3.3. Symptom severity

The mixed model analysis for symptom severity, with *reactive* testosterone levels as predictor showed main effects of Time (pre, post), *Estimate* = −8.71(1.61), F(1,66) = 29.37, $p < .001$, indicating symptom reduction from pre- to post treatment ($M_{pre} = 74.80$, $SD = 22.73$; $M_{post} = 66.01$, $SD = 24.58$), and Gender, *Estimate* = −9.62(2.77), F(1,68) = 12.07, $p < .001$, indicating higher symptom levels for females ($M = 75.63$, $SD = 23.91$), compared to males ($M = 57.53$, $SD = 18.85$). Consistent with our hypothesis, testosterone reactivity significantly modulated the effect of Time as indicated by a significant Time x Testosterone Reactivity interaction, *Estimate* = −.56(0.25), F(1,66) = 5.08, $p = .027$. As can be seen in Fig. 2a, higher testosterone reactivity was associated with stronger reductions in symptom severity relative to lower testosterone reactivity.

In addition, we repeated the same mixed models analysis but now with *baseline* testosterone. This analyses showed a main effect of Time, again confirming efficacy of exposure, *Estimate* = −9.14(1.62), F(1,67) = 31.81, $p < .001$. Specifically, symptom levels reduced from pre- to post-treatment. A main effect of Gender, *Estimate* = −9.45(2.74), F(1,69) = 11.91, $p < 0.001$ on the LSAS, indicated higher symptom levels for females compared to males. The interaction of baseline testosterone and Time, *Estimate* = 4.03(1.71), F(1,67) = 5.57, $p = .021$, showed that stronger reductions in symptom severity were related to lower baseline testosterone levels (see Fig. 2b for an illustration of this effect).

The fact that the baseline testosterone levels predicted symptom severity reduction, but in the direction opposite from what we predicted may suggest that it is the relative dynamics of the HPG-axis system rather than the absolute testosterone levels in the system that are important for exposure therapy success. In order to test this further, we additionally checked whether effects for reactive testosterone would disappear without controlling for the initial testosterone levels (thus subtracting testosterone sample 1 from sample 3, without controlling for the initial levels at sample 1) and found that this was the case (*Estimate* = −2.79(2.12), F(1,66) = 1.73, $p = 0.19$). This finding suggests that it is the *relative* and not the *absolute* reactivity of the HPA-axis system that positively relates to treatment outcome.

4. Discussion

In this proof-of-concept study, we demonstrated that reactivity of the HPG-axis constitutes a promising biomarker of response to exposure therapy in social anxiety disorder. Specifically, we showed that those patients who displayed relatively high pre-exposure testosterone reactivity (e.g., rises in testosterone in anticipation of a challenging situation) showed better outcomes following a standardized session of exposure therapy. The finding that low pre-treatment baseline testosterone levels were also associated with better outcome was unexpected and may suggest that the relative reactivity of the HPG-axis contributed to the success of the exposure session, rather than the absolute testosterone levels in the system. This interpretation was further supported by the finding that outcomes were specific for *relative reactivity* (baseline controlled) and not the *absolute reactivity* (absolute increase) of the HPG-axis to the treatment-preparation session. Together, these findings support the social challenge hypothesis (Wingfield et al., 1990), which posits that rises in testosterone in preparation to a challenging encounter lead to approach behavior and corresponding reductions in anxiety (Archer, 2006; Bos et al., 2012).

We hope that this early work stimulates further research in this area that has the potential to facilitate the goal of improving exposure

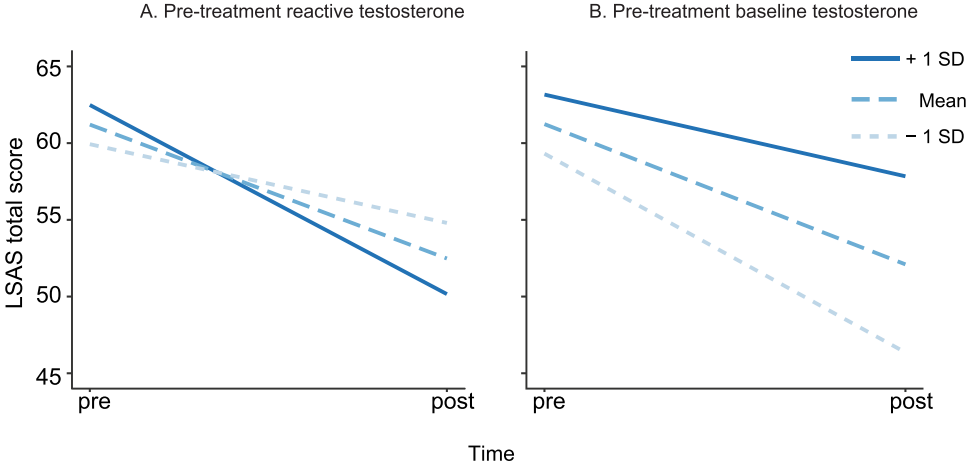

Fig. 2. Self-reported social anxiety symptoms (LSAS-total score) decreased more from pre- to post treatment in those patients who displayed stronger pretreatment testosterone increases (**Panel A)** and in those patients with low pre-treatment baseline testosterone levels (**Panel B**). For display purposes only, testosterone levels are presented in three groups; with – 1 SD referring to those with testosterone levels of 1 SD or less below the mean, and with +1 SD referring to those participants that had high testosterone levels more than 1 SD above the mean.

therapy outcomes for SAD. One important follow-up to the current study is the testing of the putative pathway for the observed relation. We did not index approach behavior in the current study. Establishing increased approach behavior during exposure therapy as a behavioral consequence of pre-treatment testosterone reactivity and understanding the nature of the relations between pre-treatment testosterone reactivity, approach behavior and exposure outcome, respectively, may guide the development of targeted augmentation strategies. Critical to this type of research is complementing the correlational approach with experimental research. In the parent trial (Davis et al., 2017), we attempted to engage testosterone reactivity using a simple behavioral strategy (i.e. power posing), but failed. Other work from our group suggests that testosterone administration to patients with SAD alleviates social avoidance and promotes prosocial behavior (Enter et al., 2016a), as well as reduces automatic threat bias to angry faces (Enter et al., 2016b; van Peer et al., 2017). Currently, our group is conducting a study testing whether administration of 0.5 mg of testosterone to females with SAD prior to an exposure session can improve exposure success by reducing avoidance behavior.

The finding that exposure-anticipatory testosterone levels predicted reductions in social anxiety *symptoms*, but not in *fear* experienced during the exposures, is in line with previous work showing that anticipatory physiological anxiety responses to a speech exposure were associated with social anxiety symptoms but not to the in-session fear levels (Cornwell et al., 2006). It also supports theoretical models that frame SAD as a problem of threat anticipation in specific (Clark and Wells, 1995; Rapee and Heimberg, 1997). We extend these notions by providing an objective marker of testosterone reactivity in the anticipation of threat.

Together these finding may also imply that, during exposure, the social motivational properties of testosterone are more relevant compared to its anxiolytic properties (e.g. promoting direct approach behavior rather than reducing fear). This interpretation is in line with the findings of a vast amount of testosterone administration studies (Enter et al., 2016b, 2016a; van Peer et al., 2017) showing that testosterone directly influenced approach behavior and reduced threat avoidance in patients with SAD. In turn, approach behavior during exposure treatment may be a more important predictor of exposure efficacy, whereas fear reductions during the exposure are not necessary for good exposure outcome. For example, studies testing predictions from Emotional Process Theory (Foa et al., 2005; Foa and Kozak, 1986) have found no relation between reductions in subjective reported distress during an exposure session and exposure outcomes in different anxiety disorders (Baker et al., 2010; Hendriks et al., 2018; Kozak et al., 1988; Meuret et al., 2012; Van Minnen and Hagenaars, 2002). Thus, fear reductions during exposure sessions do not seem to be a reliable predictor of exposure outcomes (Craske et al., 2008, 2014).

There are some limitations that deserve note. First, this study reports on correlations and therefore we cannot make inferences with respect to causality. Second, although proven useful for testing mechanisms of action and augmentation strategies (Rodebaugh et al., 2013), the use of a standardized single-session approach leaves open the question whether the observed findings translate to multiple-session protocols that are standard in practice. Third, the sample size was not sufficient to detect small effects. Fourth, our sample was unbalanced with respect to gender although we could confirm that the effect held tested in women alone (supplementary materials), we were underpowered to examine whether similar effects would hold for men alone.

In summary, pre-treatment endogenous testosterone levels were predictive of efficacy of an exposure session in patients with social anxiety disorder. The finding that low baseline testosterone levels as well as high reactive testosterone levels prior to the exposure session predicted treatment outcome in SAD, suggest that good reactivity of the HPG-axis may be a promising marker for symptom-reducing effects of exposure therapy. These findings support the further investigation into exposure-enhancing effects of testosterone in patients with SAD.

Declaration of Competing Interest

Dr. Smits is a paid Clinical Advisor for Big Health and receives royalty payments from Oxford University Press and Academic Press. This work was supported by a VICI grant (#453-12-001) from the Dutch Research Council (NWO) and a consolidator grant from the European Research Council (ERC_CoG-2017_772337) awarded to Dr. Roelofs. The other authors report no financial interests or potential conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104612.

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