SPEAK UP!

The effects of testosterone on exposure therapy for social anxiety disorder

MONIEK HUTSCHEMAEKERS

Behavioural
Science
Institute

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Moniek Helena Maria Hutschemaekers

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Moniek Helena Maria Hutschemaekers
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Promotor

Prof. dr. K. Roelofs

Copromotoren

Dr. M. Kampman (Pro Persona)
Dr. R.A. de Kleine (Universiteit Leiden)

Manuscriptcommissie

Prof. dr. I. Tendolkar
Prof. dr. S.M. Bögels (Universiteit van Amsterdam)
Prof. dr. S.G. Hofmann (Boston University, Verenigde Staten)

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Chapter 1

General introduction

Imagine giving a speech in front of a large audience. While standing there looking at all those faces in front of you, your heart rate will likely rise, you may feel a bit warm and think *"my cheeks may turn red"* or *"I hope they will like my talk."* These are normal reactions to a socially challenging event. However, for some people those feelings turn into a dominating fear, far more challenging and burdensome:

Meet Rose. Rose is a 21-year-old bachelor student. Despite the fact that she has good grades and enjoys studying, she experiences problems in completing her bachelor. She did not pass her exam as she is terrified of giving a speech in front of a public. She worries she will tremble, blush and stutter. The thought of a complete blackout and people laughing at her may not reflect a realistic scenario, but feels very real and provokes anxiety. To prevent this scenario from happening, she avoids giving speeches entirely for a few years now. Her fear of public speaking started during secondary school where she had to give a plenary presentation. Although she prepared it very well, she saw some of her classmates laughing which she attributed to her behavior. The thought of doing something stupid made her nervous which in turn made it hard to find the right words. After this experience, she avoided future presentations and became increasingly scared of public performances. Recently, she started to avoid other social situations as well, such as parties, even of good friends. In these situations, she also noticed anxiety symptoms, feeling scared not knowing what to say and being disliked. Rose symptoms meet the diagnostic criteria of Social Anxiety Disorder. As her anxiety symptoms are more and more hindering here social and academic functioning, she seeks treatment to deal with her anxiety.

Like Rose, many other individuals are suffering from Social Anxiety Disorder (SAD). This impairing mental disorder is characterized by persistent fear and avoidance of social and performance situations. SAD is one of the most common mental health disorders and it persists when untreated with high levels of impairment in social or occupational function (Aderka et al., 2012; Bruce et al., 2005; Kessler et al., 2005). It can be treated effectively with cognitive behavioral therapy. However, around 45-55% of the individuals with SAD do not profit sufficiently from treatment (Carpenter et al., 2018; Loerinc et al., 2015). Therefore, various (pharmacological) enhancement strategies have been examined to boost therapy outcomes for SAD (Guastella, Howard, Dadds, Mitchell, & Carson, 2009; Hofmann, Fang, & Gutner, 2014; Smits, Rosenfield, et al., 2013a). Based on pre-clinical research, the steroid hormone testosterone might specifically yield promise to enhance exposure therapy effects for those suffering from SAD. This hormone is an important regulator of social behavior both in males and females (Hermans & Van Honk, 2006). Relatively low levels of testosterone have been linked to social fear and avoidance

(Enter, Spinhoven, & Roelofs, 2014; Giltay et al., 2012), presumably because the hormone is particularly relevant for preparing a person for socially challenging situations (Wingfield, Hegner, Dufty, & Ball, 1990).

Therefore, the overarching aim of this dissertation is to test the potential of testosterone as an enhancer for exposure treatment efficacy for SAD. With this dissertation I aimed to build on fundamental research within the field of neuroendocrinology and to link the acquired knowledge to clinical experimental psychology. Concretely, I aimed to translate well-established experimental findings on the social approach-promoting properties of testosterone in healthy individuals and SAD (Enter et al., 2014; Enter, Spinhoven, & Roelofs, 2016; Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2016; Radke et al., 2015; Terburg et al., 2016) to a clinical application in order to improve exposure therapy efficacy in SAD.

In this general introduction I will first present a description of SAD, followed by an explanation of exposure therapy and one of its proposed mechanisms of action. Next, I will focus on social avoidance. Firstly by discussing avoidance in the maintenance and treatment of SAD, secondly by providing a neuroendocrinal model of avoidance. Following that model, I will zoom in on the potential of testosterone as an enhancer for exposure therapy for SAD. Finally, I will present the rationale and specific aims of this dissertation and I conclude with an outline of the chapters.

Social anxiety disorder

Social anxiety disorder (SAD) is the most common and burdensome of all anxiety disorders with a lifetime prevalence of 13% and long-term disability (Aderka et al., 2012; Bandelow & Michaelis, 2015; Bruce et al., 2005; Hendriks et al., 2016). SAD is characterized by an intense fear of social situations in which the individual may be scrutinized by others such as interpersonal interactions (e.g., a conversation), being observed (e.g., eating in public) and performing in front of others (e.g., giving a speech). SAD can be specified into a *performance only* variant when the fear is restricted to public speaking. These social situations are usually avoided or endured with intense fear or anxiety (American Psychiatric Association, 2013). Individuals with SAD fear that their behavior, physical sensations or appearance will be negatively evaluated by others. They realize that their fear is excessive or unrealistic. SAD usually develops during early childhood or adolescence (Kessler et al., 2005; Schneier, Luterek, Heimberg, & Leonardo, 2004) and typically follows an enduring course without treatment. Moreover, compared to healthy individuals, individuals with SAD have a greater risk of developing comorbid disorders

such as depression and other anxiety disorders (Fehm, Beesdo, Jacobi, & Fiedler, 2008; Lépine & Pélissolo, 2000).

Several theoretical models try to explain the maintenance of SAD (Clark & Wells, 1995; Heimberg & Rapee, 1997; Hofmann, 2007; Spence & Rapee, 2016; Wong & Rapee, 2016). All these models share similar aspects, for example increased self-focused attention, negative self-perception and attention to (perceived) social threat. Moreover, these models emphasize the maintaining role of behavioral processes. Specifically, avoidance and safety behaviors. Avoidance refers to the behavioral strategy to prevent exposure to the feared social situation (e.g., avoiding giving a speech or going to a party) whereas safety behaviors refer to all actions aimed to reduce or eliminate social threat while being in the social situation (e.g. holding notes or avoiding eye contact). Avoidance and safety behaviors are thought to play a crucial role in these models since they create a negative feedback loop by which the (social) anxiety remains or increases. That is, when individuals with SAD engage in these behaviors they try to prevent being rejected resulting in a momentary reduction of fear. However, as a result it becomes impossible to critically evaluate their feared outcomes and therefore the anxiety is maintained (Hofmann, 2007; Wong & Rapee, 2016).

Cognitive behavioral therapy and exposure for SAD

Cognitive behavioral therapy (CBT), is one of the psychological treatments of choice for SAD (Canton, Scott, & Glue, 2012; Pilling et al., 2013, Dutch Treatment Guidelines). CBT can be delivered individually or in a group and is considered the most efficacious and empirically supported treatment for SAD and other anxiety disorders (Hofmann & Smits, 2008a; Norton & Price, 2007; Tolin, 2010). Long term studies show that CBT can have long lasting effects (Leichsenring et al., 2014; Willutzki, Teismann, & Schulte, 2012). However, still many adults with SAD do not benefit from CBT. Response rates (the percentage of the treatment group that is classified as a "responder") vary between 45–55% (Loerinc et al., 2015).

In line with the proposed theoretical models of SAD, CBT aims to challenge dysfunctional beliefs about the likelihood of anticipated social danger (e.g., social rejection), by using cognitive techniques (e.g., keeping thought records and challenging automatic thoughts) as well as behavioral techniques, such as exposure (Hofmann, 2008; Smits, Julian, Rosenfield, & Powers, 2012). Exposure is thought to be one of the most crucial interventions in CBT protocols (Hofmann & Smits, 2008a; Norton & Price, 2007). Exposure treatment involves repeated confrontation with feared stimuli in the absence of the feared outcome. The process of fear extinction, which can be seen as a laboratory

analogue for exposure therapy, helps us understand the underlying mechanism contributing to the effects of exposure therapy (Bouton, Mineka, & Barlow, 2001; Craske et al., 2008; Vervliet, Craske, & Hermans, 2013). In this process a conditional stimulus (CS) that was previously paired with an aversive outcome (unconditional stimulus, US) is repeatedly presented without being followed by the US, leading to the loss of the fear response. According to inhibitory learning theory (ILT), a recent exposure model, inhibitory learning plays an important role in extinction learning (Craske et al., 2008; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014a; Craske, Treanor, Zbozinek, & Vervliet, 2022; Lang, Craske, & Bjork, 1999). It is stated that the original CS – US association does not disappear during exposure, but a new association: US does not predict the US (no US), is learned in addition to the original association (Bouton & King, 1983). After extinction, the CS is associated with two meanings, the original fear association (lower pathway figure 1.1) and the inhibitory meaning (upper pathway figure 1.1).

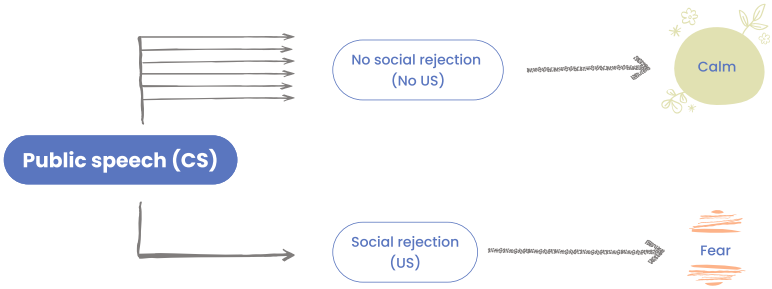

Figure 1.1 An illustration of an exposure model for Social Anxiety Disorder. Exposure treatment is characterized by repeated confrontation with the feared situation (e.g., the public speech, the CS) in absence of the feared outcome (No-US), reflected by the multiple arrows in the upper route. Within exposure, the individual learns a new association between the social situation and the absence of danger. Retrieval of this new association in a social situation results in feeling calmer. According to Inhibitory Learning Theory (ILT), both the original association (lower pathway) as well as the new association (upper pathway) remain. The new association competes for retrieval with the original association.

Moreover, both the new association (CS – no US) and the original association (CS – US) compete for retrieval. Inhibitory learning can be maximized by expectancy violation in which the harm expectancies of the individual are altered. For example, Rose would be repeatedly exposed to her feared situation such as giving a public speech (CS) in order to test her explicitly stated harm expectancy: *"people will laugh at me and reject me"*

(US) when she gives a speech in different contexts (see Figure 1.1). When she experiences that she is not being rejected repeatedly, she no longer expects social rejection to occur when giving a public speech.

Avoidance behavior

Avoidance and safety behaviors in SAD

As described in the different psychological models (Clark & Wells, 1995; Heimberg & Rapee, 1997; Hofmann, 2007; Spence & Rapee, 2016; Wong & Rapee, 2016), avoidance behavior plays a crucial role in the maintenance of SAD. As mentioned above, avoidance is a behavioral strategy to prevent exposure to the feared situation, which can take different forms. For example, an individual with SAD could completely avoid social situations such as a party, a speech or a job interview. Also, avoidance behavior can take more subtle forms in which individuals aim to reduce their distress or hide it, such as avoiding eye contact, speaking quietly, or taking somebody with them to the feared situation (those types of more subtle avoidance behaviors can also be classified as safety behaviors, Cuming et al., 2009; Wells et al., 1995). Although avoidance can be adaptive in threatening situations (e.g., *running away when being attacked by a dangerous animal)*, and can result in temporary relief in the short run, avoidance is unnecessary in absence of real danger and can become maladaptive in the long run. Critically, systematic avoidance behavior hinders the individual to learn that fears are unsubstantiated. Rose can never experience that she can chat at a party and might not be rejected if she avoids going there at all. The same is true when she uses safety behaviors such as looking away when people make eye contact. Moreover, successful avoidance usually results in a temporary reduction in anxiety and is therefore reinforcing and persistent (Wong & Moulds, 2011). As such, social avoidance behavior is a major factor that maintains fear of social situations in individuals with SAD (Arnaudova, Kindt, Fanselow, & Beckers, 2017; Clark & Wells, 1995).

Therefore, reducing avoidance behavior is the core target of treatment for SAD. Safety and subtle avoidance behaviors are typically discouraged during exposure sessions through modeling by the therapist and by providing explicit instructions (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014b; Hofmann & Otto, 2017), since they may also hamper the efficacy of exposure therapy (Wells et al., 1995). A therapist might for instance stimulate Rose not to use notes during the speech neither holding something in her hands, stimulating effective approach behavior. Otherwise, Rose may feel that she could only give the speech because of her notes in her hand (Piccirillo, Taylor Dryman, & Heimberg, 2016). In fact, the use of safety behaviors could increase the likelihood of

feared outcomes (e.g., people may actually reject her because she avoids looking at them; Cuming et al., 2009; Piccirillo, Taylor Dryman, & Heimberg, 2016). A number of studies indeed showed that the use of safety behaviors impedes exposure efficacy in SAD (McManus et al., 2009; Morgan & Raffle, 1999; Piccirillo et al., 2016). However, studies in other anxiety disorders show that the controlled use of safety behaviors may be beneficial in the context of exposure treatment (Milosevic & Radomsky, 2008), as it might help individuals in taking the first steps in exposure and it promotes acceptability.

Avoidance tendencies

Individuals with SAD do not only show overt social avoidance such as avoiding a social event, they also show more automatic and implicit avoidance tendencies that can be picked up in speeded experimental tasks. An example is the biased information processing toward threatening stimuli. This is usually characterized by initial increased attention to negative emotional information, such as angry faces followed by attentional avoidance of these stimuli, specifically avoidance of the eyes, in order to regulate anxiety provoked by the initial registration of threat (the CS), (Chen & Clarke, 2017). In addition, biased action tendencies seem to play a particular role in individuals with SAD. These action tendencies can for instance be assessed by means of social Approach Avoidance Tasks (AAT: Rinck & Becker, 2007), in which participants respond to social stimuli (for example faces) by pushing (avoidance) or pulling (approach) a joystick. Socially anxious individuals typically show avoidance of social stimuli; i.e. stronger avoidance tendencies compared to approach tendencies toward angry, but also happy faces (Heuer, Rinck, & Becker, 2007; Loijen, Vrijsen, Egger, Becker, & Rinck, 2020; Roelofs, Putman, et al., 2010; Roelofs, van Peer, et al., 2009a) and even neutral faces, compared to non-social stimuli and healthy controls (Kuckertz, Strege, & Amir, 2017). Angry faces communicate potential threat and neutral and happy faces are ambiguous to individuals with SAD and therefore labeled as threatening, and thereby activating avoidance mechanisms (Heuer et al., 2007; Roelofs et al., 2010). Previous work showed that automatic avoidance tendencies assessed by an AAT could predict real life avoidance in specific phobia (Rinck & Becker, 2007). These findings indicate that automatic avoidance tendencies may underly overt avoidance behavior, an important maintaining factor in SAD. Moreover, it could be hypothesized that these avoidance tendencies hinder successful exposure, presumably since they prevent individuals with SAD to really engage in the exposure.

Neuroendocrinal regulation of social avoidance

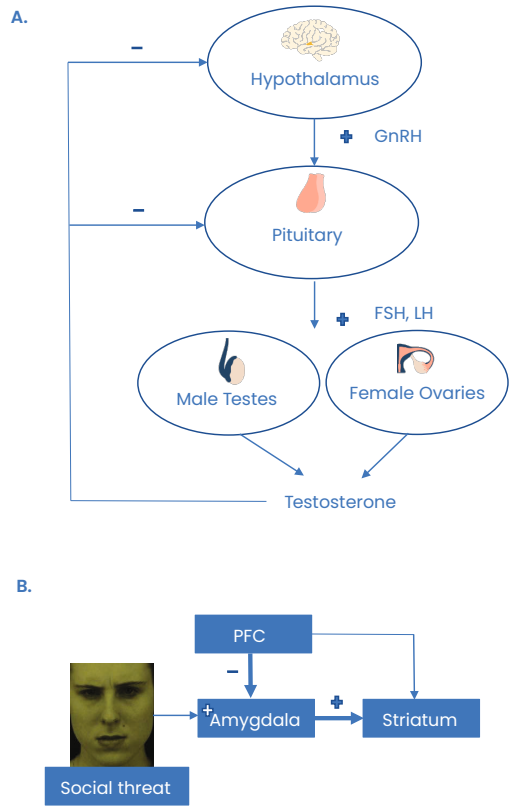

Figure 1.2. Illustration of the HPG-axis and a simplified model of the approach enhancing properties of testosterone. A: Illustration of the hypothalamic–pituitary–gonadal axis (HPG-axis) activity of which leads to testosterone production. **B:** Simplified neural model of the proposed threat approach enhancing properties of testosterone based on neuroimaging studies verifying effects of testosterone administration during social threat challenges (such as visual presentation of an angry facial expression): testosterone enhances amygdala activity, dopaminergic projections from the amygdala to the striatum, and has been associated with reduced connectivity between the prefrontal cortex (PFC) and the amygdala.
Note. GnRH = Gonadotropin-releasing hormone, FSH = Follicle-stimulating hormone, LH = Luteinizing hormone. + indicates excitatory connection; - indicates inhibitory connections.

Testosterone

Produced by the Hypothalamus-Pituitary-Gonadal (HPG)-axis (see figure 1.2A), testosterone constitutes an important regulator of social motivational behavior in both males and females, including social approach and avoidance behavior (Hermans & Van Honk, 2006; Mazur & Booth, 1998). Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus, which stimulates the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary gland. This triggers the production of testosterone and estradiol in the gonads (i.e., testes and ovaries). Testosterone follows a diurnal cycle with the highest levels upon wakening (Diver, Imtiaz, Ahmad, Vora, & Fraser, 2003). Next to the pre- and early postnatal organizational effects on the brain structure, testosterone also affects emotion, motivation and behavior later in life (Lombardo et al., 2012; McHenry, Carrier, Hull, & Kabbaj, 2014). Endogenous testosterone levels can be assessed by blood samples (Serum Testosterone), but also less invasively via (passive drool) saliva samples (Salivary Testosterone). There is intra- variability in testosterone levels, depending on for example, time of the day and menstrual cycle as well as inter-individual variability (e.g., age and sex). Typically, the lower end of the serum concentration range is 4-5 times higher in healthy males compared to the upper end of the healthy female range (Clark et al., 2019; Kanakis, Tsametis, & Goulis, 2019). In addition to testosterone assessment, testosterone can be administrated to individuals (exogenous testosterone) through different methods (i.e., sublingually or using gels or via injections), enabling determination of a causal relationship between testosterone reactivity and its effects on social motivational behavior. Within this dissertation, we applied a single dose (0.5 mg) sublingual testosterone administration. This well-established method has been used in healthy and anxious individuals, showing consistent psychophysiological and behavioral effects approximately 4-6 hours after administration (Bos, Panksepp, Bluthé, & Honk, 2012; Tuiten et al., 2000).

Testosterone interacts with other neurotransmitters and -peptides such as oxytocin, vasopressin and dopamine (de Souza Silva, Mattern, Topic, Buddenberg, & Huston, 2009). Moreover, the HPG-axis works in antagonism with the Hypothalamus-Pituitary-Adrenal (HPA)-axis. Specifically, cortisol (end-product of the HPA-axis) has an inhibitory effect on the production and actions of testosterone and vice versa (Toufexis, Rivarola, Lara, & Viau, 2014). Both the HPG and HPA-axis are important in the regulation of social motivational behavior (Mehta & Josephs, 2010; Roelofs, van Peer, et al., 2009b), but within this dissertation I will focus on the HPG-axis and its end product testosterone.

Social challenge hypothesis

Social events are usually associated with a temporary surge in testosterone levels. The social challenge hypothesis (Wingfield et al., 1990), originally based on testosterone and aggression associations in monogamous birds (Wingfield, Lynn, & Soma, 2001) and later also established in primates (Muller & Wrangham, 2004) and humans (Bateup, Booth, Shirtcliff, & Granger, 2002; Neave & Wolfson, 2003), is the most predominant theory of testosterone reactivity. It states that testosterone levels rise in preparation to a challenging encounter in which social status might be threatened, such as giving a speech in front of a public, and thereby initiating approach motivation and reducing fear (Archer, 2006; Bos, Panksepp, et al., 2012). Following this hypothesis, a rise in testosterone levels in preparation to public speaking, may stimulate Rose to fully approach this challenging situation, rather than using safety behaviors or avoiding it completely.

Consistent with this hypothesis, in both animal and human studies, low levels of endogenous testosterone have been linked to socially submissive, anxious, and avoidant behaviors (Archer, 2006; Josephs, Sellers, Newman, & Mehta, 2006; Sapolsky, 1991), whereas high basal testosterone levels are related to social dominance and approach behavior (Maner, Miller, Schmidt, & Eckel, 2008; Mazur & Booth, 1998). 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, Yeap, Hankey, Jamrozik, & Flicker, 2008; Giltay et al., 2012). The threat-approach facilitating properties of testosterone have been linked to its effects on the amygdala (and its connectivity with the prefrontal cortex, PFC) and striatum: biasing the amygdala toward reward anticipation and threat approach (see figure 1.2B, Hermans et al., 2010; Volman, Toni, Verhagen, & Roelofs, 2011; Radke et al., 2015).

Testosterone as a possible enhancer of exposure therapy in SAD

Experimental studies using testosterone administration

The social motivational enhancing effects of testosterone have been established not only by correlational but also by more causal testosterone administration studies. For example, administration of a single dose testosterone (0.5 mg sublingual vs placebo) to healthy (female) participants prior to exposure to a threat cue, has been shown to reduce fear, to enhance reward sensitivity and to promote social approach motivation (Bos, van Honk, Ramsey, Stein, & Hermans, 2012; Enter et al., 2014; Terburg et al., 2016). Critically, administration of a single dose of testosterone (0.5 mg sublingually versus placebo), specifically in females with SAD, prior to an eye-tracking experiment, result-

ed in alleviation of gaze avoidance toward angry facial expressions in individuals with SAD (Enter, Terburg, et al., 2016). Participants showed less aversion of gaze towards the eye-regions of negative facial expressions after testosterone versus placebo. Moreover, testosterone administration led to increased approach behavior toward social threat (e.g., angry faces) on a social approach avoidance task (Enter, Spinhoven, et al., 2016). Finally in an EEG study, it was found to result in reduced automatic threat processing of angry faces in individuals with SAD versus healthy controls (van Peer, Enter, van Steenbergen, Spinhoven, & Roelofs, 2017). In light of these consistently established prosocial and approach enhancing properties of testosterone in general and specifically in SAD, testosterone may be a potential candidate to boost exposure effects in SAD, by targeting within session avoidance behavior. This is exactly what I aim to investigate using the current dissertation.

Boosting treatment effectiveness by means of testosterone would extend a vast line of research conducted over the past two decades on pharmacological enhancement of CBT. Among potential pharmacological enhancement methods, various randomized controlled trials have found most support for D-cycloserine (DCS), a partial N-methyl-D-Aspartate (NMDA) receptor agonist, associated with fear extinction consolidation (Hofmann et al., 2006; Rodebaugh, Levinson, & Lenze, 2013; Smits et al., 2020). Additionally, few studies examined effects of pharmacological enhancers such as Yohimbine and Oxytocin in SAD (Guastella et al., 2009; Smits, Rosenfield, Davis, et al., 2013). These enhancement strategies all aimed to target the process of (extinction) learning in SAD. Although the results are encouraging, none of these pharmacological enhancers directly acts on acute within-session social-approach behavior, essential for effective exposure. This brings me to the next section, on the potential mechanism of action of testosterone in exposure therapy.

Testosterone as a potential enhancer of exposure therapy

As mentioned, one of the proposed mechanisms of action of exposure is that individuals with SAD learn that exposure to a feared stimulus (e.g., a social situation) does not lead to their feared outcome (social rejection). To learn this, it is needed that individuals with SAD approach the feared situation, rather than avoiding it. That is, they have to approach the social interaction and have to get engaged in it. The social challenge hypothesis (Wingfield et al., 1990, 2001) states that testosterone levels rise in preparation to a socially challenging situation (such as exposure) and thereby initiates approach motivation. Testosterone reactivity may therefore be important for successful exposure treatment by stimulating within-session approach behavior. Crucially, testosterone reactivity can be experimentally manipulated for example by testosterone administration, resulting in social approach behaviors in individuals with SAD (Enter, Spinhoven, et al.,

2016; Enter, Terburg, et al., 2016). Based on these fundamental and experimental findings on testosterone, we expect that testosterone reactivity (or administration) prior to an exposure session improves engagement and approach behavior within the exposure session, resulting in improved corrective learning, as assessed by retention of learning in the following exposure session(s) (see Figure 1.3 for an illustration).

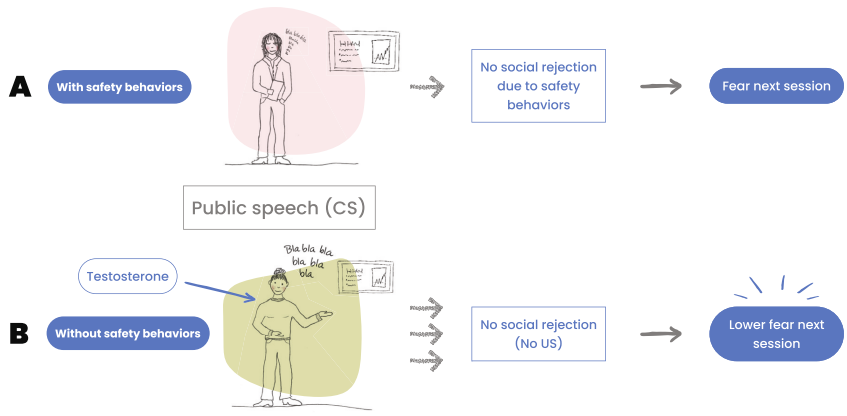

Figure 1.3 An illustration of the proposed mechanism of action for testosterone as an enhancer for exposure therapy. A. The upper part of the figure depicts subtle avoidance and safety behaviors during a speech exposure, such as holding notes, talking quietly, hiding face behind hair etc. According to Inhibitory Learning Theory this negatively affects inhibitory learning, thereby reducing the efficacy of exposure treatment. If anything, individual learns confirmation that absence of social rejection can be attributed to safety behaviors, leading to maintained fear for the next exposure session. **B.** The lower part of the picture illustrates our hypothesis that Testosterone reactivity or administration stimulates within-session approach behavior and engagement: no notes, talking at higher volume, open posture. We expect that reducing subtle avoidance and safety behaviors in individuals with SAD, results in more effective exposure and improved corrective learning.
Note. The dotted lines reflect hypotheses rather than established findings.

Aim of this dissertation

To summarize, SAD is one of the most common anxiety disorders with detrimental consequences when left untreated. It is characterized by avoidance behavior, which is the core target of exposure therapy. Although efficacious, the therapy leaves ample room for improvement (response rates vary between 45–55%). Considering the anxiolytic, avoid-

ance alleviating, and prosocial properties of testosterone, testosterone might have the potential to enhance exposure treatment efficacy for SAD. Therefore, the overarching aim of this dissertation is to examine whether endogenous or exogenous testosterone increases can enhance exposure efficacy for SAD. Specifically, the first aim is to review the current scientific knowledge on social motivational properties of the HPG-axis and its potential role in social motivational deficiencies underlying affective disorders, such as SAD. Second, we aim to test 1) whether endogenous testosterone is predictive of exposure outcomes, 2) if administrating testosterone to individuals with SAD prior to exposure can improve exposure efficacy and 3), whether automatic avoidance behavior toward social stimuli may moderate the effects of exposure enhancement with testosterone.

Outline of this dissertation

Chapter 2 presents a theoretical overview of steroid hormones testosterone and cortisol and their relationship with social motivational behavior and psychopathology such as aggression related disorders and SAD in specific. **Chapter 3** describes a proof-of-concept study in which we translated the social challenge hypothesis of testosterone into the clinical practice. In a sample of 73 participants with SAD, this study sought to test whether endogenous pre-treatment testosterone increases, enhances efficacy of a standardized exposure therapy session for SAD, as measured by fear levels during exposure and change in social anxiety symptoms following one standardized exposure session. In **chapter 4** the results of a placebo controlled randomized proof-of-concept trial are presented. Concretely, we tested the augmentative potential of administrating one dose of testosterone (0.5 mg sublingual, vs placebo) prior to a speech exposure session for females with SAD (N = 55). Within session fear and social anxiety symptoms were the primary and secondary outcome measures, respectively. In **chapter 5** we tested the hypothesis that highly avoidant participants benefit more from the testosterone-enhanced therapy described in chapter 4. We measured pre-treatment automatic avoidance tendencies toward social stimuli with an approach-avoidance joystick task in the same sample as the study described in chapter 4 and tested if these tendencies moderate the effects of testosterone enhanced exposure. Additionally, we tested whether these avoidance tendencies are relatively stable or whether they vary with (testosterone enhanced) exposure efficacy. Finally, I will close with a discussion in **chapter 6,** presenting an overview and discussion of all findings in light of the existing literature, followed by an evaluation of strengths, limitations and implications for the clinical practice, as well as considerations for future research and concluding remarks.

Chapter 2

Neuroendocrinological aspects of social anxiety and aggression related disorders

Enter, D., Hutschemaekers, M. H., & Roelofs, K. (2018). Neuroendocrinological aspects of social anxiety and aggression-related disorders. In *Routledge international handbook of social neuroendocrinology* (pp. 635-655). Routledge.

Introduction

Steroid hormones, like cortisol and testosterone, play an important role in the regulation of social motivational behavior. Whereas testosterone facilitates threat approach, presumably by facilitating dopaminergic projection from the amygdala to the striatum (de Souza Silva, Buddenberg, Huston, Topic, & Mattern, 2008; Hermans et al., 2010; Radke et al., 2015), cortisol increases threat avoidance, particularly in high socially anxious individuals (van Peer et al., 2007; van Peer, Spinhoven, Dijk, & Roelofs, 2009). Interestingly, social motivational disorders, such as social anxiety, and aggression-related disorders show an imbalance in these steroid hormones: social anxiety has been associated with increased cortisol stress-responses and decreased testosterone levels (Gerra et al., 2000; Giltay et al., 2012; Roelofs, Minelli, Mars, van Peer, & Toni, 2009), while aggressive psychopathologies have been linked to increased testosterone levels (Glenn, Raine, Schug, Gao, & Granger, 2012; Montoya, Terburg, Bos, & van Honk, 2012; Volman et al., 2016). In this chapter, we discuss the role of these steroid hormones and the neuropeptide oxytocin in social psychopathologies, especially social anxiety and psychopathy. First, we will give a description of the neuroendocrine aspects of social motivational behavior, including social approach and avoidance behaviors. Then we will focus on the neuroendocrine aspects of social anxiety and aggression-related disorders. Finally, motivational and psychiatric findings will be integrated, followed by a research agenda, aiming to provide starting points for clinical applications.

Social motivational action

The term motivation reflects a broad concept related to anything that may prompt the person to act in a certain way, or to develop an inclination for specific behavior. In this chapter though, we will focus largely on social motivational actions that can be roughly divided into social approach and social avoidance (Davidson, Ekman, Saron, Senulis, & Friesen, 1990; Gray, 1994). These action tendencies involve a basic response to stimulus valence. They are mediated by primary motivational systems of the brain -whereby reward potentiates behavioral activation, while punishment promotes behavioral inhibition or avoidance - and are thought to underlie every complex emotional responding (Carver & White, 1994; Gray & MacNaughton, 2003). Successful social functioning depends on adaptive regulation of these social approach and avoidance responses.

Both automatic defensive action tendencies and more instrumental (or goal-directed) mechanisms shape an individual's behavior. When an individual encounters a social

stimulus (e.g., an angry facial expression directed at him/her), he/she will engage in an automatic defensive freeze and flight-or-fight response, a quick and automatic sequence of defensive responses stages (Bradley, Codispoti, Cuthbert, & Lang, 2001). During threat exposure in particular, an initial freezing response is activated during which the individual ceases all ongoing activity and perception is enhanced to quickly assess the situation in order to optimize subsequent fight-or flight responses (Blanchard, Griebel, Pobbe, & Blanchard, 2011; Lojowska, Gladwin, Hermans, & Roelofs, 2015; Roelofs, Hagenaars, & Stins, 2010). This is an automatic process, and the evaluation directly results in a behavioral disposition towards the stimulus: aversive stimuli generally elicit the tendency to move away from the stimulus and appetitive stimuli will elicit a tendency to move towards the stimulus (Lang, Bradley, & Cuthbert, 1997). Such automatic tendencies can also influence more complex, instrumental approach–avoidance decision making (Geurts, Huys, den Ouden, & Cools, 2013; Guitart-Masip, Duzel, Dolan, & Dayan, 2014). For instance, Ly, and colleagues (2014) tested such influence in 45 healthy human individuals using an experimental set-up in which automatic freezing reactions towards negatively (versus positively) valenced stimuli were disentangled from instrumental approach–avoidance decisions (guided by monetary rewards and punishments). Critically, the transfer of valence (and related automatic reactions) to the instrumental approach–avoidance actions were systematically tested. The valence of angry (versus happy) faces was indeed found to transfer to instrumental decision making, in such a way that it induced an instrumental avoidance bias. The extent of freezing elicited by the angry faces was significantly correlated to the instrumental avoidance bias.

Both automatic freeze–fight–flight tendencies and more instrumental approach and avoidance biases have been suggested to play a prominent role in the maintenance and perhaps even cause of psychopathology (Blanchard et al., 2011; Rudaz, Ledermann, Margraf, Becker, & Craske, 2017; Turk, Lerner, Heimberg, & Rapee, 2001; Wong & Moulds, 2011). Aggression, for instance, has been conceptualized as a defensive response system in which automatic fight - responses are triggered too easily and in which instrumental threat– approach tendencies become well-learned and rewarded (Blair, 2013; Blanchard et al., 2011; Ly et al., 2016). On the contrary, persistent avoidance in anxiety disorders has been thought of as a defensive response system in which automatic flight –response are easily triggered and in which instrumental threat– avoidance tendencies become rewarded and well learned (Blanchard et al., 2011).

Rolls, (2000) emphasized the importance of facial expressions as input for these systems, as they convey social information. When applied in social approach–avoidance tasks (AATs), healthy people show a general tendency to move away from angry expressions and to approach happy faces (Bradley et al., 2001; Chen & Bargh, 1999; Heuer et

al., 2007; Roelofs, Minelli, et al., 2009; Inge Volman, Toni, et al., 2011). Social AATs using emotional faces have therefore been used to objectively measure the motor responses that are brought about by the automatic and instrumentally driven tendency to approach or avoid a certain stimulus (Chen & Bargh, 1999; Heuer et al., 2007; Roelofs, Elzinga, & Rotteveel, 2005; Rotteveel & Phaf, 2004). A commonly used type is a manual reaction time task which requires participants to approach and to avoid socially appetitive and aversive visually presented stimuli (happy and angry faces, respectively) by pulling (approach) or pushing away a joystick (avoidance) (see Figure 2.2E). In zooming versions of the AAT, pulling or pushing the joystick increases or decreases the size of the picture respectively, giving the impression of moving towards or moving away from the participant (Heuer et al., 2007). Affect–behavior congruence (i.e., approaching happy or avoiding angry faces) leads to quicker responses than when automatic tendencies need to be overridden, as is the case with affect–behavior incongruence (i.e., approaching angry or avoiding happy faces). Highly socially anxious individuals have been shown to avoid socially threatening (i.e., angry) faces, compared to low anxious controls (Heuer et al., 2007; Roelofs, Putman, et al., 2010), while psychopathic offenders show diminished avoidance tendencies of angry faces, compared to controls (von Borries et al., 2012).

Neurobiology underlying social motivational behavior

Approach and avoidance-related behaviors are mediated by complex interacting neural networks, which can be categorized in the so-called emotional network, reward network, and cognitive control network (Cremers & Roelofs, 2016), which will be broadly described hereafter. The amygdala plays a central role in the emotional network; its subnuclei process salient information from the environment, such as emotional facial expressions, and trigger behavioral responses in response to these environmental stimuli. The basolateral amygdala (BLA) receives input from the thalamus and sensory cortices (such as fusiform gyrus, involved in face processing), whereas the central amygdala (CeA) orchestrates autonomic responses by projections to the periaqueductal gray (PAG) initiating freeze, to brainstem nuclei for release of neurotransmitters, and the hypothalamus for release of oxytocin, corticotropin releasing hormone (CRH), and gonadotropin releasing hormone (GnRH). This eventually leads to enhanced cortisol and testosterone levels, respectively. The amygdala is also connected to the reward network, which comprises the ventral tegmental area (VTA), striatum (including the nucleus accumbens (NAcc)), and medial prefrontal cortex (mPFC) (Haber & Knutson, 2010). Striatal dopamine transmission is essential for the adaptive regulation of social behavior as it is involved in reward learning

(i.e., obtaining social reward but also avoiding punishment; see Delgado, (2009), behavioral activation, and motivational behavior (Cools, 2008; Yacubian & Büchel, 2009). The anterior prefrontal cortex plays a crucial role in the cognitive control network as it is involved in the regulation of emotion (Damásio, 1994; Rolls, 1999). It also has a role in social motivational behavior as it inhibits the amygdala, making it possible to control and override automatic behavioral approach and avoidance tendencies (Roelofs, Minelli, et al., 2009; Volman, Roelofs, Koch, Verhagen, & Toni, 2011). Furthermore, it modulates mesolimbic striatal activity (Grace, Floresco, Goto, & Lodge, 2007; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). Naturally, this description is a highly simplified one, and many other brain regions partake in these networks (Cremers & Roelofs, 2016).

Hormonal regulation of social motivational behavior

Testosterone

The hypothalamus–pituitary–gonadal (HPG) axis with its end product testosterone plays a key role in the neuroendocrine regulation of social motivational behavior in both sexes. Testosterone levels follow a pulsatile, seasonal, and diurnal cycle in which levels are highest upon waking and typically decline by 50% during the day (Dabbs, 1990). Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus, which stimulates the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary gland, which in turn triggers production of testosterone and estradiol in the gonads (i.e., testes and ovaries). The secreted estradiol and testosterone in turn inhibit the hypothalamus and pituitary, thus forming a negative feedback loop. In addition, small amounts of testosterone are produced in the adrenal cortex and synthesized in the brain from cholesterol and other steroid precursors. Testosterone is able to cross the blood–brain barrier, and besides having (epigenetic) organizational effects on brain structures during pre- and early postnatal development, testosterone also influences emotion, motivation, and behavior later in life (i.e., activational effects; Lombardo et al. (2012; McHendry, Carrier, Hull, & Kabbaj. (2014). Actions of testosterone are brought about directly via androgen receptors but also via metabolites such as estradiol, dihydrotestosterone, and 3-diol, which binds to the aminobutyric acid (GABA-A) receptor (Balthazart & Ball, 2006; Wood, 2008). The effects can either be slow and long-lasting (i.e., hours–days) via a genomic pathway featuring intracellular steroid receptors, or rapid (i.e., seconds–minutes) via membrane-bound (steroid) receptors, which exert non-genomic actions in the cell. Importantly, testosterone acts through a steroid-responsive network which includes the amygdala, hypothalamus,

hippocampus, and PAG, among other limbic areas (Wood, 1996), and hence influences the flight–fight response.

Naturally, testosterone interacts with other neurotransmitters and peptides, such as serotonin (probably via estradiol), vasopressin, oxytocin, and dopamine. With regard to the latter, testosterone enhances dopamine transmission in the mesolimbic system, which in turn can lead to increased reward sensitivity and augmented motivational behavior by promoting dopaminergic projections form the amygdala to the striatum (de Souza Silva et al., 2009; Hermans et al., 2010; Welker, Gruber, & Mehta, 2015).

Baseline hormone levels are in general predictive of psychological traits and behavior (Welker et al., 2015), whereas social events are typically associated with a temporary surge or decline in hormone levels (Casto & Edwards, 2016; Maner et al., 2008; Sapolsky, 1991). The social challenge hypothesis states that testosterone levels rise in preparation to a challenging encounter in which social status might be threatened, thereby initiating approach motivation and simultaneously reducing fear (Archer, 2006; Mazur & Booth, 1998; Wingfield et al., 1990). Several studies featuring single-dose testosterone administration, which leads to a transient increase in testosterone levels, to healthy female participants confirmed the causal relationship between testosterone and its effects on the social motivational system. The findings show that testosterone administration reduces fear and sensitivity to threat and punishment, enhances reward sensitivity, and promotes social approach motivation aimed at achieving social status (i.e., social reward; see for a review (Bos, Panksepp, et al., 2012; Enter et al., 2014). These actions have been suggested to be brought about by anxiolytic effects (GABA, androgen receptors; (McHenry et al., 2014) and upregulation of the dopaminergic system (de Souza Silva et al., 2009), in addition to biasing the amygdala towards threat approach (Radke et al., 2015) and reducing prefrontal control over the amygdala (Schutter & van Honk, 2004; van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010; Volman, Toni, et al., 2011). Although associated with aggression (Montoya et al., 2012), the effects of testosterone on social motivational behavior depend on social context and individual differences and thus do not entail aggressive behavior per se, but could also lead to prosocial behavior when this is more appropriate to ensure an increase in social status (Boksem et al., 2013; Carré et al., 2017; Eisenegger, Haushofer, & Fehr, 2011; Mehta & Josephs, 2010; Stanton & Schultheiss, 2009; van Honk, Terburg, & Bos, 2011; sample sizes in these studies ranged from n = 54 to n = 121).

Cortisol

For decades cortisol has been a popular biomarker to index acute and chronic social and psychological stress (Hellhammer, Wüst, & Kudielka, 2009). Individual differences in the

diurnal pattern are associated with psychopathology (Adam et al., 2017); however, most research has focused on stress-induced cortisol surges. Like testosterone, this hormone follows a pulsatile and diurnal pattern, in which levels are high in the morning, surging within 30–40 minutes after waking, followed by a steep drop for a few hours and a steady decline until the lowest point at bedtime. Cortisol is the end product of the hypothalamus–pituitary–adrenal (HPA) axis. The hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACH); this travels via the bloodstream to the adrenal cortex where it stimulates the production of cortisol. Cortisol in turn inhibits the pituitary and the hypothalamus, forming a negative feedback loop, and is able to exert both rapid non-genomic and slow genomic effects in the brain (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006). Cortisol binds to glucocorticoid and mineralocorticoid receptors in brain areas important in regulating the fight–flight response, such as frontal areas, amygdala, and hippocampus (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). It has an important role in regulating homeostatic systems, affecting arousal, metabolic processes, and the immune system (Sapolsky, Romero, & Munck, 2000). During the initial phase of the stress response, epinephrine from the adrenal medulla triggers norepinephrine release in the basolateral amygdala, among other regions, which induces an increase in vigilance by prioritizing sensory processing and activation of the amygdala (Osborne, Pearson-Leary, & McNay, 2015). Subsequent cortisol release regulates the stress response by downregulating amygdala responsivity and decreasing anxiety-driven selective attention to threat (Henckens, van Wingen, Joëls, & Fernández, 2010, *n* = 72; Putman & Roelofs, 2011; van Peer et al., 2009, *n* = 21, small effect sizes), besides affecting activity in areas involved in the planning and execution of motor responses (Montoya, Bos, Terburg, Rosenberger, & van Honk, 2014, *n* = 20). Animal research has shown that higher cortisol levels are associated with social avoidance behavior (Sapolsky, 1990). Studies featuring stress-induced cortisol surges and cortisol administration in healthy humans extend these findings by showing that elevated levels of cortisol are associated with increased avoidance of social threat on the AAT (Roelofs et al., 2005, *n* = 22, small to medium effect sizes; van Peer et al., 2007, *n* = 40, lare effect sizes).

The HPG axis works in antagonism with the hypothalamus–pituitary–adrenal (HPA) axis, in such a way that the end product of the latter (i.e., cortisol, released in response to stress) disrupts production and inhibits actions of testosterone, which in turn inhibits the stress-induced activation of the HPA axis at the hypothalamus (Viau, 2002). Both neuroendocrine axes are important in the regulation of social–motivational behavior and show a complex interaction: basically, higher basal cortisol levels, and low testosterone, are associated with social subordination stress and avoidance behavior, whereas higher basal testosterone and low cortisol facilitate social dominance and approach behavior

(Bedgood, Boggiano, & Turan, 2014; Mehta & Josephs, 2010; Mehta, Lawless DesJardins, van Vugt, & Josephs, 2017; Sapolsky, 1990, 1991; van Honk et al., 1999).

Oxytocin

Originally considered as having a key role in labor and lactation, in the past decade the neuropeptide oxytocin has gained more and more interest as a modulator of social cognition and behavior. Oxytocin has a very similar structure to vasopressin, and both neuropeptides are synthesized in the supraoptic and paraventricular nuclei of the hypothalamus (Johnson & Young, 2017). From there they are released, via the anterior pituitary, in the bloodstream. In addition, there are projections to the amygdala, lateral septum, nucleus accumbens, hippocampus, and ventral tegmental area (Ross & Young, 2009), which are areas involved in the fight–flight response. Oxytocin inhibits the output of the central amygdala to the PAG, whereas vasopressin excites this pathway (Huber, Veinante, & Stoop, 2005). There are indications that oxytocin attenuates the cortisol stress response (Cardoso, Kingdon, & Ellenbogen, 2014) and it is thought to have anxiolytic effects (Heinrichs & Domes, 2008). In addition, oxytocin enhances the salience of social information by increasing attention towards social cues and also increases the reward value of social stimuli (see for a review Crespi (2016)). During the control of social approach–avoidance behavior, oxytocin decreases amygdala responses during threat approach as a result of its anxiolytic properties (Radke et al., 2017, $n = 57$). Also, oxytocin administration promotes threat approach in low socially anxious men (Radke, Roelofs, & de Bruijn, 2013, $n = 24$, medium to large effect sizes). It has been proposed that oxytocin and testosterone have opposite effects on social cognition and behavior: where testosterone facilitates a dominance-related approach strategy which serves individual status defense, oxytocin promotes social exploration and in-group protection (Bos, Panksepp, et al., 2012; Reimers & Diekhof, 2015). It is important to note that the effects of oxytocin depend on social context and individual differences and can have both positive and negative social effects. Shamay-Tsoory & Abu-Akel, (2016) argue that oxytocin increases the salience of safety signals in a positive and supportive context, but on the other hand triggers orienting responses to threat and enhances anxiety in an unpredictable and threatening situation. It is likely that interactions between phasic dopaminergic signaling and oxytocin in the ventral tegmental area, nucleus accumbens, and amygdala modulate the effects of context and individual differences. Interactions of oxytocin and serotonin in the nucleus accumbens have been shown to be important in social interaction (Dölen, Darvishzadeh, Huang, & Malenka, 2013).

Before discussing the potential role of these hormones and peptides in social psychopathologies, we will introduce two types of pathologies that show marked alterations in social motivational processing: social anxiety and psychopathy.

Social psychopathology

Social anxiety disorder

Social anxiety disorder (SAD) is one of the most common mental health disorders (e.g., Bandelow & Michaelis, 2015). SAD is characterized by an intense fear of social situations in which the individual may be scrutinized by others (American Psychiatric Association, 2013). The affected individual fears that he/she will behave, or show anxiety symptoms, in a way that will be negatively evaluated and will lead to rejection by others. Social situations, such as social interactions, are therefore avoided or endured with intense fear or anxiety. Avoidance behavior plays a crucial role in the persistence of the disorder and hinders extinction of fear in social situations as it reduces the opportunity for accommodation to and reevaluation of the situation (Clark & Wells, 1995). In addition, when engaging in social interaction, someone with SAD typically tends to avoid eye contact (Stein & Stein, 2008). As eye contact is important in social communication, this characteristic hinders social interactions and influences how others respond to the person with SAD, reinforcing the social fear–avoidance cycle. Furthermore, there is evidence that SAD persists because of biased processing of social information, favoring disorder-relevant information, which leads to interpretation of the situation as more negative than it was in reality (Heeren, Lange, Philippot, & Wong, 2014; Stein & Stein, 2008). With a lifetime prevalence rate of 7–12%, SAD is the most common anxiety disorder and among the most common psychiatric disorders (Kessler et al., 2005). Onset occurs in childhood or early adolescence, and SAD affects more women than men. The disorder typically leads to significant distress and, when left untreated, tends to follow a chronic, unremitting course leading to substantial impairments in vocational and social functioning. Treatment of SAD consist of pharmacotherapy and/or psychotherapy, mainly cognitive behavioral therapy aiming at acquiring the behavioral and cognitive skills to function effectively. Exposure therapy is part of the latter and aims at fear extinction by repeated or prolonged exposure to feared social situations, leading to a reduction of anxiety and avoidance behavior. Notwithstanding the efficacy of current evidence-based psychological and pharmacological treatments in SAD, nonresponse rates in large clinical trials have been up to 50% (Hofmann & Bögels, 2006; Stein & Stein, 2008), leaving considerable room for improvement.

Neuroendocrinology of social anxiety disorder

Social anxiety disorder (SAD) is associated with deviations in the neuroendocrine brain circuits underlying social motivational behavior. Unfortunately, evidence is inconsistent, and may be due to relatively small sample sizes (averaging 12 patients per group) and differences in methods and analyses. Nevertheless, several meta-analytic studies have consistently shown a hyperactive amygdala in response to social threat, probably reflecting enhanced processing of and attention to threat (Brühl, Delsignore, Komossa, & Weidt, 2014; Cremers & Roelofs, 2016; Fouche, van Der Wee, Roelofs, & Stein, 2013). In addition, prefrontal structures are also more active than in healthy controls; however, prefrontal–amygdala connectivity seems to be reduced (Brühl et al., 2014; Cremers & Roelofs, 2016; Fouche et al., 2013), indicating an inability to regulate subcortical regions. Interestingly, pharmaco- and psychotherapy seem to "normalize" deviating activation patterns in SAD (Fouche et al., 2013; Freitas-Ferrari et al., 2010). Studies also show alterations in striatal functioning in SAD, but findings are mixed (Freitas-Ferrari et al., 2010). A recent fMRI study in patients with SAD (n = 20) compared to healthy controls (n = 20) reported reduced striatal activity in anticipation of social reward and relative increased striatal activity for avoiding social punishment (Cremers, Veer, Spinhoven, Rombouts, & Roelofs, 2015). These findings suggest that patients with SAD show a reduced motivation to obtain social reward and relative increased motivation to avoid social punishment compared to healthy controls. In addition, patients with SAD showed a reduced pattern of fronto-striatal connectivity during reward and punishment anticipation, relative to healthy controls.

Patients with SAD (n = 18) show an increased cortisol response to social stress, compared to healthy participants (n = 22) and patients with Post-Traumatic Stress Disorder (PTSD; n = 17; small to medium effect sizes), and this response was associated with social avoidance behavior (large effect sizes) (Roelofs, van Peer, et al., 2009b). Studies combining cortisol administration with electroencephalography (EEG) in patients with SAD confirmed a causal relationship between cortisol and increased early processing of emotional faces during social avoidance (van Peer et al., 2009, n = 21, large effect size), and modulation of early threat processing depending on motivational context and symptom severity (van Peer, Spinhoven, & Roelofs, 2010, n = 18). In addition, both higher baseline levels of cortisol and exogenous cortisol are associated with EEG wave activity patterns related to anxiety and behavioral inhibition (Schutter & Van Honk, 2005, n = 28; van Peer, Roelofs, & Spinhoven, 2008, n = 40), whereas testosterone has an opposite effect (Schutter & van Honk, 2004, n = 16).

Studies on testosterone in SAD are scarce, and although previous results on the relation between SAD and baseline testosterone levels show no differences (Gerra et al.,

2000, *n* = 40 ; Maner et al., 2008, *n* = 64), recent findings from a large cohort study show reduced testosterone levels in women with SAD compared to women without a lifetime history of anxiety or depressive disorders. (Giltay et al., 2012, *n* = 2102, small-medium effect size). Interestingly, testosterone administration to women with SAD promotes social threat approach on the AAT (Enter, Spinhoven, & Roelofs, 2016, *n* = 17) and socially dominant gaze behavior. For example, patients with SAD have been shown to display reduced fixations on the eye-regions of angry (versus neutral) faces (Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2016, SAD *n* = 18, HC *n* = 19; Horley, Williams, Gonsalvez, & Gordon, 2004, SAD *n* = 22, HC *n* = 22). Administration of 0.5 mg testosterone in 18 patients with SAD resulted in normalization of the gaze pattern, increasing the number of first fixations to the eye-region of angry faces (see Figure 2.1). These results suggest that testosterone is able to promote social dominant behavior by its anxiolytic and reward-promoting properties, presumably by influencing early automatic mechanisms (van Peer, Enter, van Steenbergen, Spinhoven, & Roelofs, 2017, SAD *n* = 19, HC *n* = 19).

Quite a few studies have focused on the role of oxytocin in SAD, but the results should be interpreted with caution, as statistical power tends to be low (Walum, Waldman, & Young, 2016). There is no evidence of altered baseline levels in SAD compared to healthy controls, although higher levels of oxytocin were associated with more severe social anxiety symptomatology and less satisfaction in social relationships in one study (*n* = 46) (Hoge, Pollack, Kaufman, Zak, & Simon, 2008). Oxytocin administration studies in SAD (18 SAD patients versus 18 healthy controls) have shown that this neuropeptide is able to dampen heightened amygdala and prefrontal responses (Labuschagne et al., 2010; Labuschagne et al., 2012) and to normalize amygdala–frontal connectivity during resting state (Dodhia et al., 2014, *n* = 36). Interestingly, oxytocin administration promotes other-oriented reward motivation, but only in patients with generalized SAD who have less severe social interaction anxiety (Fang, Treadway, & Hofmann, 2017, *n* = 52).

In sum, individuals with SAD show alterations in the regulation of social motivational behavior characterized by persistent social avoidance, reduced testosterone levels, increased cortisol responses, enhanced threat sensitivity, and probably reduced reward processing, a pattern that is associated with socially submissive behavior. Results on the role of oxytocin in SAD are inconclusive and need to be elucidated in future research.

Psychopathy

Psychopathy is a multidimensional personality condition which overlaps partly with anti-social personality disorder, sharing an anti-social lifestyle, but distinguished by affective–interpersonal impairments (Brazil, van Dongen, Maes, Mars, & Baskin-Sommers, 2016). The full clinical manifestation affects less than 1% of the general population, and ap-

proximately 15–25% of the prison population. Characterization in the literature is inconsistent, but psychopathy has often been defined based on the Hare Psychopathy Checklist, which describes psychopathy along two distinct dimensions: Factor 1 (comprising lack of guilt and empathy, shallow affect, and pathological lying), and Factor 2 (including impulsivity, anti-social behavior, and sensation-seeking) (Hare & Neumann, 2008). Alternatively, a distinction between primary and secondary psychopathy has been made, with similar symptoms but a difference in anxiety levels (Anderson & Kiehl, 2012; Brazil et al., 2016; Van Honk & Schutter, 2006). Whereas primary psychopathy is characterized by low anxiety, secondary psychopathy is defined by higher anxiety levels. Both show pronounced problems in emotional processing (e.g., reduced guilt, empathy, etc.), increased goal directed behavior, instrumental aggression (i.e., goal-oriented self-serving aggression), and an increase in impulsive behavior and uncontrolled aggression after emotional provocation.

Psychological and behavioral interventions give mixed results in effectiveness, depending on different types of anti-social individuals (Brazil et al., 2016). In general, psychopathic individuals are not responsive to treatment due to the specific characteristics of the disorder and a lack of motivation to seek treatment.

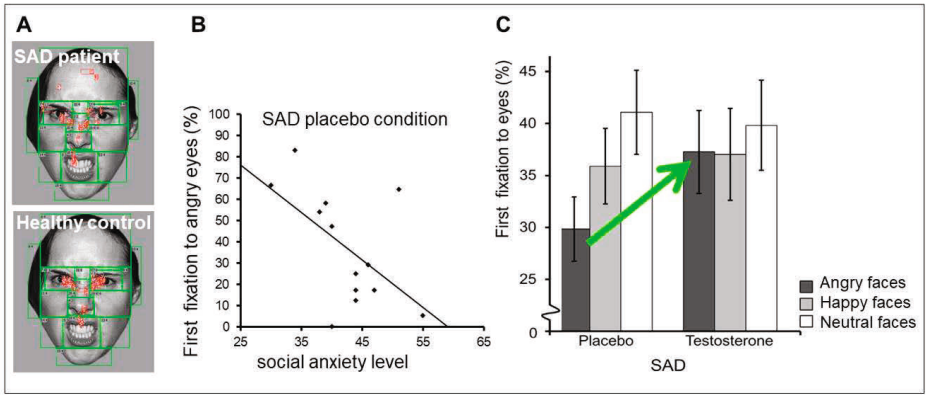

Figure 2.1 Illustration of gaze path, correlation between Social Anxiety symptoms and first fixations on angry eyes and effects of testosterone administration on gaze avoidance in individuals with SAD. Adapted from Enter, Terburg, Harrewijn, Spinhoven, and Roelofs (2016). Panel A provides an illustration of a gaze path measured using eye-tracking while a patient with social anxiety disorder (SAD) and a healthy control participant (HC) were viewing an angry facial expression. Panel B: A correlation between percentage first fixations on angry eyes and LSAS social anxiety scores (Liebowitz, 1987) in the placebo condition indicated that SAD participants with stronger anxiety symptomatology showed increased gaze avoidance of angry eye contact (r = -.561, p = .046). Panel C: Testosterone administration, compared to placebo, alleviated gaze avoidance of angry eye contact by increasing the percentage first fixations towards angry eyes.

Neuroendocrinology of psychopathy

Research on the neuroendocrinological underpinnings of psychopathy suffers from small sample sizes, especially for individuals scoring high on the psychopathy checklist, and also from lack of one clear definition of the different subtypes of psychopathy (Brazil et al., 2016; Koenigs, Baskin-Sommers, Zeier, & Newman, 2011). One of the few studies with psychopathic offenders (n = 17) featuring the AAT indicates that this maladaptive behavior already stems from early automatic mechanisms by showing reduced avoidance tendencies towards angry faces compared to healthy control participants (n = 15) (von Borries et al., 2012). In addition, this effect was related to higher levels of instrumental aggression and reduced feelings of discomfort when observing another's negative experiences. Neuroimaging studies of psychopathic offenders show structural deviations in several brain areas, including reduced amygdala and prefrontal volume, increased striatal volume, and an abnormal shape of the hippocampus (Koenigs et al., 2011). In addition, psychopathy is associated with impaired amygdala–prefrontal connections in a relatively small sample (n = 22) and a larger sample (n = 147); (Hoppenbrouwers et al., 2013; Wolf et al., 2015, respectively). On a functional level, the amygdala seems to respond less to aversive stimuli, fearful faces, and pictures of moral violations (Anderson & Kiehl, 2012; Decety, Chen, Harenski, & Kiehl, 2015, n = 155), in addition to atypical activity of the aPFC during various tasks. Interestingly, striatal activity is enhanced, suggesting an increase in reward sensitivity (Van Honk & Schutter, 2006). During the control of social approach–avoidance behavior, psychopathic offenders (n = 15) show reduced anterior prefrontal cortex activity and less anterior prefrontal cortex–amygdala connectivity (versus 19 healthy controls), and this was modulated by endogenous testosterone levels (Volman et al., 2016). These findings suggest that higher testosterone levels are associated with less prefrontal control over amygdala-driven actions.

Because of its social dominance-promoting effects, including dampening of punishment sensitivity and increasing reward sensitivity, testosterone has been in the picture with regard to the biological underpinnings of psychopathy. Psychopathy is associated with social dominance (Lobbestael, Arntz, Voncken, & Potegal, 2018). Studies yielded mixed results in trying to relate endogenous testosterone to psychopathy scores (e.g., Glenn et al., 2012, n = 178; Welker, Lozoya, Campbell, Neumann, & Carré, 2014), and rather found relationships with typical personality traits associated with psychopathy, such as impulsivity and antisocial aspects (Stålenheim, Eriksson, von Knorring, & Wide, 1998, n = 61). Importantly, several studies showed that the relation between testosterone and psychopathy was modulated by cortisol (Glenn et al., 2012; Loomans, Tulen, de Rijke, & van Marle, 2016; Welker et al., 2014, sample sizes were respectively 178, 166, 237). A combination of high testosterone levels and low cortisol levels tends to be associated

with psychopathy (Loomans et al., 2016), which predisposes individuals to aggressive behavior (Montoya et al., 2012; Terburg, Morgan, & van Honk, 2009). However, Welker et al. (2014) showed a different pattern of results in which the positive relationship between testosterone and psychopathy only emerged when cortisol was high. High testosterone levels in utero, during adolescence, and in response to stress might predispose to psychopathy, in combination with individual and environmental risk factors (Yildirim & Derksen, 2012). Testosterone likely dampens oxytocinergic effects on social empathy (see Yildirim & Derksen, 2012 for a review). Both lower oxytocin levels and certain variations in oxytocin receptor polymorphisms are related to risk factors for developing psychopathy (e.g., callous– unemotional traits and conduct problems) in children and adolescents (Dadds et al., 2014; Levy et al., 2015).

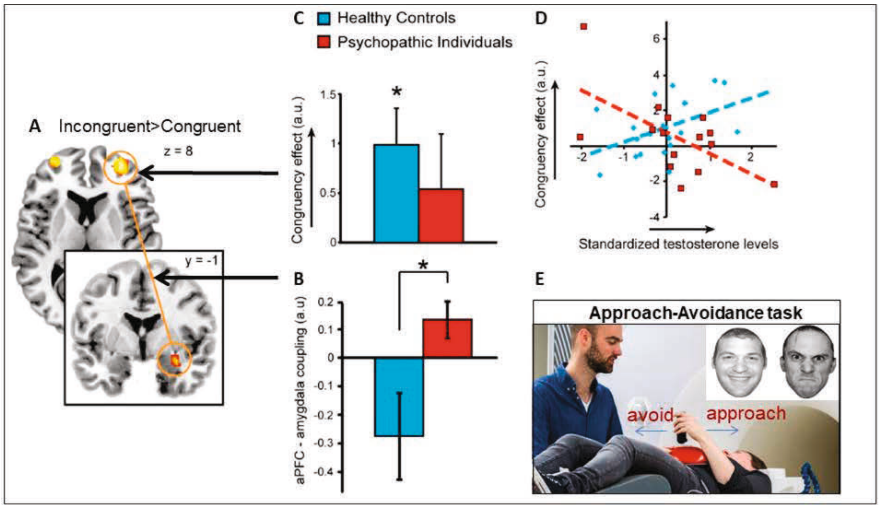

Figure 2.2 Summary of results of approach – avoidance tendencies on the Approach Avoidance Task in psychopathic individuals and healthy controls. Adapted from Volman et al., 2016 . Panels A and B: Psychopathic individuals (violent offenders with high psychopathic traits) show reduced pattern of negative coupling between the anterior prefrontal cortex (aPFC) and the amygdala when they have to control their emotional actions on the approach– avoidance task (AAT, see panel E), compared to healthy matched controls. Panel C: Psychopathic individuals (compared to controls) show reduced local activity in the aPFC when they exert approach-avoidance control, and this effect is modulated by their testosterone levels (Panel D). Panel E: fMRI adapted AAT– people make affect-congruent reactions by pulling a joystick towards their body when detecting a happy face or by pushing the joystick away from their body in response to an angry face. In contrast, affect-incongruent responses involved the opposite reactions to the emotional faces, which requires AA control and is typically associated with increased reaction times and increased aPFC activity (Volman et al., 2011 , 2013).

Taken together, the pattern that emerges is that psychopathy is associated with structural and functional brain deviations. Alterations in social approach–avoidance behavior, such as decreased social threat avoidance, are likely associated with reduced prefrontal control over limbic structures. Testosterone plays a role in modulation of prefrontal–amygdala connectivity, and is associated with psychopathic tendencies, a relationship that seems to be modulated by cortisol. Lower oxytocin seems to be related to psychopathy as well. However these findings should be considered with caution, due to small sample sizes and differences in definitions of psychopathy.

Clinical implications and future directions

The above-described neuroendocrine aspects and effects on social motivational behavior in social anxiety and psychopathy give rise to various directions for future research aiming to provide starting points for the enhancement of interventions into these persistent disorders.

It would be of theoretical and clinical interest to test whether hormone administration could benefit the treatment of SAD. Exposure therapy is part of first-line treatment of SAD and aims at fear extinction by repeated or prolonged exposure to feared social situations, which should lead to a reduction in fear and avoidance behavior. Although exposure therapy has proven effective, nonresponse rates in large clinical trials have been 50% or higher (Hofmann & Bögels, 2006), and many patients do not achieve remission. In an attempt to enhance exposure therapy efficacy, research has explored the augmentation effects of pharmacological agents thought to enhance the underlying mechanisms of action (e.g., extinction learning) of exposure therapy, for example using D-cycloserine, yohimbine hydrochloride, glucocorticoids, and cortisol and brain-derived neurotrophic factor. This approach has potential, as was shown by studies featuring a variety of pharmacological agents (e.g., Hofmann, Fang, & Gutner, 2014; McGuire, Lewin, & Storch, 2014). Despite initially promising findings, the working mechanisms are still not entirely clear and studies yield mixed findings. It is possible that these cognitive enhancers do not target the most optimal mechanism for enhancement of exposure therapy, and an alternative approach targeting social motivational mechanisms directly might provide a more effective solution. Considering the alleviating effects of testosterone on actual social avoidance behavior in SAD, and considering that exposure therapy is also aimed at reduction of avoidance behavior, it would be of relevance to test whether single-dose testosterone administration – applied only a few times to enhance efficacy of the first few exposure sessions – can enhance therapy efficacy for SAD. Nevertheless, it should

be noted that there is still much unclear about the working mechanisms of testosterone and of pharmacological add-ons in exposure therapy. Testosterone could have beneficial effects on dopamine transmission and glucocorticoid mechanisms, but its effects on the GABA system might, apart from being anxiolytic, also potentially interfere with extinction learning, which should be elucidated in future research (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015).

The anxiolytic effects of oxytocin administration have prompted research on its suitability for SAD treatment (Kirsch, 2015). It should be noted that there is still controversy around the effects of intranasal oxytocin, and that there is likely a publication bias (Lane, Luminet, Nave, & Mikolajczak, 2016; Leng & Ludwig, 2016). Intranasal oxytocin administration improved self-evaluation of performance during exposure therapy sessions, and thus was able to counteract the typical exaggerated negative mental representations after social performance in SAD; however, no long-term effects could be established, which warrants further research (Guastella, Howard, Dadds, Mitchell, & Carson, 2009, n = 25). In addition, intranasal oxytocin administration seemed to reduce "physical discomfort" in patients with SAD during a Trier Social Stress Test (Heinrichs et al., 2006). The ability of oxytocin to make social interactions more rewarding might be beneficial for certain subtypes of SAD patients who lack the motivation to engage in social interaction (Van Honk, Bos, Terburg, Heany, & Stein, 2015).

Finally, an initial study with cortisol administration to promote extinction learning during exposure sessions in 40 patients with SAD led to reduced heart rate and self-reported anxiety during the sessions (Soravia et al., 2006). However, long-term effects of cortisol on persistent avoidance tendencies in SAD have not been investigated.

Another interesting approach would be to explore whether the automatic avoidance tendencies in SAD and psychopathy could be diminished by approach–avoidance training on the AAT. Research on this topic across various disciplines has shown positive results (Eberl, Wiers, Becker, Lindenmeyer, & Rinck, 2011; for a review see Woud & Becker, 2014). Two studies featuring socially anxious participants showed that, after being required to approach positive social stimuli on the AAT (e.g., smiling faces), they subsequently showed more approach behavior during social interactions, elicited more positive reactions by their interaction partners (Taylor & Amir, 2012, n = 47, large effect sizes), and reported better mood and less anxiety after a social challenge (Rinck et al., 2013, n = 40, medium to large effect sizes). A study featuring approach–avoidance training in alcoholism (20 alcohol dependents versus 17 healthy controls) showed that amygdala activity reduced in response to alcohol-related stimuli (Wiers et al., 2014). This finding suggests that the affective evaluation of the stimulus was altered by the training; however, the mechanisms behind approach–avoidance training are not clear to date. It is

also possible that the training increases prefrontal control over amygdala-driven approach–avoidance responses. Single-dose testosterone administration in SAD might aid the training process by biasing the brain towards social approach, although this effect is likely specific for social threat faces.

All these studies would be helped by increased clarity on the neuroendocrine mechanisms underlying deviations in social motivational behavior in SAD. Research featuring neuroimaging techniques should shed more light on this matter. Future studies could combine social approach and avoidance tasks with fMRI, endogenous hormone/neuropeptide measurements (e.g., Volman, Toni, et al., 2011), and/or testosterone/oxytocin administration to find out how these neuroendocrine agents modulate social approach/avoidance behavior in SAD. In addition, it would be interesting to try to probe the functioning of the emotion-network (e.g., fear reduction by GABA-ergic mechanisms) and the reward network (dopaminergic mechanisms) in SAD, by using single photon emission computed tomography (SPECT) or positron emission tomography (PET) scanning (e.g., Schneier, Kent, Star, & Hirsch, 2009; van der Wee et al., 2008), in combination with testosterone administration. It would be particularly interesting to add genotyping for androgen and dopaminergic receptor genes. Furthermore, future research featuring EEG or magnetoencephalography (MEG) should further elucidate the temporal dynamics of these processes in both healthy individuals and those with SAD.

The same line of reasoning holds for aggression-related disorders, such as psychopathy, for which identification of biological underpinnings of subtypes and adaptive treatment is still in its infancy (e.g., Brazil et al., 2016). More research is needed to obtain starting points for studying the effects of hormone manipulations on the management of social aggression. Pioneering evidence in autism spectrum disorders, where aggression plays an important role as well, suggests that oxytocin has the potential to enhance motivation and attention to social cues in patients with autism spectrum disorder (Yamasue, 2016). A recent review and meta analyses concluded that studies on autism did show significant effect sizes (combined effect size of d = 57, e.g., medium effect); however, oxytocin seemed less effective in other psychopathologies (Bakermans-Kranenburg & Van IJzendoorn, 2013; Guastella & Hickie, 2016). On the other hand, no significant meta-analytic effect of oxytocin on the social domain in autism was found by Ooi and colleagues (Ooi, Weng, Kossowsky, Gerger, & Sung, 2017). More sophisticated and targeted clinical trials are required, due to a limited number of studies and small sample sizes. Whether similar interventions may be effective for aggression-related disorders, such as psychopathy, remains to be determined. It is conceivable that the social salience enhancing effects of oxytocin could make matters worse in psychopathy.

Finally, increasing evidence suggests that the effects of testosterone, cortisol, and oxytocin on social motivational behavior are modulated by individual differences and social context. For example, the effects of testosterone and oxytocin on social motivational behavior in healthy individuals were modulated by social anxiety scores (Enter et al., 2014; Radke et al., 2013). Future research should address these influences in order to optimize indications for potential hormonal interventions.

Conclusion

Steroid hormones, like cortisol and testosterone, and neuropeptides such as oxytocin, play a role in the regulation of social motivational behavior. Both social anxiety and aggression-related disorders show an imbalance in these steroid hormones. Given the important interaction between steroid hormones and the main motivational systems in healthy individuals as well as individuals with social psychopathologies, future studies should explore the potential role of hormones in enhancing therapy efficacy.

Chapter 3

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

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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 the 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.

Introduction

Social anxiety disorder (SAD) is one of the most common anxiety disorders, with a lifetime prevalence rate of 13% (Bandelow & Michaelis, 2015). Persistent avoidance behavior in SAD is a major factor that hinders extinction of fear during social situations (Arnaudova et al., 2017; Clark & 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., 2005). 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 & 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 & Wrangham, 2004) and humans (Bateup et al., 2002; Neave & Wolfson, 2003) 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, Panksepp, et al., 2012). Consistent with this hypothesis, high endogenous testosterone has been associated with social dominance and approach behavior (Maner et al., 2008; Mazur & 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 (Gutiérrez-García, Contreras, Vásquez-Hernández, Molina-Jiménez, & Jacome-Jacome, 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 and 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, Terburg, et al., 2016). In addition, testosterone administration reduces automatic threat bias to angry faces in SAD patients (Enter, Spinhoven, et al., 2016; van Peer et al., 2017). These findings converge to suggest that enhanced testosterone-reactivity prior to exposure therapy may facilitate its outcomes (Enter, Hutschemaekers, & Roelofs, 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.

Materials and Methods

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 ex-

posure 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, Dreber, Johannesson, Sul, & Weber, 2016; Simmons & 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.

Exposure session

Participants all completed one standardized exposure session, based on the protocol developed by Rodebaugh, Levinson, and Lenze (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 minutes) 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, Smits, & Telch, 2004; Ressler et al., 2004; Sloan & Telch, 2002; Smits, Rosenfield, Otto, et al., 2013b; Telch et al., 2014; Wolitzky & Telch, 2009).

Outcome Measures

In-session fear

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

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).

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 Figure 3.1: T1) 50 minutes prior to exposure (30 minutes after arrival), T2) 25 minutes prior to exposure, see procedure paragraph), T3) directly prior to exposure, T4) after exposure (around 20 minutes after start exposure) and T5) 50 minutes after the start of exposure.

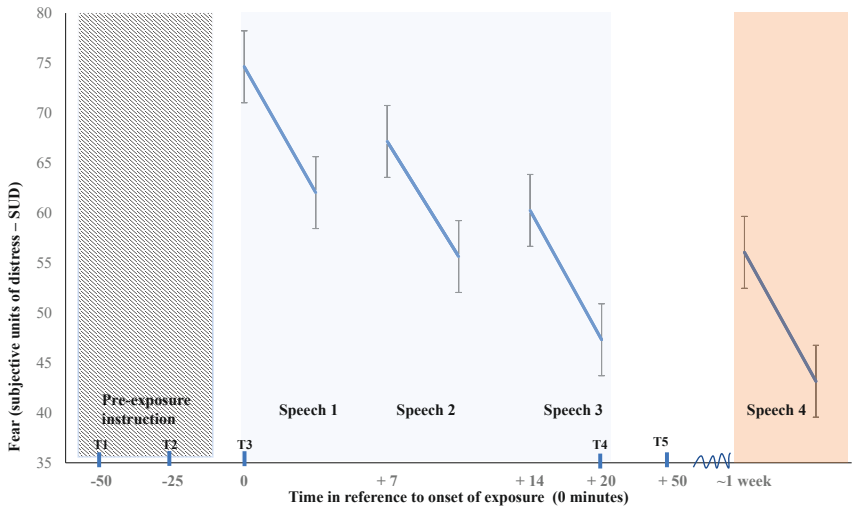

Figure 3.1 The mean peak and mean end Subjective Units of Distress (SUDs) per Speech exposure from all 73 participants (speech 4, N= 66). The Figure also depicts the timing of testosterone samples (T1-5) relative to the Speech exposures that took place either during the exposure session (in the middle (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.

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 methodology used by this laboratory, see: (Miller, Plessow, Kirschbaum, & Stalder, 2013; Reardon, Herzhoff, & Tackett, 2016).

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 the Saliva Measures section 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.

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, Maechler, Bolker, & Walker, 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 & Flora, 2018), we report unstandardized effect sizes (i.e. the estimates).

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).

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.

Results

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, Toni, Roelofs, Volman, & Smeekens (2016)). Means and standard deviations of the non-transformed data are presented in Table 3.1. 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 chapter 3 – appendix 2 for details).

Table 3.1 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*)
Testosterone sample 1	21.95 (*17.34*)	169.60 (*94.50*)	
Testosterone sample 2	22.45 (*24.87*)	145.23 (*84.33*)	
Testosterone sample 3	19.93 (*21.99*)	146.10 (*106.26*)	
Testosterone sample 4	19.68 (*20.35*)	147.28 (*95.54*)	
Testosterone 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*)	

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

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 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 Chapter 3 - Appendix 2 for details). Analyses testing the predictive effects of baseline testosterone yielded similar results to those for reactive testosterone (see Chapter 3 - Appendix 2 for details).

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 **Figure 3.2A**, higher testosterone reactivity was associated with stronger reductions in symptom severity relative to lower testosterone reactivity.

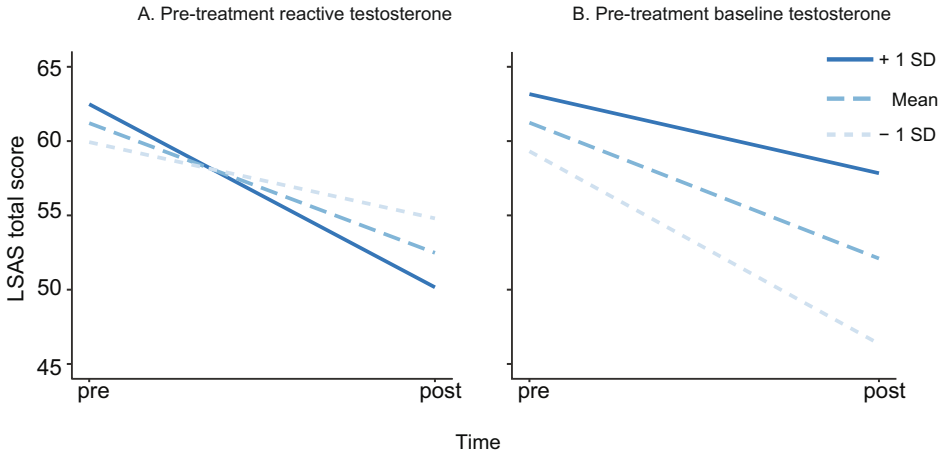

Figure 3.2 Social anxiety symptoms over time (pre – post) as a function of pre-treatment reactive testosterone (A) and pre-treatment baseline testosterone (B). Self-reported social anxiety symptom (LSAS-total score) decreased more from pre- to post treatment in those patients who displayed stronger pretreatment testosterone increases (Panel A) and in those patients with low pretreatment 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.

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 Figure 3.2B 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.

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, Panksepp, 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 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 be-

tween 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, Spinhoven, et al., 2016), as well as reduces automatic threat bias to angry faces (Enter, Terburg, et al., 2016; 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, Johnson, Berardi, & Grillon, 2006). It also supports theoretical models that frame SAD as a problem of threat anticipation in specific (Clark & Wells, 1995; Heimberg & Rapee, 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, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016; 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 & Kozak, 1986; Foa, Huppert, & Cahill, 2005) 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, Kleine, Broekman, Hendriks, & Minnen, 2018; Kozak, Foa, & Steketee, 1988; Meuret, Seidel, Rosenfield, Hofmann, & Rosenfield, 2012; Van Minnen & 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, al-

though 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 (Chapter 3 - Appendix 2), 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.

Chapter 3 - appendix 1

Table. *Means and standard deviations of raw testosterone samples*

Variable	M (SD)			Statistic
	Power (*n* = 26)	Submissive (*n* = 27)	Neutral (*n* = 20)	
Testosterone sample 1	54.24 (62.11)	91.19 (112.77)	41.54 (56.70)	*p* = .104
Testosterone sample 2	49.18 (47.17)	80.76 (99.88)	37.92 (56.29)	*p* = .113
Testosterone sample 3	41.71 (44.89)	83.91 (115.32)	37.71 (55.94)	*p* = .086
Testosterone sample 4	46.71 (46.71)	79.47 (108.55)	37.82 (54.53)	*p* = .149
Testosterone sample 5	44.14 (49.60)	64.90 (79.87)	37.35 (55.45)	*p* = .249

Chapter 3 - appendix 2

Further details reported analyses

Details analysis reactive testosterone and fear levels at post-assessment

This analysis showed a main effect of Time, *Estimate* = -6.27(0.89), F(1,67) = 49.53, *p* <.001). Peak SUD scores reduced from speech 1 (*M* = 74.63, *SD* = 16.56) to the post-assessment (*M* = 56.05, *SD* = 20.02), confirming that exposure resulted in the expected within-session reductions in fear levels. A main effect of gender was also found, *Estimate* = -5.37(1.96), F(1,67) = 7.46, *p* =.008. SUD scores are higher for females (*M* = 69.23, *SD* = 18.84), compared to males (*M* = 57.03, *SD* = 22.08). However, no interaction effect of Time x Testosterone reactivity was found, *Estimate* = -0.15(.14), F(1,64) = 1.33, *p* = .25. Reductions in fear levels from speech 1 (of the speech exposure session) to post-assessment were not dependent of testosterone reactivity.

Details analysis baseline testosterone and fear levels

This analysis showed a main effect of Time, *Estimate* = -7.19(0,95), F(1,71) = 59.92, *p* <.001. Specifically, peak SUDs reduced 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). An additional main effect of Gender, *Estimate* = -4.55(1.82), F(1,68) = 6.02, *p* = 0.02 showed that fear levels were higher for females (*M* = 70.04., *SD* = 16.19) compared to males (*M* = 60.65, *SD* = 17.72). However, contrary to our hypothesis there was no significant modulation of this effect by baseline testosterone levels, as indicated by a non-significant Time x Testosterone interaction, *Estimate* = -0.24(1.02), F(1,71) = 0.06, *p* = 0.81.

Details analysis baseline testosterone and fear levels at post-assessment

This analysis showed a main effect of Time, *Estimate* = -6.30(0.88), F(1,68) = 50.81, *p* <.001. Fear levels reduced from speech 1 (*M* = 74.63, *SD* = 16.56) to post-assessment (*M* = 56.05, *SD* = 20.02). Moreover, a main effect of Gender was found, *Estimate* = -5.98(1,96), (F(1,69) = 8.15, *p* <.001). Fear levels are higher for females (*M* = 69.23, *SD* = 18.84), compared to males (*M* = 57.03, *SD* = 22.08). However, no interaction effect of Time x Baseline testosterone was found, *Estimate* = 0.92(0.93), F(1,67) = 0.97, *p* = .33. Reductions in fear levels from speech 1 tot post-assessment were not dependent of baseline testosterone.

Additional analyses

Fear levels and absolute testosterone reactivity

The mixed model analysis for the peak SUDs at the speech exposure session as dependent variable, with Absolute testosterone reactivity (sample 3 – sample 1) and Time (speech 1, speech 2, speech 3, in the speech exposure session) as predictors, showed a main effect of Time, confirming that exposure resulted in the expected within-session reductions in fear levels, *Estimate* = -7.18(0.97), F(1,70) = 55.40, p <.001. More specifically, 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). An additional main effect of gender, *Estimate* = -4.45(1.87), F(1,67) = 5.51, p = 0.02 showed that fear levels are higher for females (*M* = 70.04, *SD* = 16.19) compared to males (*M* = 60.65, *SD* = 17.72). In comparison to the analysis for *relative* reactivity, there was no significant modulation of this effect by *absolute* testosterone reactivity levels, as indicated by a non-significant Time x Testosterone reactivity interaction, *Estimate* = -0.55(1.27), F(1,70) = 0.20, p = 0.66.

Similar effects were found for post-assessment fear levels. A main effect of Time was found, *Estimate* = -6.18(0.89), F(1,67) = 47.83, p <.001. More specifically, fear levels diminished from speech 1 (in the speech exposure session) to post assessment ($M_{speech-pre}$ = 74.63, *SD* = 16.56; $M_{speechpost}$ = 56.05, *SD* = 20.02. An additional main effect of gender, *Estimate* = -5.54(1.99), F(1,67) = 7.74, p = 0.01 showed that fear levels are higher for females (*M* = 69.23, *SD* = 18.84) compared to males (*M* = 57.03, *SD* = 22.08). Again, conforming the effects of the *relative* testosterone reactivity, there was no significant modulation of this effect by absolute testosterone reactivity levels, as indicated by a non-significant Time x Testosterone reactivity interaction, *Estimate* = -0.39(1.15), F(1,65) = 0.12, p = 0.73.

Social anxiety symptom levels and absolute testosterone reactivity

The mixed model analysis for LSAS as dependent variable, with absolute testosterone reactivity (sample 3 – sample 1) and Assessment (pre/post) as predictors, showed a main effect of Assessment, again confirming efficacy of exposure, but now with respect to the social anxiety *symptom* levels *Estimate* = -8.76(1.64), F(1,67) = 29.09, p <.001. In specific, LSAS scores reduced from pre to post-assessment (M_{pre} = 74.80, *SD* = 22.73; M_{post} = 66.01, *SD* = 24.58). A main effect of Gender, *Estimate* = -9.60(2.77), F(1,68) = 12.04, p < 0.001 on the LSAS, also indicated again higher scores for females (*M* = 75.63, *SD* = 23.91) compared to males (*M* = 57.53, *SD* = 18.85). Most critically, an interaction of absolute testosterone reactivity and assessment was not found, *Estimate* = -2.79(2.12), F(1,66) = 1.73, p = 0.19. Contrary to our results for *relative* reactivity, reductions in social anxiety symptoms were not dependent of *absolute* testosterone reactivity.

Analysis baseline testosterone and social anxiety symptoms with winsorized data

The mixed model analysis for LSAS as dependent variable, with baseline testosterone (winsorized) and Assessment (pre/post) as predictors, showed a main effect of Assessment, again confirming efficacy of exposure, but now with respect to the social anxiety *symptom* levels, *Estimate* = -9.14(1.63), F(1,67) = 31.29, p <.001. In specific, LSAS scores reduced from pre to post-assessment (M_{pre} = 74.80, *SD* = 22.73; M_{post} = 66.01, *SD* = 24.58). A main effect of Gender, *Estimate* = -9.40(2.75), F(1,68) = 11.68, p < 0.001 on the LSAS, also indicated again higher scores for females (*M* = 75.63, *SD* = 23.91) compared to males (*M* = 57.53, *SD* = 18.85). Most critically, an interaction of baseline testosterone and assessment *Estimate* = 3.87(1.86), F(1,67) = 4.33, p = .04, showed that stronger reductions in symptom levels were related to low baseline testosterone levels (see figure chapter 3 - Appendix 3, panel A for a scatter plot).

Analysis reactive testosterone and social anxiety symptoms with winsorized data

We repeated the same mixed models analysis but now with *reactive* testosterone level (winsorized) and Assessment (pre/post) as predictors. In line with our hypothesis, reactive testosterone levels were positively related to the pre-to post assessment decrease in social anxiety symptoms: apart from the main effects of Assessment (pre, post), *Estimate* = -8.63(1.60), F(1,66) = 29.26, p <.001 and Gender, *Estimate* = -9.73(2.76), F(1,68) = 12.41, p <.001, testosterone reactivity significantly modulated the effect of Assessment as indicated by a significant Time x Assessment interaction, *Estimate* = -1.63(0.64), F(1,66) = 6.42, p = .014. In specific, high reactive testosterone levels were associated with relatively stronger reductions in symptom levels pre to post assessment (see figure chapter 3 Appendix 3, panel B for a scatter plot).

Analyses separated per gender

Although we found no significant moderation by gender of the testosterone by assessment (pre- to post-intervention social anxiety symptom severity) interaction, we explored *post-hoc* whether similar patterns of results to the total sample held for females alone, with a disclaimer that we were underpowered to detect small effects.

Fear levels females

The mixed model analysis for the peak SUDs at the speech exposure session as dependent variable, with baseline testosterone and Time (speech 1, speech 2, speech 3, in the speech exposure session) as predictors, showed a main effect of Time, confirming that exposure resulted in the expected within-session reductions in fear levels, *Estimate* = -7.75(1.19), F(1,50) = 42.65, p <.001. More specifically, SUDs diminished over the

three speeches ($M_{speech1}$ = 78.06, *SD* = 14.85; $M_{speech2}$ = 69.52, *SD* = 12.91; $M_{speech3}$ = 62.56, *SD* = 16.92). However, contrary to our first hypothesis there was no significant modulation of this effect by baseline testosterone levels, as indicated by a non-significant Time x Testosterone interaction, *Estimate* = 0.89(3.50), F(1,50) = 0.06, *p* = 0.80.

The same results were found for the post-assessment SUDs. Time, *Estimate* = -6.14(1,05), F(1,48) = 34.69, *p* <.001. Peak SUD scores reduced from speech 1 (*M* = 78.06, *SD* = 14.85) to post-assessment (*M* = 59.67, *SD* = 18.13); Time x Baseline testosterone *Estimate* = 3.40(3.09), F(1,48) = 1.21, *p* = .28. Reductions in fear levels from speech 1 tot post-assessment were not dependent of baseline testosterone.

Fear levels males

The mixed model analysis for the peak SUDs at the speech exposure session as dependent variable, with baseline testosterone and Time (speech 1, speech 2, speech 3, in the speech exposure session) as predictors, did not show a main effect of Time for males. Fear levels did significantly diminish over speeches, *Estimate* = -5.81(1.53), F(1,19) = 14.33, *p* = .001 ($M_{speech1}$ = 66.14, *SD* = 17.93; $M_{speech2}$ = 61.29, *SD* = 15.17; $M_{speech3}$ = 54.52, *SD* = 18.73). Moreover, there was no significant interaction between baseline testosterone levels and Time, *Estimate* = -.712(7.04), F(1,19) = 1.03, *p* = .32.

Similar effects were found for the post-assessment SUDs: Time, *Estimate* = -6.70(1.71), F(1,17) = 15.18, *p* = .001. Fear levels reduced from speech 1 (*M* = 66.14, *SD* = 17.93) to post-assessment (*M* = 46.39, *SD* = 22.10); Time x Baseline testosterone, *Estimate* = 0.82(7.47), F(1,16) = 0.01, *p* = .91. Reductions in fear levels from speech 1 tot post-assessment were not dependent of baseline testosterone.

Social anxiety symptom levels females

The mixed model analysis for LSAS as dependent variable, with baseline testosterone and Assessment (pre/post) as predictors, showed a main effect of Assessment, again confirming efficacy of exposure, but now with respect to the social anxiety *symptom* levels, *Estimate* = -9.73(1.84), F(1,48) = 27.86, *p* <.001. In specific, LSAS scores reduced from pre to post-assessment (M_{pre} = 80.33, *SD* = 22.35; M_{post} = 70.74, *SD* = 24.62). Most critically, an interaction of baseline testosterone and assessment *Estimate* = 15.76(5.44), F(1,48) = 8.39, *p* = .006, showed that stronger reductions in symptom levels were related to low baseline testosterone levels. Females show the same results as found in the total sample.

We repeated the same mixed models analyses but now with *reactive* testosterone levels as predictors for females only. Apart from the typical main effect of Assessment (pre, post), *Estimate* = -9.22(1.92), F(1,47) = 22.99, *p* <.001, testosterone reactivity did not modulate the effect of Assessment as indicated by a non-significant Time x Assessment

interaction, *Estimate* = -11.08(7.36), F(1,47) = 2.27, p = .13. In specific, reactive testosterone levels were not associated with reductions in symptom levels pre to post assessment for females only.

Social anxiety symptom levels males

The mixed model analysis for LSAS as dependent variable, with baseline testosterone and Assessment (pre/post) as predictors, showed a main effect of Assessment, again confirming efficacy of exposure, but now with respect to the social anxiety *symptom* levels, *Estimate* = -7.25(3.28), F(1,17) = 4.87, p = .04. In specific, LSAS scores reduced from pre to post-assessment (M_{pre} = 61.09, *SD* = 17,58; M_{post} = 53.59, *SD* = 19.86). However, an interaction of baseline testosterone and assessment was not found, *Estimate* = 0.05(14.44), F(1,17) = 0.00, p = .99. For males, reductions in symptom levels were not related to baseline testosterone levels. We also conducted a mixed model analysis with *reactive* testosterone levels as predictor for males only. Apart from the typical main effect of Assessment (pre, post), *Estimate* = -7.35(3.27), F(1,17) = 5.03, p = 0.38, testosterone reactivity did not modulate the effect of Assessment as indicated by a non-significant Time x Assessment interaction, *Estimate* = -.34(45.85), F(1,17) = 0.01, p = .94. In specific, reactive testosterone levels for males were not associated with reductions in symptom levels pre to post assessment.

Chapter 3 - appendix 3

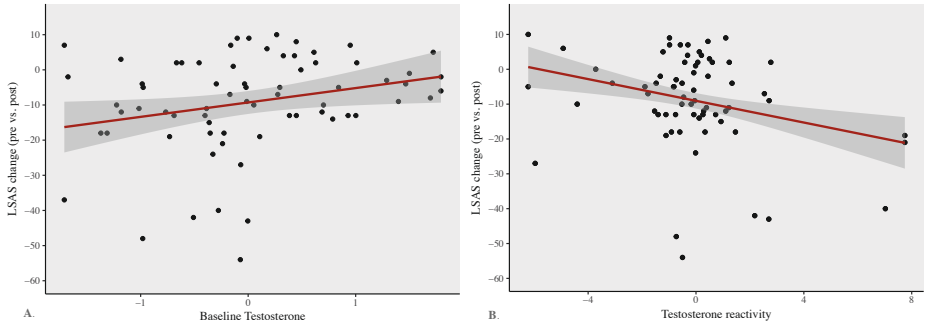

Figure. Correlation between change in social anxiety symptoms (LSAS- post minus pre-treatment) on the one hand and Baseline Testosterone (Left panel) and Reactive Testosterone (Right panel) on the other hand (z-scores, winsorized) with a regression line and confidence interval.

Chapter 4

The enhancing effects of testosterone in exposure treatment for social anxiety disorder: a randomized proof-of-concept trial

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Abstract

Individuals with social anxiety disorder (SAD) show hypofunctioning of the hypothalamus-pituitary-gonadal (HPG) axis, which is linked to social fear and avoidance behavior. As testosterone administration has been shown to facilitate social approach behavior in this population, it may enhance the effectiveness of exposure treatment. In this proof-of-concept study, we performed a randomized clinical assay in which 55 women diagnosed with SAD received two exposure therapy sessions. Session 1 was supplemented with either testosterone (0.50 mg) or placebo. Next, transfer effects of testosterone augmentation on within-session subjective fear responses and SAD symptom severity were assessed during a second, unenhanced exposure session (session 2) and at a one-month follow-up, respectively. The participants having received testosterone showed a more reactive fear pattern, with higher peaks and steeper reductions in fear levels in session 2. Post-hoc exploration of moderating effects of endogenous testosterone levels, revealed that this pattern was specific for women with high basal testosterone, both in the augmented and in the transfer session. In contrast, the participants with low endogenous testosterone showed reduced peak fear levels throughout session 1, again with transfer to the unenhanced session. Testosterone did not significantly affect self-reported anxiety. The effects of testosterone supplementation on fear levels show transfer to non-enhanced exposure, with effects being modulated by endogenous testosterone. These first preliminary results indicate that testosterone may act on important fear mechanisms during exposure, providing the empirical groundwork for further exploration of multi-session testosterone-enhanced exposure treatment for SAD.

Introduction

With a lifetime prevalence of 13% and long-term disability, social anxiety disorder (SAD) is the most common and burdensome of all anxiety disorders (Bandelow & Michaelis, 2015; Bruce et al., 2005; Hendriks et al., 2016). Persistent avoidance is the main factor hindering extinction of fear during social situations, which is why reducing avoidance behavior is the core target of exposure therapy, the treatment of choice for SAD (Arnaudova et al., 2017; Clark & Wells, 1995). However, with response rates of 45-55%, the intervention leaves room for improvement (Carpenter et al., 2018; Hofmann & Smits, 2008b; Loerinc et al., 2015; Young et al., 2019). Particularly, augmentation strategies aimed at alleviating social avoidance and promoting social approach have the potential to boost core mechanisms assumed to underlie the effects of exposure in SAD.

Previous attempts to enhance the therapy's efficacy with pharmacological agents (e.g., d-cycloserine (DCS), yohimbine, oxytocin) targeted the process of extinction learning in SAD (Guastella et al., 2009; Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Hofmann et al., 2006; Rodebaugh et al., 2013; Smits et al., 2020; Smits, Rosenfield, Otto, et al., 2013a). Although the results are encouraging, no pharmacological enhancer has been tested that directly acts on acute within-session social approach behavior, essential for effective exposure.

Testosterone, the end product of the hypothalamus-pituitary-gonadal (HPG) axis, is important in the regulation of social motivational behavior, including approach and avoidance behavior (Hermans & Van Honk, 2006). In both animal and human studies, low endogenous testosterone has been linked to socially submissive, anxious, and avoidant behaviors (Archer, 2006; Josephs et al., 2006; Sapolsky, 1991), while high basal testosterone is related to social dominance and approach behavior (Maner et al., 2008; Mazur & Booth, 1998).

Importantly, in individuals with SAD (Giltay et al., 2012) and other social avoidance-related disorders such as depression (Almeida et al., 2008; Giltay et al., 2012) reduced levels of endogenous testosterone have been found. Moreover, relatively high pre-treatment testosterone concentrations predicted a better outcome of exposure therapy in terms of larger symptom reductions (Hutschemaekers et al., 2020). Additionally, causal studies on the relationship between testosterone and avoidance behavior further confirm the social avoidance-reducing and approach-facilitating properties of testosterone (Enter et al., 2014; Enter, Spinhoven, et al., 2016; Terburg et al., 2016). Testosterone acts on dopaminergic projections from the amygdala to the striatum and its administration was shown to bias amygdala activity towards social threat approach in humans (Hermans et al., 2010; Radke et al., 2015). At a behavioral level, testosterone

administered to healthy participants prior to threat exposure was found to reduce fear, enhance reward sensitivity, and promote social approach motivation (Bos, Panksepp, et al., 2012; Enter et al., 2014; Terburg et al., 2016). Eye-tracking and electrophysiological studies by our group showed that, when administered to women with SAD, testosterone reduced automatic threat bias to angry faces (Enter, Terburg, et al., 2016; van Peer et al., 2017), diminished social avoidance, and promoted prosocial behavior, including approach towards angry faces (Enter, Spinhoven, et al., 2016). Although preliminary, these findings provide promising evidence that testosterone administration prior to exposure therapy enhances the treatment's efficacy by promoting social approach, one of its main goals (Enter et al., 2018; Hutschemaekers et al., 2020).

Given its profound role in the regulation of social motivational behavior, in the present proof-of-concept study we test the augmentation effects of testosterone on exposure in women coping with SAD in a randomized clinical assay, comparing a single testosterone-enhanced session (0.50 mg) with a placebo-supplemented session. To assess the transfer of testosterone-induced effects, the participants engaged in a similar but unenhanced exposure session one week later. We hypothesized that, compared to the placebo group, testosterone-enhanced exposure would induce steeper reductions in subjective fear during the repeated exposure session, as an index of retention of extinction learning during session 1 (Craske et al., 2008; Foa & Kozak, 1986a; Foa, Huppert, & Cahill, 2005). In addition, we verified whether testosterone supplementation would affect self-reported pre-to-post-treatment social anxiety symptoms. Finally we explored physiological effects of acute testosterone administration by assessing within-session Heart rate (HR). Given the recent insight into the moderating effects of endogenous testosterone on exogenous testosterone (Carré et al., 2015a; van Honk et al., 2011; Welling, Moreau, Bird, Hansen, & Carré, 2016) and the efficacy of exposure treatment for SAD in particular (Hutschemaekers et al., 2020), we explored endogenous testosterone as a moderating factor in our analyses.

Methods and Materials

Participants

Participants were recruited from an outpatient clinic specializing in the treatment of anxiety disorders, from the Radboud University Nijmegen, and from the community from 2017 through 2019. Inclusion criteria were: 1) woman, 2) age: 18-45 years, 3) primary diagnosis of SAD (as assessed with the Mini International Neuropsychiatric Interview (MINI; (Sheehan et al., 1998)), with a predominant fear of public speaking, and 4) score > 30 on

the Liebowitz Social Anxiety Scale (LSAS; (Liebowitz, 1987)). We focused exclusively on women because the pharmacodynamics of the currently used testosterone administration methods have as yet been established in women only (Tuiten et al., 2000). Exclusion criteria were: A) prior non-response to speech exposure therapy for SAD, B) other predominant mental disorder(s), C) (current or lifetime) psychosis or delusion disorders, D) significant suicidal ideation or behavior within 6 months prior to screening, E) intellectual disability, F) substance or alcohol dependence, G) somatic illness, H) unwillingness to use an active form of birth control during the trial, I) pregnancy or lactation, J) infertility, K) antipsychotic medication, L) unstable regimen of antidepressants or benzodiazepines within 6 weeks prior to enrollment, M) insufficient proficiency in the Dutch language, N) current use of contraceptives containing cyproterone acetate. All participants received 70 Euros for their participation. Ethical approval for this study was granted by the local (Arnhem-Nijmegen) Review Board.

In total, 55 women suffering from SAD (M_{age} = 23.31, *SD* = 5.63, *range* = 18-43) were included in the study sample. One participant dropped out before the first exposure session (due to illness). She was replaced by another participant to ensure equal group sizes, resulting in 55 participants receiving the allocated drug (testosterone/placebo) and 54 the exposure sessions: 27 per group (see Chapter 4 – Appendix 2 for CONSORT flowchart). After study completion, one participant (testosterone group) divulged she had been on atypical antipsychotic quetiapine, which fact she had not mentioned during eligibility screening. Since she had consistently used a low, stable dose (25 mg) for the last 18 months, we decided not to exclude her from the analyses.

Medication and randomization

The pharmacist providing the study solutions randomly assigned participants to testosterone (T) or placebo (P) in blocks of four (no stratification). T was suspended in a clear solution (0.5 ml) with 0.5 mg hydroxypropyl-beta-cyclodextrin, 0.005 ml ethanol 96%, and distilled water. P contained the same ingredients, barring T. Participants held the liquid under their tongues for 60 seconds. In women, this dose yields a sharp increase in plasma testosterone concentrations within 15 minutes and declines to baseline within 90 minutes (van Rooij et al., 2012). Pharmacodynamic effects can be assayed 4-6 hours after intake (Bos, Panksepp, et al., 2012; Tuiten et al., 2000). Researchers, therapists, and participants were blinded to the group allocation until completion of the primary outcome analyses.

Exposure intervention

The participants engaged in two 90-minute public-speaking exposure sessions delivered one week apart in accordance with the protocols developed by Rodebaugh and colleagues (Rodebaugh et al., 2013; Smits, Rosenfield, Davis, et al., 2013). The sessions were standardized with respect to exposure length (6-8 minutes), preparation time (max. 5 minutes), reaction of the experimenter (neutral), and the availability of notes and speech topic. On the morning of the first day, the participants received psychoeducation about SAD and exposure, with the first session starting after four hours. In both sessions, psychoeducation was repeated and personalized harm expectancies and goals were assessed. Then, the participants presented their prepared speech in front of a therapist, two confederates, and a camera. They reviewed their videotaped performance afterwards together with the therapist. The therapists were psychology students in their last year of training (BA and MA level) trained and supervised by experienced, board-certified psychologists (M.H.M.H. and M.K). To guarantee adherence to the protocol, the therapists were instructed to fill out a checklist of all protocol components and to report any deviations from the protocol. The checklists and reports on deviations showed that 96.3% of the sessions were delivered in accordance with the protocol.

Outcome measures

Within-session fear (primary outcome)

Participants rated their fear levels on a subjective units of distress (SUD) scale ranging from 0: No fear to 100: Extreme fear (Wolpe & Lazarus, 1966). SUDs were collected after psychoeducation (initial SUD), immediately prior to each exposure session (baseline SUD), immediately prior to the speech (start SUD), every two minutes during, and immediately after the speech (endpoint SUD).

Symptom severity (secondary outcome)

Social anxiety symptoms were assessed with the Social Phobia Scale (SPS; (Mattick & Clarke, 1998)), a self-report measure assessing the fear of being observed or watched during social or performance situations. The scale has shown good internal consistency ((Mattick & Clarke, 1998); $\alpha = .94$; Dutch translation; (Beurs, Tielen, & Wollmann, 2014), $\alpha = .91$, current study $\alpha = .86$). Participants completed the SPS at baseline, after the second exposure session (post-treatment) and at the one-month follow-up (FU) assessment.

Saliva samples

To determine endogenous testosterone levels, saliva samples were collected (2 ml passive drool saliva by Salicap; Hamburg, Germany) at eight time points (see **Figure 4.1**): (1) at baseline, (2) prior to T/P intake, (3) prior to exposure session 1, (4) immediately after speech delivery in session 1, (5) 30 minutes after speech delivery in session 1, (6) prior to exposure session 2, (7) immediately after speech delivery in session 2, and (8) 30 minutes after speech delivery in session 2. Participants were asked to conform to certain directives regarding food and drink intake to prevent pollution of the saliva samples. Samples were stored at -20 °C until radio-immune assays were performed at Dr. Kirschbaum's laboratory (Dresden, Germany); for descriptions of the methodology, see (Miller et al., 2013; Reardon et al., 2016).

Procedure

After having provided their informed consent, participants were screened online for eligibility, to which end they filled out the LSAS and answered general screening questions (e.g., age, treatment status, infertility, menstrual cycle). Eligible participants were telephoned for further screening (MINI, check in/exclusion criteria), after which they learned whether they would be participating in the study (see Chapter 4 - Appendix 1 for details). All other assessments and the exposure sessions took place at the treatment facility. After enrollment (see **Figure 4.1** for timing and procedure), participants completed the baseline assessment[1], with the first exposure session being scheduled within one week. The morning of the session, the participants took a pregnancy test, saliva was collected, and psychoeducation provided, after which the participants completed a non-speech-related SUD and received T/P (administered by a research assistant). After four hours, during which time participants were instructed to avoid physically and psychologically straining activities and heavy meals, they returned for the exposure session, before which saliva was collected and resting HR was recorded. Another saliva sample was collected immediately upon completion of the speech and 30 minutes thereafter. During the speech, SUDs and HRs were collected. At the end of the session, the therapist checked for adverse drug effects by asking participants about physical complaints. A week later, the second exposure session took place, with all steps being identical to those of the first session barring supplement administration. After a 30-minute break, participants took the post-exposure assessment comprising the SPS and a computer task (reported elsewhere). We asked all participants to refrain from using alcohol, drugs

1 Computerized tasks were also part of the baseline assessment, but outcomes will be reported elsewhere.

or medication (except from their stable dose of psychotropic medication) during testing days. One month later, participants once again completed the SPS online. This study was registered in the Dutch Trial Register: https://www.trialregister.nl/trial/6238 and at EudraCT (2014-004475-23).

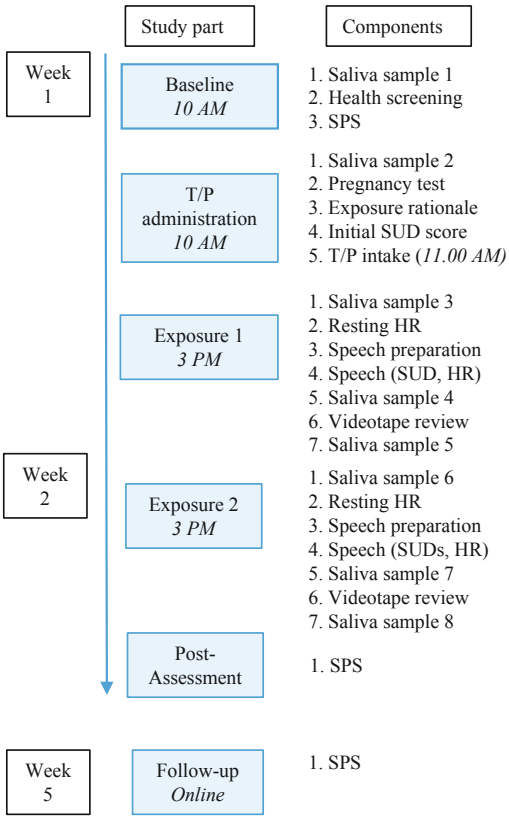

Figure 4.1 Timing and procedure of the study protocol. SPS Social Phobia Scale, SUD subjective units of distress, T testosterone, P placebo, HR heart rate. Since pregnancy was a reason for exclusion, the pregnancy test was to ascertain that none of our participants was pregnant prior to the start of the testosterone-enhanced session

Statistical analyses

To test the effects of testosterone augmentation on subjective fear, we used mixed models. A sample size of 52 participants was deemed necessary to detect group differences with at least a moderate effect size and a power of 80%. We tested acute effects of

the enhancement (session 1) and transfer to unenhanced exposure (session 2) separately. Its effects on SAD symptoms (SPS scores) were tested in an additional model (see below). Moreover, we explored augmentation effects on HR in similar models as subjective fear (see Chapter 4 - Appendix 1 for details). We used the Lme4 package in R (Bates et al., 2013) and *p*-values were calculated using the likelihood ratio tests (Afex package) (Singmann, 2013). Independent continuous predictors were centered and sum-to-zero contrasts used. Consistent with the recommendations for mixed models (Pek & Flora, 2018), we report unstandardized effect sizes (estimates).

More specifically, to determine whether the added testosterone had affected self-reported fear (SUDs) during session 1, group (T/P) and time (start, 2 min, 4 min, 6 min, 8 min, end) were included as predictors. Linear, quadratic, and cubic time terms were modeled since we expected that the SUD scores would not necessarily follow a linear pattern (e.g. they could increase first [fear activation] before they decrease). Participant was included as random intercept and time (linear, quadratic, cubic) as random slope. Initial SUDs (rated after psychoeducation) were included as a fixed factor to control for variance unrelated to time or group; see also (Rodebaugh et al., 2013). To examine whether enhanced exposure affected fear patterns during the second unenhanced session, we ran the same analysis used for the first session. As regards the effects of the enhancement on symptom severity, we modeled SPS scores, with group (T/P) and time (pre/post/FU) as predictors and participant as the random intercept.

Finally, in light of recent insights into the role of endogenous testosterone on the effects of exposure treatment for SAD (Hutschemaekers et al., 2020), we conducted post-hoc tests, re-running all our analyses now including basal testosterone levels (mean samples 1 and 2). Since we detected some outliers (visual inspection of boxplots) in the baseline data, we repeated the analyses after winsorizing (i.e., setting extreme baseline testosterone values to the second and 98th percentile to thus reduce the effects of spurious outliers). The results remained unchanged. Also, given that age and hormonal birth control are known factors affecting endogenous testosterone, we checked if the observed effects would hold after correcting for these variables in all models.

Results

Attrition

One participant receiving placebo dropped out before the first exposure due to illness (see participant section). Another participant in the same group dropped out during the first session (3.6%). All other participants completed both sessions and the follow-up.

Sample characteristics

The data of 54 participants was analyzed (M_{age} = 23.31, *SD* = 5.64, range = 18-43; see Table 4.1). There were no significant between-group differences on any of the baseline measures. The manipulation was successful: compared to the placebo group, testosterone levels after testosterone administration (sample 3) were significantly higher in the enhanced group, moreover blinding was successful, participants were unaware if they received T or P (see Table 4.1).

Table 4.1 *Sample characteristics (N = 54)*

	Total sample N = 54	Placebo *n* = 27	Testosterone *n* = 27	t or χ^2, *p*
Demographics				
Age	23.31 (5.64)	24.00 (6.85)	22.61 (4.12)	0.90, *.372*
SPS total score	30.20 (11.85)	32.15 (13.82)	28.26 (9.35)	1.21, *.232*
LSAS total score	63.06 (19.24)	64.70 (18.96)	61.41 (19.73)	0.62, *.534*
Initial SUD score	20.59 (13.86)	20.44 (11.33)	20.74 (16.21)	0.77, *.938*
Testosterone sample 1	15.17 (16.41)	15.94 (19.65)	15.47 (12.76)	.105, *.917*
Testosterone sample 2	16.21 (15.45)	17.57 (16.83)	14.80 (14.06)	.650, *.518*
Testosterone sample 3	252.49 (380.03)	15.29 (16.19)	489.68 (421.06)	-5.85, <.001
Educational level				
High school	7	2	5	1.15, *.680*
Intermediate vocational	6	3	3	
Higher vocational	11	6	5	
University	30	16	14	
Psychological treatment (n)	9	5	4	.13, *.715*
Psychotropic medication use (n)	4	2	2	*2.00, .572*
Contraceptive use (n)				1.29, *.733*
Hormonal	30	15	15	.00, *1.00*
Non-hormonal	24	12	12	
Comorbid anxiety disorder (n)	18	11	7	1.39, *.239*
Comorbid depressive disorder (n)	5	2	3	.22, *.639*
Participants believed to be in T group (n)	12	7	5	

Note. For testosterone sample 2 in the testosterone group n = 26 as one participant had a missing value. Testosterone values are in picograms per milliliter (pg/ml). SPS = Social Phobia Scale; LSAS = Liebowitz Social Anxiety Scale; SUD = subjective units of distress.

Adverse events

The testosterone and placebo arms did not differ with respect to adverse events; no serious events were reported in either group (for details see Chapter 4 - Appendix 1).

Acute effects of testosterone augmentation (session 1)

Fear

Before reporting on the critical transfer session (2), we first describe acute effects of testosterone on fear scores in session 1. Fear scores decreased over time (linear and quadratic), with exposure resulting in the expected within-session reduction: *Estimate*(linear) = -81.96 (16.76), F(1,51) = 23.89, *p* <.001, *Estimate*(quadratic) = −85.12(13.99), F(1,51) = 36.95, *p* < .001. The interaction between time (linear or quadratic) and group was not significant: *p*-values > .618 (for details see Chapter 4 – Appendix 3).

In the post-hoc model including baseline testosterone levels, the effects of time were confirmed: *p*-values < .049. There was no significant time x group interaction, *p*-values > .562 (see Chapter 4 - Appendix 1), but the effect for time(quadratic) x group x baseline-T effect was significant: *Estimate* = 2.26(.94), *F*(1,48) = 5.72, *p* = .021. As to fear patterns as a function of endogenous testosterone, in the placebo group, fear was not moderated by baseline testosterone: *Estimate* = .82(1.14), *F*(1,24) = .52, *p* = .476. In contrast, in the testosterone group, fear patterns in the participants with higher baseline testosterone were relatively more reactive, showing higher peaks followed by stronger reductions, than in those with lower values, where fear responses were characterized by relatively blunted peaks followed by weaker reductions: *Estimate* = -3.73(1.48), *F*(1,24) = 6.32, *p* = .019 (Figure 4.2A). Inclusion of age or hormonal contraceptives did not improve the fit of any of the models, so these were dropped from the analyses.

Transfer effects of testosterone augmentation (session 2)

Fear

Next, we tested effects for the critical unenhanced session (2). Fear reduced over time[2]: *Estimate*(linear) = -62.95(15.42), F(1,50) = 16.66, *p* <.001, *Estimate*(quadratic) = -48.32(11.03), F(1,50) = 19.18, p < .001, *Estimate*(cubic) = -36.76(8.90), F(1,50) = 17.01, *p* <.001. Critically, there was a Group x Time(quadratic) interaction: *Estimate* = 23.68(11.03), F(1,50) = 4.61, *p* = .037. Compared to participants having received placebo, the participants in the testos-

2 The residuals of the models for the SUDs in session 2 and the SAD symptom scores showed one standardized value > 3; therefore, the models were re-run without this outlier. Since our primary outcomes were similar, the results presented include all data points.

terone group showed a more reactive fear pattern (higher SUDs) with a steeper decline at the end of the session (See Figure Chapter 4 – Appendix 3).

The post-hoc observation that testosterone administration had resulted in steeper fear reductions in participants with high baseline testosterone (session 1) was again made in the second, non-enhanced session, with fear levels showing a similar time(quadratic) x group x baseline-T interaction: *Estimate* = 1.53(.74), F(1,47) = 4.22, p = .045. In the placebo group, session-2 fear levels followed the same quadratic pattern regardless of baseline testosterone: *Estimate* = .77(.82), F(1,23) = .94, p = .357. In the testosterone group they showed higher peaks followed by stronger reductions for participants with high baseline testosterone, whereas for those with low baseline testosterone peak fear levels flattened: *Estimate* = -2.29(1.27), F(1,23) = 3.26, p = .084 (**Figure 4.2B**).

Post-hoc exploration of Heart Rate in session 1 and 2[3]

Our results so far suggest that testosterone may have acute impact on exposure mechanisms, boosting a steeper fear-decline in individuals with high baseline testosterone levels in session 1, which could be relevant for the subsequent transfer to session 2. To deepen our understanding of potential mechanisms affected during session 1, we post-hoc explored whether psychophysiological reactivity (HR) mimics the acute effects of testosterone administration on fear levels. HR patterns largely mimicked those of the subjective fear patterns in session 1: There was a non-significant trend towards a time(-linear) x group x baseline-T interaction: *Estimate* =.80(.42), F(1,44) = 3.64, p = .063. In the placebo group HR decline followed the same slope regardless of baseline testosterone: *Estimate* = .04(.03), F(1,23) = .056, p = .461, while in the testosterone group HR reduced more for the participants with higher baseline testosterone levels: *Estimate* = -.12(.06), F(1,21) = 3.89, p = .061. These acute psychophysiological effects did not transfer to the non-enhanced transfer session, indicating that they may support the acute fear reactivity, but that it is the subjective fear pattern that is longer term affected (for full analyses see Chapter 4 – Appendix 1 and Appendix 4 for a Figure).

3 We initially modeled linear, quadratic, and cubic time terms in all HR analyses but dropped the cubic term as it did not improve the model fit.

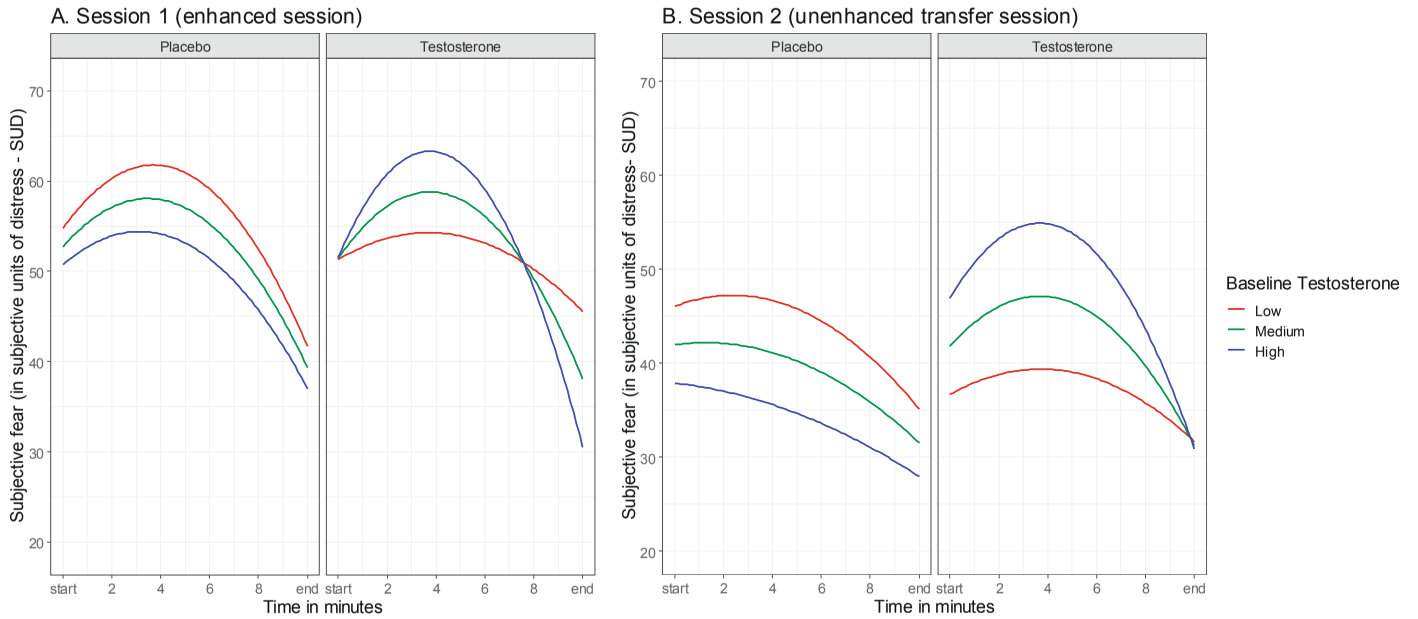

Figure 4.2 Subjective fear levels during exposure as a function of baseline testosterone per group (T/P). The figure illustrates the evolution of subjective fear levels during exposure with placebo (P) or testosterone (T) (session 1; A) and unenhanced exposure indicating transfer after P and T (session 2; B). Fear, expressed in subjective units of distress (SUDs), is displayed over time as a function of baseline-T. In order to visualize the interaction effect between baseline-T and time, we subdivided baseline-T into low (-1 SD), medium (mean), and high (+1 SD) values. Thus, the plot shows model-based predicted values, illustrating that high baseline-T is associated with higher SUD reactivity during testosterone-enhanced exposure (session 1), a pattern that largely transfers to the second unenhanced exposure (session 2). Note that for both groups there is no correlation between baseline-T and start SUDs in session 1 ($r_{placebo}$ = −0.07, p = 0.73; $r_{testosterone}$ = 0.06, p = 0.788) or session 2 ($r_{placebo}$ = −0.03, p = 0.873; $r_{testosterone}$ = 0.14, p = 0.472), indicating that effects are not driven by differences in start SUDs as a function of baseline-T but merely reflect differences in within-session fear patterns.

The effect of testosterone administration on social anxiety symptoms

SAD symptoms decreased from pre- to post-treatment (M_{pre} = 30.20 vs. M_{post} = 28.04), *Estimate* = -2.22(1.23), t(1,102) = -1.80, p = .074 and significantly so from pre- to FU: *Estimate* = -7.02(1.23), t(1,102) = -5.70, $p < .001$ (M_{pre} = 30.20, M_{FU} = 23.26). There was no group x time interaction: pre-post, *Estimate* = -0.48(1.23), t(1,102) = -.39, p = .697, pre-FU: *Estimate* = -2.10(1.23), t(1,102) = -1.70, p = .092, indicating that symptom severity after treatment discontinuation did not show any of the effects of testosterone enhancement on within-session fear. There was no effect of baseline testosterone.

Discussion

Seeking to test the effects of testosterone-augmented exposure treatment for individuals with SAD, we compared a single exposure session with testosterone supplementation (0.50 mg) to an exposure session with placebo, assessing fear levels in an unenhanced second session and SAD severity after one month. The exposure sessions were successful in reducing fear, HR, and SAD symptoms, independent of group. Foremost, testosterone augmentation was associated with higher peaks followed by a steeper decline in fear at the end of the second unenhanced session. Post-hoc analyses revealed this pattern was most pronounced in participants with higher baseline testosterone and evident in both the enhanced (session 1) and the transfer session (session 2). Peak fear levels in the participants with low basal testosterone remained lower throughout both sessions. Testosterone enhancement did not significantly change SAD symptom severity. Our proof-of-concept results provide preliminary support that testosterone may act on important mechanisms of exposure, meriting further examination of multiple-session testosterone-enhanced exposure therapy for SAD.

The effects of testosterone partly coincide with several studies supporting avoidance-reducing and social-approach-facilitating properties of the hormone (Enter et al., 2014; Enter, Spinhoven, et al., 2016; Terburg et al., 2016). Moreover, the SUD patterns (increase prior to a decrease) are in line with Emotional Processing Theory (EPT) positing that fear needs to be activated first and only after prolonged exposure, fear levels will drop. Such reactive pattern is deemed essential for learning and, hence, transfer in the long run (Foa & Kozak, 1986b; Foa et al., 2005). By boosting initial engagement with the feared stimulus, testosterone may affect important learning mechanisms reinforcing transfer (e.g., initial engagement to the feared stimulus in session 1 that transfers to fear levels in a second unenhanced session). Such interpretation is in line with the threat-ap-

proach boosting effects of testosterone in patients with social anxiety disorder (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016).

Then again, acute testosterone-augmentation effects depended on endogenous testosterone levels. This is consistent with evidence showing that individual differences in basal testosterone and proxies of fetal testosterone exposure (2D:4D ratio) moderate the effects of exogenous testosterone on various pertinent behavioral processes, including social approach, aggression, dominance, and risk-taking (Carré et al., 2015b; Carré & Robinson, 2020; Geniole & Carré, 2018; van Honk et al., 2011; Welling et al., 2016).

The interaction between endogenous testosterone levels and exogenous testosterone administration was interesting and deepen our understanding of the primary results, in that exclusively the participants with low baseline concentrations having received testosterone reported blunted peak fear levels. This is in line with earlier findings regarding the anxiolytic properties of testosterone (Bos, Panksepp, et al., 2012; Hermans, Putman, Baas, Koppeschaar, & van Honk, 2006). Together, these findings suggest that women with relatively low endogenous testosterone show lowered threat response following testosterone supplementation that transfers to the non-enhanced session. In contrast, although the women with higher basal testosterone reported similar fear levels at the end of the enhanced session, they arrived there via a different, more fear-reactive route that appeared to be transferred to the unenhanced session. Arguably, the testosterone-induced effects (e.g., higher peak fear) in women with higher endogenous levels could be interpreted as negative. However, in theoretical accounts of exposure therapy (i.e., EPT (Foa & Kozak, 1986b; Foa et al., 2005) and inhibitory learning theory (Craske et al., 2008, 2014b)) high fear levels during (initial) exposure sessions are deemed beneficial for a good response, prompting the hypothesis that, it may facilitate essential exposure mechanisms in those with high basal levels. In the present proof of concept study we cannot yet verify such qualification of patterns as beneficial or not, particularly as our single session-enhancement did not result in lower SUD levels at the end of the second exposure, in the testosterone compared to placebo group. We can only make speculations based on theoretical grounds and clearly, treatment protocols with more exposure sessions are needed to further elucidate the effects of exogenous testosterone on fear activation and reduction within and across exposure sessions.

That endogenous testosterone moderates the effects of exogenous testosterone may be explained by trait factors, including individual differences in the sensitivity of the androgen receptor (AR), where relative AR insensitivity has been reported for people with low basal concentrations (Hogervorst, Bandelow, & Moffat, 2005; Holland, Bandelow, & Hogervorst, 2011). Moreover, testosterone administration can lead to AR downregulation

in hypogonadal mice and human males, while long lasting effects of endogenous testosterone may upregulate its expression (Sader et al., 2005).

The observed effects of exogenous testosterone on fear levels did not generalize to SAD symptoms. Although we extend previous observations that a single dose of testosterone can affect threat-approach behavior in SAD in an experimental context (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016), to fear-reactivity in a clinical context, we do not observe an effect on clinical outcomes. This may be a result of the fact that our symptom outcome measure (SPS) only has one item measuring speech anxiety. We recommend future studies to use a measure more sensitive to changes in speech anxiety. On the other hand, research testing other pharmacological enhancers demonstrated that repeated doses yielded better exposure outcomes than did a single dose (Rosenfield et al., 2019; Smits et al., 2020). So, future investigations comprising more testosterone-enhanced sessions are necessary to establish whether testosterone can improve SAD symptoms.

As to the strengths of our study, we can say that with a comparative randomized clinical assay we were able to establish that the administration of a single dose of testosterone was safe and tolerable; there were no adverse events or augmentation-related drop-out. Moreover, by comparing effects in two successive sessions, we were able to examine the direct effects of the enhancement and their transfer in a relatively quick and cost-effective manner. However, since we only included women because the administration method we used has as yet only been applied in women (Tuiten et al., 2000), we cannot say whether our findings will generalize to men. Also, due to inclusion restrictions (e.g. birth control types, pregnancy) and because women with relatively low endogenous testosterone were relatively underrepresented, it remains to be tested whether findings generalize to a broader group and replication in a larger, more varied sample is needed. Furthermore, although all our participants met the SAD criteria, their baseline severity scores were somewhat lower than those reported in other exposure enhancement studies (Guastella et al., 2007; Hofmann et al., 2019; Smits et al., 2020). Even though our findings show that exogenous testosterone already exerts effects in a population with relatively mild symptoms, it needs to be shown whether they generalize to more severely impaired populations.

To conclude, testosterone-augmented exposure differentially affects in-session fear levels, partly depending on baseline testosterone levels of individuals with SAD. It reduced self-reported peak fear levels in individuals with low baseline testosterone, and increased reactive patterns in individuals with high baseline testosterone. Because both patterns may be relevant for long term extinction learning, we hope this study inspires investigation of the longer-term effects of repeated testosterone-enhancements in SAD.

Chapter 4 – appendix 1

Details Methods and Materials

Heart rate (explorative outcome)

We adopted HR (as measured with a Polar RS800CX) as a psychophysiological index of the effects of exposure (see supplementary materials for details). The raw data of the R-R (inter-beat) intervals was analyzed using Kubios HRV Analysis Software (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014). Artefacts in R-R data were corrected using the software threshold-based artefact correction (0.25 seconds). Mean HR was calculated for a 2-minute resting period 15 minutes prior to each session and for 2-minute samples during speech delivery, resulting in a maximum of four samples per speech per participant (0-2 min, 2-4 min, 4-6 min, 6-8 min).

Details on eligibility screening

The raters conducting the MINI were psychology students who after having fulfilled courses of their bachelor or Master degree did an internship at treatment facility in Nijmegen and who as part of their training underwent a MINI training. The MINI administration was supervised by a registered psychologist, with whom they discussed every MINI administered in biweekly supervision meetings

Procedure heart rate measurements

A Polar RS800CX was used to assess resting-state heart rate (HR), i.e., during a 2-minute period before the start of the exposure sessions, and during speech delivery in each session (6-8 minutes). The device uses a transmitter consisting of a polyamide case with electrodes attached to an elastic belt fixated to the chest. Interbeat (R-R) intervals (IBI) were measured at a frequency of 1000 Hz in order to provide a temporal resolution of 1 ms for each R-R interval. The Polar RS800CX has been found to be an accurate, reliable, and valid measure to record heart rate variability (Tsitoglou, Koutedakis, Dinas, & Khadka, 2019; Vasconcellos et al., 2015; Williams et al., 2017). Polar Pro Trainer 5 software was used to transfer the HR recordings to a computer. The raw data was analyzed using Kubios HRV analysis software (Tarvainen et al., 2014). Artefacts in the R-R data were corrected using the threshold-based artefact correction of the Kubios software. This algorithm compares every IBI value to a local average interval. The local average is obtained by median filtering the IBI time series, and thus, the local average is not affected by single outliers in IBI time series. A threshold value of .25 seconds was selected.

Mean HR was calculated for the baseline measurement (2 minutes recorded 15 minutes before each session) and for the 2-minute samples during each exposure, resulting in a maximum of four samples per speech per participant (0-2 minutes, 2-4 minutes, 4-6 minutes, 6-8 minutes). Because not every participant presented a speech lasting more than 6 minutes, we had only 3 HR samples for some participants.

Statistical analyses HR

To examine whether enhanced exposure affected psychophysiological reactivity we post-hoc analyzed heart rate responses with the same mixed models as fear levels in session 1 and session 2), including baseline HR as a fixed factor.

Details results

Adverse events (AEs)

Compared to the placebo (P) group, the testosterone (T) group reported fewer AEs, including mild headache (T: *n* = 1, P: *n* = 4), mild nausea (T: *n* = 0, P: *n* = 2), and mild stomachache (T: *n* = 0, P: *n* = 1). Around 1.5 weeks after the post-treatment assessment, one participant in the T-group, who was on hormonal birth control, reported a breakthrough bleeding lasting several days. On request of her GP, our attending psychiatrist de-blinded her randomization (with the condition remaining blinded for the researchers) three days before the one-month follow-up. No serious AEs were reported.

Details analyses of the effects of testosterone-administration on exposure (session 1)

Fear

The model showed that the linear and quadratic terms were significant predictors (each added significantly to the model fit). SUD scores reduced over time, confirming that the enhanced exposure resulted in the expected within-session reduction in fear: *Estimate* (linear) = -81.96 (16.76), F(1,50) = 23.89, *p* <.001, *Estimate* (quadratic) = −85.12(13.99), F(1,50) = 36.95, *p* < .001. Of main interest here, was the Group x Time effect. The interaction was not significant: *Estimate*(linear) = -.86(16.76), F(1,51) = .003, *p* = .959, *Estimate*(quadratic) = 7.01(13.99), F(1,51) = .251, *p* = .619, which showed that the fear patterns observed for the enhanced session did not differ per group. Accordingly, both groups followed the same quadratic pattern (Figure chapter 4 – appendix 3, panel A).

In the post-hoc model in which the baseline testosterone data were included, the effects of Time were confirmed: *Estimate*(linear) = -82.23(16.96), F(1,48) = 23.45, *p* <.001, *Estimate*(quadratic) = −87.07(13.69), F(1,48) = 40.39, p <.001, *Estimate*(cubic) = -23.42(11.54), F(1,47) = 4.09, *p* = .049. No Time x Group interactions were found: *Estimate*(linear) = -1.00(16.96), F(1,48) = .00, *p* = .995, *Estimate*(quadratic) = 8.01(13.69), F(1,47) = .341, *p* = .619, *Estimate*(cubic) = -1.08(11.45), F(1,47) = .01, *p* = .926. We did find a significant Time(quadratic) x Group x Baseline-T effect: *Estimate* = 2.26(.94), *F*(1,48) = 5.72, *p* = .021. The fear patterns recorded per group depended on baseline testosterone levels. For the P group, fear scores showed the same quadratic pattern regardless of basal testosterone: *Estimate* = *.82*(1.14), *F*(1,24) = .52, *p* = *.476*, while in the T group fear levels showed a higher peak which then reduced more sharply for the participants with high endogenous testosterone levels, while for the participants with low endogenous testosterone the peak fear levels flattened and reduced less: *Estimate* = -3.73(1.48), *F*(1,24) = 6.32, *p* = .019 (Figure 4.2, main manuscript, panel A).

Details analyses of transfer to unenhanced exposure (session 2)

The model showed that fear levels reduced over time (linear, cubic, and quadratic), confirming that the unenhanced exposure resulted in the expected within-session reduction: *Estimate* (linear) = -62.94(15.42), F(1,50) = 16.66, *p* <.001, *Estimate* (quadratic) = −48.32(11.03), F(1,50) = 19.18, p < .001, *Estimate* (cubic) = -36.76(8.90), F(1,50) = 17.01, *p* <.001. Of main interest was the Group x Time effect; a significant interaction was found between Time (quadratic) and Group: *Estimate* = 23.68(11.03), F(1,50) = 4.61, *p* = .037, indicating that the fear patterns differed per group. Compared to the P group, the participants in the T group reported higher fear levels throughout the session, with a steeper decline towards the end of the session (see Figure chapter 4 – appendix 3, panel B).

Details HR analyses session 1 and session 2

Session 1

Baseline HR was a significant predictor: *Estimate* = .65(.11), F(1,49) = 32.72, *p* <.001, i.e., the higher the baseline HR, the higher the HR during exposure. The linear and quadratic time terms were both significant predictors and each added to the model fit: *Estimate* (linear) = -26.51(6.44), F(1,49) = 16.89, *p* < .001; *Estimate* (quadratic) = 7.50(3.09), F(1,49) = 5.84, *p* = .019. Mean HR during exposure reduced over time, confirming that exposure resulted in the expected within-session reduction. We did not find a significant interaction between Time and Group: *Estimate*(linear) = 5.43(6.44), F(1,49) = .71, *p* = .404,

Estimate(quadratic) = -1.86(3.09), F(1,48) = .36, *p* = .551. The HR patterns in the T group did not differ from those in the P group.

With baseline testosterone in the post-hoc-model we again found Time effects: *Estimate* (linear) = -28.07(6.29), F(1,47) = 19.85, *p* < .001, *Estimate*(quadratic) = 7.68(3.23), F(1,46) = 5.63, *p* = .022, and the absence of Time x Group interactions were confirmed: *Estimate*(linear) = 6.22(6.29), F(1,47) = .97, *p* = .329, *Estimate*(quadratic) = -2.44(3.23), F(1,46) = .57, *p* = .456. There was a non-significant trend towards a Time(linear) x Group x Baseline T interaction: *Estimate* =.80(.42), F(1,44) = 3.64, *p* = .063. Partly in line with the fear patterns, the HR reductions following P showed the same slope regardless of baseline T: *Estimate* = .04(.03), F(1,23) = .056, *p* = .461, while after T HRs reduced more so for the participants with higher basal T: *Estimate* = -.12(.06), F(1,21) = 3.89, *p* = .061 (see Figure chapter 4 – appendix 4).

Session 2

The model showed that baseline HR was a significant predictor: *Estimate* = .70(.10), F(1,51) = 43.90, *p* <.001, signifying that the higher the baseline HR, the higher the HR during exposure. The linear time term was a significant predictor. Mean HR during exposure reduced over time, confirming that exposure resulted in the expected within-session reduction: *Estimate* = -12.77(4.59), F(1,50) = 7.70, *p* = .008. We did not find a significant interaction between Time x Group: *Estimate(linear)* = 6.91(4.59), F(1,50) = 2.26, *p* = .139, *Estimate (quadratic)* = -1.82(2.36), F(1,48) = .587, *p* = .447. This means that the HR patterns recorded in session 2 did not differ for the two groups.

The same effects were noted with baseline T in the model: Time(linear): *Estimate* = -13.92(4.66), F(1,46) = 8.87, *p* = .005; Time x Group *Estimate*(linear) = 8.11(4.66), F(1,47) = 3.01, *p* = .089, *Estimate*(quadratic) = 1.49(2.38), F(1,45) = .386, *p* = .537. Unlike subjective fear, the psychophysiological HR indices per group were independent of baseline testosterone: Time(linear) x Group x Baseline-T, *Estimate* =.10(.31), F(1,45) = .10, *p* = .759.

Chapter 4 – appendix 2

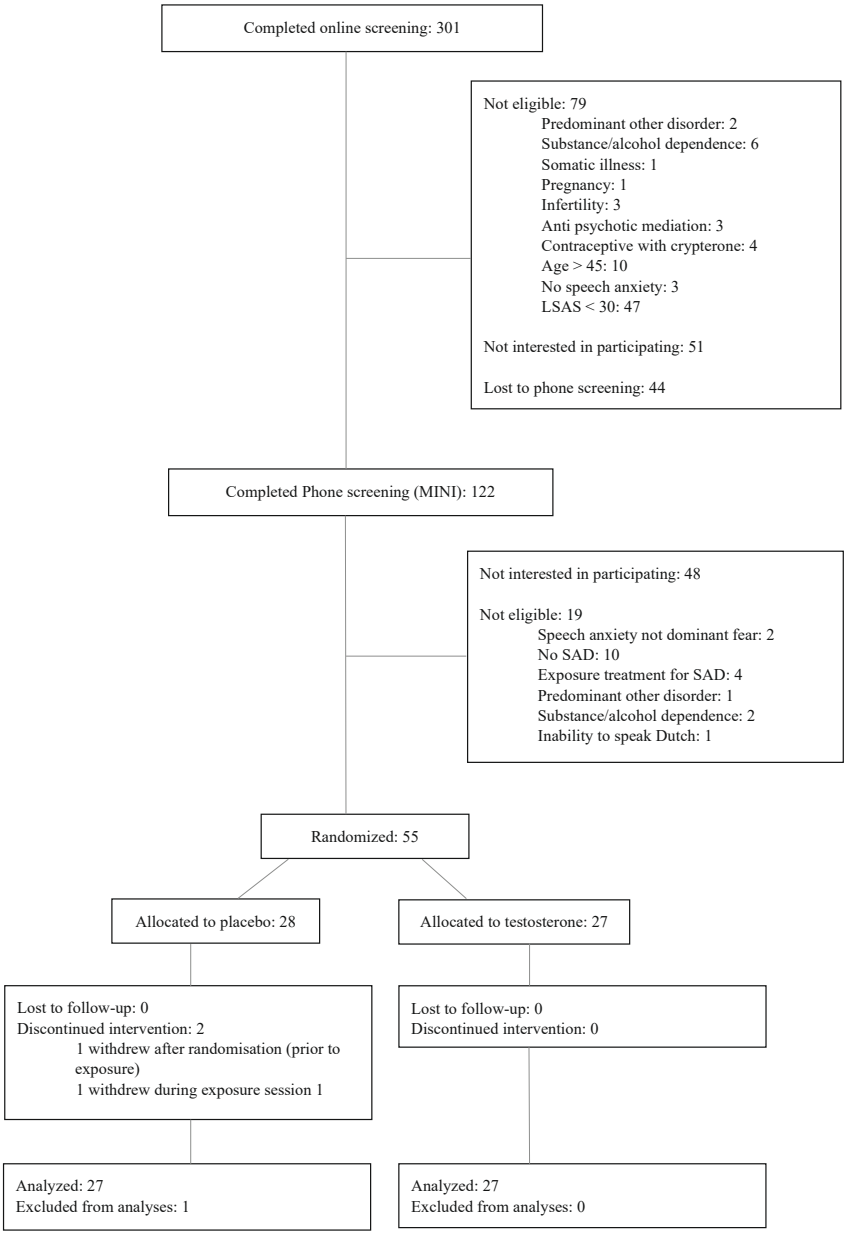

Figure. *CONSORT* flowchart

Chapter 4 – appendix 3

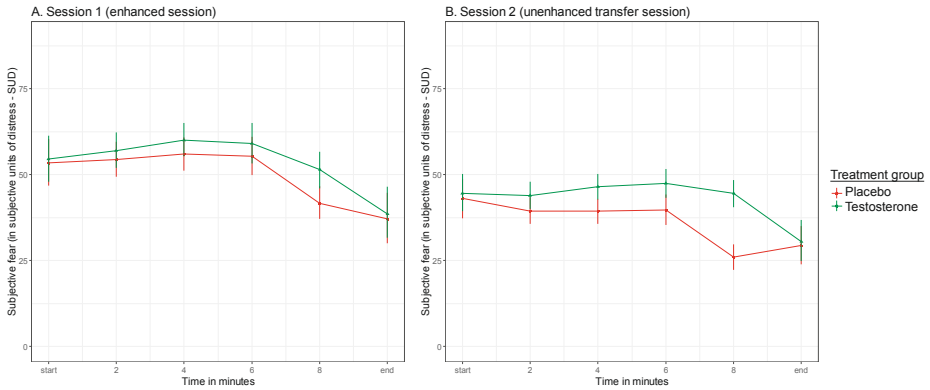

Figure. SUDs over time as a function of group. The left panel (A) depicts the scores recorded for the enhanced session and the right panel (B) the scores for the second, unenhanced session. In the first session SUDs have a similar pattern over time in both groups. In the second session SUDs show a more reactive pattern with a steeper decline towards the end for the participants in the testosterone group. Plots are based on the modeled data, including fixed and random effects. SUD = subjective units of distress.

Chapter 4 – appendix 4

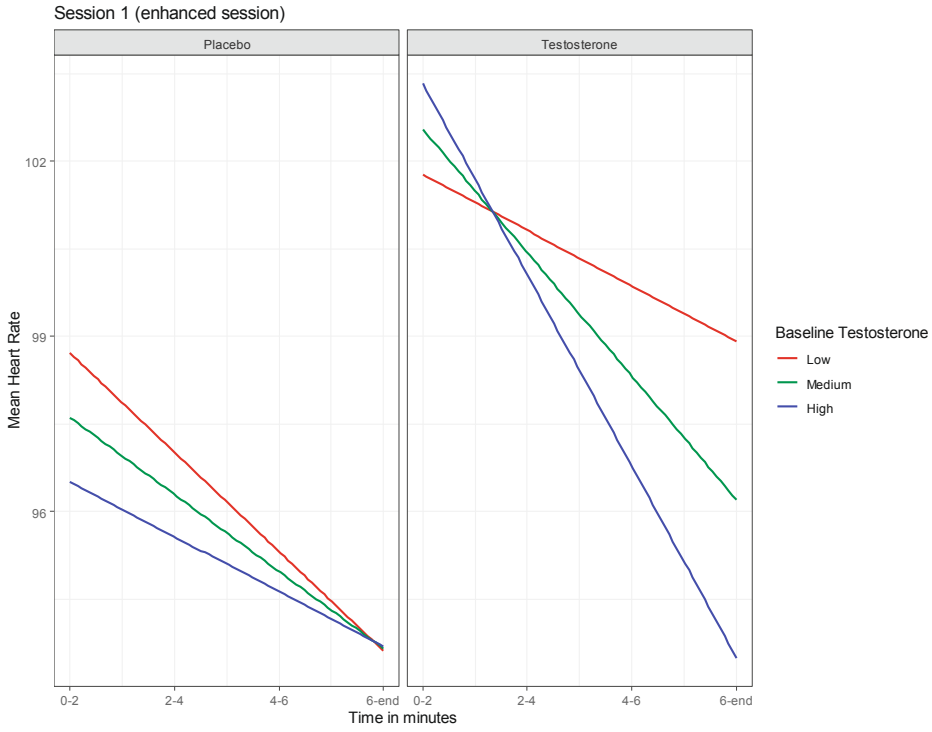

Figure This plot shows the mean heart rates (HRs) during the first exposure session, with placebo [left panel] and with testosterone [right panel]). Mean HRs are displayed over time as a function of baseline testosterone. In order to visualize the interaction effect between baseline Testosterone and Time we divided baseline testosterone in low (-1 SD), medium (mean) and high (+1SD) values. The plot illustrates the finding that high baseline testosterone is associated with a steeper decrease in HR during the testosterone-enhanced exposure. There is no correlation between start HR and baseline testosterone levels for either group ($r_{placebo}$ = -.14, p = .507 ; $r_{testosterone}$ = .04 , p = .851).

Chapter 5

Social Avoidance and Testosterone Enhanced Exposure Efficacy in Women with Social Anxiety Disorder: A Pilot Investigation

Hutschemaekers, M.H.M., de Kleine, R.A., Kampman, M., Smits, J.A.J., & Roelofs, K. (in press). Social Avoidance and Testosterone Enhanced Exposure Efficacy in Women with Social Anxiety disorder: A Pilot Investigation. *Psychoneuroendocrinology.*

Abstract

Social avoidance has been associated with more persistent social anxiety disorder (SAD) symptoms and low testosterone levels in individuals with SAD. We tested whether pre-treatment avoidance tendencies moderate the efficacy of testosterone-augmented exposure therapy. Fifty-five females with SAD received two exposure sessions during which fear levels were assessed. Session 1 was augmented with testosterone (0.50mg) or placebo. Avoidance tendencies and symptom severity were assessed pre- and post-exposure. Participants showed stronger avoidance for social versus non-social stimuli and this tendency remained stable over time. Stronger pretreatment avoidance tendencies were associated with larger fear reduction in the testosterone but not the placebo condition. This effect did not transfer to the second non-enhanced session or symptom severity. The findings support the hypothesis that individuals suffering from SAD with relatively stronger pretreatment avoidance tendencies benefit more from testosterone-augmentation, pointing to a potential behavioral marker for testosterone enhancement of exposure therapy.

Introduction

Social anxiety disorder (SAD) is the most common of all anxiety disorders with a lifetime prevalence of 13% (Bandelow & Michaelis, 2015; Bruce et al., 2005; Hendriks et al., 2016). Social avoidance is a major factor that prevents fear to extinguish in individuals with SAD (Arnaudova et al., 2017) and is therefore a main target in exposure therapy (Clark & Wells, 1995). Based on the well-established social approach-promoting and avoidance-reducing effects of testosterone (Enter, Spinhoven, et al., 2016; Hermans & Van Honk, 2006; Maner et al., 2008), researchers have started to study the use of testosterone interventions to boost the effects of exposure therapy in SAD (Hutschemaekers et al., 2020; Hutschemaekers, de Kleine, Hendriks, Kampman, & Roelofs, 2021). Although initial findings are promising, it remains unclear whether social avoidance tendencies in individuals with SAD influence the efficacy of testosterone-enhanced exposure interventions. Identification of social avoidance tendencies as a behavioral marker for the efficacy of these interventions is relevant for the optimization of (personalized) treatments. In the present study, we tested social avoidance tendencies before and after an exposure-based treatment intervention in SAD. In half of the participants the exposure was augmented with testosterone, offering the unique opportunity to explore whether pretreatment social avoidance tendencies would moderate testosterone augmentation effects.

Cognitive models of SAD imply that attentional processes and social avoidance behaviors play an important role in the etiology and maintenance of SAD (Clark & Wells, 1995). In addition to more overt avoidance behaviors such as avoiding social situations or eye contact, individuals with SAD also show more implicit automatic avoidance (biased action tendencies). Those automatic social avoidance tendencies can be measured using Approach Avoidance Tasks (AAT: Rinck & Becker, 2007), which instruct participants to respond to visual stimuli by pushing or pulling a joystick. Socially anxious individuals typically show automatic avoidance of social stimuli - i.e., stronger avoidance tendencies compared to approach tendencies toward angry, but also happy faces (Heuer et al., 2007; Loijen et al., 2020; Roelofs, Putman, et al., 2010; Roelofs, van Peer, et al., 2009a) and even neutral faces, compared to non-social stimuli and healthy controls (Kuckertz et al., 2017). Cross-sectional studies show that these avoidance tendencies toward social threat relate to higher SAD symptom levels (Enter, Spinhoven, et al., 2016), and to the onset and chronic course of social anxiety symptoms (Struijs et al., 2018). Although not all evidence points to such predictive value of symptom development (Kampmann, Emmelkamp, & Morina, 2018b; Struijs et al., 2017), the observation of relative avoidance tendencies to threathening cues on AAT-tasks is robust in individuals with SAD (for review see Loijen et al., 2020).

Testosterone enhances social approach behavior in socially challenging situations where social status may be threatened (Maner et al., 2008; Mazur & Booth, 1998; Terburg & Van Honk, 2013). A recent study showed that pre-treatment rises in testosterone were predictive of better exposure outcomes in terms of larger symptom reduction for individuals with SAD (Hutschemaekers et al., 2020). Moreover, single-dose testosterone administration in individuals with SAD increases automatic approach behavior toward threatening (angry) faces on an AAT (Enter, Spinhoven, et al., 2016) and reduces biased processing (van Peer et al., 2017) and gaze-avoidance toward angry faces (Enter, Terburg, et al., 2016), together suggesting that testosterone can alleviate automatic avoidance behavior in individuals with SAD. Indeed neuroimaging studies have indicated that testosterone administration increases amygdala activation, specifically when one has to approach an angry face and not during threat avoidance (Radke et al., 2015). It does so presumably by enhancing (largely dopaminergic) projections from the amygdala to the ventral striatum, relevant for motivated action (Hermans et al., 2010). Together, these findings suggest that testosterone can stimulate approach behavior in healthy individuals and importantly in highly avoidant individuals with SAD. Translated to clinical application, testosterone administration may be a viable augmentation strategy for exposure therapy for SAD. Indeed, the increased engagement in an exposure session afforded by testosterone may facilitate corrective learning and thereby optimize outcomes (Hutschemaekers et al., 2021).

Toward the goal of personalizing treatment, the present study sought to test the hypothesis that testosterone-enhancement of exposure-therapy would be most effective among individuals with SAD who present with high (as opposed to lower) levels of automatic social avoidance tendencies. We tested this hypothesis using data from a clinical trial involving 55 females with SAD who completed a session of exposure therapy and were randomized to receive either a single dose of testosterone (0.5 mg) or placebo prior to this session (Hutschemaekers et al., 2021). To assess the transfer of testosterone effects, the participants engaged in a second exposure session one week later that did not involve testosterone administration.

We predicted that social avoidance tendencies as measured at baseline would moderate testosterone augmentation effects, such that those showing stronger social avoidance tendencies at baseline and receive testosterone would profit more compared to those participants that receive placebo. Finally, we tested whether social avoidance tendencies changed over time with (testosterone enhanced) exposure therapy.

Materials and Methods

Participants

A complete description of the sample and procedures has been provided elsewhere (Hutschemaekers et al., 2021) and in Chapter 5 – Appendix 1. In short, the sample included 55 females suffering from SAD (M_{age} = 23.31, *SD* = 5.63, range = 18–43). Participants were recruited at an outpatient clinic specialized in anxiety disorders, at the Radboud University Nijmegen, and from the community. We focused exclusively on females because the pharmacodynamics of the currently used testosterone administration methods have as yet been established in females only (Tuiten et al., 2000). Exclusion criteria were: A) Prior non-response to speech exposure therapy for SAD, B) other predominant emotional disorder(s) C) Psychosis or delusion disorders (current or lifetime), D) Significant suicidal ideations or behaviors within 6 months prior to screening, E) Intellectual developmental disorder , F) Substance or alcohol dependence, G) Somatic illness, H) Females unwilling to use an active form of birth control during the trial, I) pregnancy or lactation, J) Infertility, K) Antipsychotic medication, L) Unstable dose of Antidepressants or Benzodiazepines within 6 weeks prior to enrollment, M) Insufficient proficiency of Dutch language, N) Use of contraceptive containing cyproterone acetate. Ethical approval for this study was granted by the local Review Board (Arnhem-Nijmegen).

Medication and randomization

Participants were randomly assigned to testosterone (T) or placebo (P) treatment. T was suspended in a clear solution (0.5 ml) with 0.5 mg hydroxypropyl-beta-cyclodextrin, 0.005 ml ethanol 96%, and distilled water. P contained the same ingredients, except the T. Participants held the liquid under their tongue for 60 seconds (4 hours prior to the first exposure). Participants and researchers were blind to treatment condition until completion of the primary outcome analyses of the parent trial.

Exposure intervention

Participants received 2 public speaking exposure sessions of 90 minutes, at two separate days, based on the protocol developed by Rodebaugh, Levinson, and Lenze (2013). The second session, one week later, followed the same protocol as the first session without drug administration. Therapists were advanced Bachelor- or Master-level psychology students trained and supervised by authors M.H. and M.K. Adherence to the protocol was checked during supervisions and deviations from the protocol were reported by the therapists. Adherence was good as 96.3% of the sessions was performed according to the protocol.

Outcome measures

Symptom severity

Social anxiety symptoms were assessed with the Social Phobia Scale (SPS; Mattick & Clarke, 1998), a self-report measure assessing the fear of being observed or watched during social or performance situations. The scale has shown good internal consistency; α = .94 (Mattick & Clarke, 1998); Dutch translation; α = .91 (Beurs et al., 2014); current study α = .86. The SPS was completed at baseline, post-assessment (after the second exposure session) and at one month follow-up (online).

Fear levels

Participants rated their fear levels, using a Subjective Units of Distress (SUDs) scale ranging from 0: no fear to 100: extreme fear (Wolpe & Lazarus, 1966). SUDs were assessed after the psychoeducation (initial SUDs), at the beginning of each exposure session (baseline SUDs), immediately prior to the speech (start SUDs), every 2 minutes during and immediately after the speech (end SUDs).

Approach avoidance task

To assess approach-avoidance tendencies toward facial expressions we used the Approach Avoidance Task (AAT; Rinck & Becker, 2007). In the AAT participants responded to emotional stimuli: happy, angry, neutral facial expressions presented on a computer screen by either pulling a joystick toward themselves or pushing it away as quickly as possible with their dominant hand. Instructions were indirect based on the color of the picture (grey or sepia). By doing this, the size of the picture increased (pulling movement) or decreased (pushing movement). After making a complete correct movement, the picture disappeared from the screen. Participants then moved the joystick back to its central position and, by pressing the fire button of the joystick, they initiated a new trial. The stimuli were selected from the Karolinska Directed Emotional Faces database based on quality of emotional expression (Goeleven, De Raedt, Leyman, & Verschuere, 2008; Lundqvist, Flykt, & Öhman, 1998). The three types of emotions were taken from the same models (5 females and 5 males). The task also included 20 checkerboards as control stimuli, resulting in 80 different pictures presented in random order twice. Reaction times (RTs) were recorded in ms. Relative faster execution of the push response compared to the pull response reflects heightened behavioral avoidance of the specific type of stimulus. In general, response latencies for affect-congruent (e.g., happy-approach and angry-avoid) are shorter compared to affect incongruent responses (e.g., happy-avoid and angry-approach). The task consisted of 30 practice trials (with different models) and

160 experimental trials. The AAT was performed at baseline and at post-assessment (30 minutes post the second exposure session).

Before calculating mean reaction times for each picture type (happy, angry, neutral and control) and movement (push, pull), we removed all incorrect trials (on average 2%) and outliers (fastest and slowest 1%). We computed a combined AAT effect score from these RTs in which mean RTs for pulling were subtracted from pushing RTs (all facial combined) corrected for the control stimuli ([Push RTs – Pull RTs of all facial expressions combined]/3 – [checkerboards Push – checkerboards Pull]), resulting in a score that reflects the direction of the response tendency. For this AAT effect score negative values indicate stronger avoidance then approach (see also see Chapter 5 – Appendix 1).

Saliva samples

To assess endogenous testosterone levels, saliva samples were collected (2 ml passive drool saliva by Salicap; Hamburg, Germany) at eight time points: (1) at baseline, (2) prior to T/P intake, (3) prior to exposure session 1, (4) immediately after speech delivery in session 1, (5) 30 min after speech delivery in session 1, (6) prior to exposure session 2, (7) immediately after speech delivery in session 2, and (8) 30 min after speech delivery in session 2.). These timepoints were similar for all participants to control for fluctuations of testosterone levels during the day, see also procedure section. For the current study only the first three samples were relevant. Sample 1 (at baseline) and sample 2 (prior to drug intake) were used to assess endogenous baseline testosterone levels and sample 3 (prior to exposure session 1) was assessed as a manipulation check. Samples were stored at -20 °C until radio immune assays were performed by Dr. Kirschbaum's laboratory (Dresden, Germany), for descriptions of methodology, see Miller, Plessow, Kirschbaum, and Stalder (2013), Reardon, Herzhoff, and Tackett (2016).

Procedure

Participants first completed the baseline assessment (between 9 and 11 AM), including questionnaires, saliva collection and the pre-exposure AAT. The first exposure session was scheduled within the week of the baseline session. Participants began this session by taking a pregnancy test, followed by saliva collection, psychoeducation, a baseline SUDS rating, and administration of study drug commensurate with group assignment (always between 9 and 11 AM). Participants returned 4 hours later for a salivary sample and the first exposure session. SUDs were collected during exposure and AEs were assessed at the end of the session. The second exposure session took place one week later (at the same time of the day as the first exposure session) and was, apart from the study medication administration, identical to the first exposure session. Participants

completed the post-exposure assessment, which included the SPS and the post-exposure AAT, 30 minutes after the second session. One month later, participants completed an online follow-up assessment which included the SPS. The original parent trial was registered in the Dutch trial register (https://www.trialregister.nl/trial/6238) and at EudraCT (2014–004475–23).

Data analytic strategy

The research questions, hypotheses and data analytic procedures were pre-registered at Open Science Framework (OSF): see https://osf.io/3cxsv. At two minor points the analyses we performed deviated from the pre-registration (https://osf.io/3cxsv), which are discussed in Chapter 5 – Appendix 1. For the parent trial a sample size of 52 participants was deemed necessary to detect group differences with at least a moderate effect size and a power of 80%. Consistent with pre-registration, we ran preparatory analyses to further specify our AAT predictors in our analyses. Specifically, we first tested if participants showed an avoidance bias toward facial expressions and if this bias was affected by picture type with a Repeated Measures ANOVA with factor picture type (happy, angry, neutral, control) and response direction (push, pull) on the AAT reaction times. Based on the results of this analysis (e.g., all facial stimuli showed faster push than pull RTs (see Chapter 5 – Appendix 1 for details), we decided to analyze AAT reaction times for social stimuli (i.e., all facial expressions) versus non-social stimuli (i.e., the checkerboards).

To test the moderator hypothesis, we conducted mixed model analyses for the first (enhanced, with testosterone (T) or placebo (P)) and second (unenhanced) session separately. More specifically, to determine whether avoidance tendencies toward facial expressions moderated (testosterone enhanced) exposure efficacy in terms of self-reported fear (SUDs) during the exposure sessions, AAT combined effect score, group (T/P) and time (start, 2 min, 4 min, 6 min, 8 min, end) were included as predictors. Because we found that SUD scores did not follow a linear pattern (Hutschemaekers et al., 2021), we included linear and quadratic time terms. Participant was included as random intercept. Initial SUD scores were included as a fixed factor to control for variance in fear levels unrelated to time or group. To test the relation between social avoidance tendencies and symptom severity, we modeled SPS scores, with AAT combined effect score, group (T/P) and time (pre/post/FU) as predictors and participant as the random intercept.

To test the effects of treatment condition on pre- to post changes in avoidance tendencies toward facial expressions, we modelled AAT RTs with Time (pre-post exposure), Group (T/P), Response direction (Push/Pull) and Picture type (Social/Non-social) as predictors. Participant and Stimulus model (e.g., the model presented on the stimulus) were included as random intercepts. Response direction and time (and their interaction)

were included as random slopes for participant. Response direction, Time and Group (and their interactions) as random slopes for Stimulus model.[1]

Per registration, see also parent trial Hutschemaekers et al. (2021), we included endogenous baseline testosterone as an additional control variable (mean saliva sample 1 and 2) in all models. We used the Lme4 package in R (Bates et al., 2013) and *p*-values were calculated using the likelihood ratio tests in the Afex package (Singmann, 2013). The confidence intervals were determined using Lme4's confint function using Bootstrapping (1000 simulations). Continuous predictor variables were centered, and sum-to-zero contrasts used. Consistent with the recommendations for mixed models (Pek & Flora, 2018), we report unstandardized effect sizes (estimates).

Results

Sample characteristics

The data of 54 participants were analyzed (M_{age} = 23.31, SD = 5.64, range = 18–43) since one participant receiving placebo dropped out before the first exposure due to illness. Another participant in the same group dropped out during the first session (3.6%). All other participants completed both sessions and the follow-up. A full overview of the sample characteristics have been described elsewhere (Hutschemaekers et al., 2021). There were no baseline differences between the placebo and testosterone group on any of the AAT reaction times, all *p*-values > .257 (see Table 5.1).

1 We aimed to test a maximum random effect structure (picture type, response direction and time and their interactions as random slopes for the random intercept of participant and random slopes of response direction, time and group for the random intercept of stimulus model) but this model did not converge due to model estimation problems. Therefore, we ran simpler random effects models by dropping random slopes step by step and comparing the AIC after each step. As is common with mixed models, some of the simplified models also resulted in convergence warnings, but these warnings are more often false positives. In line with the recommendations by Bolker (2022), we used different optimizers (allFit function) and compared the estimates which all showed the same results and highly similar estimates. As such we decided to report the results of the model with the best fit (i.e. lowest AIC) and most extended random effect structure that was modeled.

Table 5.1 *Mean AAT reaction times in ms and Standard deviations depending on group (placebo/testosterone), picture type, response direction and measurement time.*

Measure	Picture type							
	Angry		Neutral		Happy		Control	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Testosterone group (*n* = 27)								
Push	643.66 (86.21)	622.75 (88.58)	641.77 (74.74)	626.89 (81.85)	647.98 (77.14)	638.59 (76.87)	679.08 (82.13)	651.08 (91.03)
Pull	659.13 (83.1)	648.98 (94.70)	672.50 (81.16)	651.27 (92.98)	647.03 (64.73)	639.89 (83.62)	664.48 (81.51)	635.99 (70.52)
Placebo group (*n* = 27)								
Push	631.59 (81.38)	605.80 (60.95)	627.23 (56.52)	609.30 (63.20)	632.80 (65.04)	611.23 (48.77)	688.10 (81.65)	639.62 (62.45)
Pull	649.67 (58.09)	628.39 (73.79)	649.56 (65.02)	618.41 (57.40)	652.93 (79.96)	627.70 (68.86)	658.52 (81.26)	626.67 (61.09)

N = 54

Predictive effects of automatic avoidance tendencies on exposure success

Only effects relevant for the current research questions are reported. For a full overview of the results of the exposure and testosterone administration, we refer the reader to the report of the parent trial (Hutschemaekers et al., 2021).

Fear levels

Session 1 (Enhanced session)

The three-way interaction of AAT effect score x Time (linear) x Group approached statistical significance, *Estimate*(linear) = -.44(.23), 95% CI [-.89, -.02]; F(1, 213) = 3.85, *p* = .051 (see step 1, table 5.2, also for the quadratic time term). To further test our hypothesis that those who show greater social avoidance tendencies at baseline and receive testosterone would profit better compared to those participants that receive placebo, we post-hoc decomposed this result. This follow-up analyses revealed a significant two-way interaction of AAT effect score X Time (linear) for the testosterone group: *Estimate* = .66(.33), 95% CI [-.01, 1.27]; F(1,106) = 4.04, *p* = .047, but not for the placebo group, *Estimate* = -.23(.31), 95% CI [-.82, .39]; F(1, 107) = .538, *p* = .465. Simple slope analyses further showed that, among those assigned to the testosterone condition, participants with lower AAT effect scores (- 1 SD, relative avoidance) reported greater reductions of fear (*Estimate* = -126.72(27.58), 95% CI [-187.13, -71.55]; *t*(106) = -4.60, *p* < .001) relative to those with higher AAT effect scores (mean + 1SD, relative approach; *Estimate* = -53.46(23.84), 95% CI [-103.61, -7.16], *t*(106) = -2.24, *p* = .03). These differential effects were not observed for the placebo group (see Figure 5.1). The inclusion of baseline testosterone as a control variable did not change the results (step 2, see Table 5.2).

Session 2 (Non-enhanced)

There was no significant three-way interaction effect between AAT effect score, time and group (see Table 5.2)[2] or two-way interaction between AAT effect score and time. Inclusion of baseline testosterone in the model showed a significant interaction of AAT effect score with Group: *Estimate* = .09(.04), 95% CI [.001, .17]; F(1,45) = 4.44, *p* =.041. For the testosterone group, avoidance scores were not associated with fear levels while for the placebo group, stronger avoidance scores were associated with lower overall fear levels. All other main effects and interaction with AAT effect scores remained non-significant (see step 2, Table 5.2).

2 The residuals of the model did show some deviations from normality. However, log or square root transformations did not improve the distribution of the data.

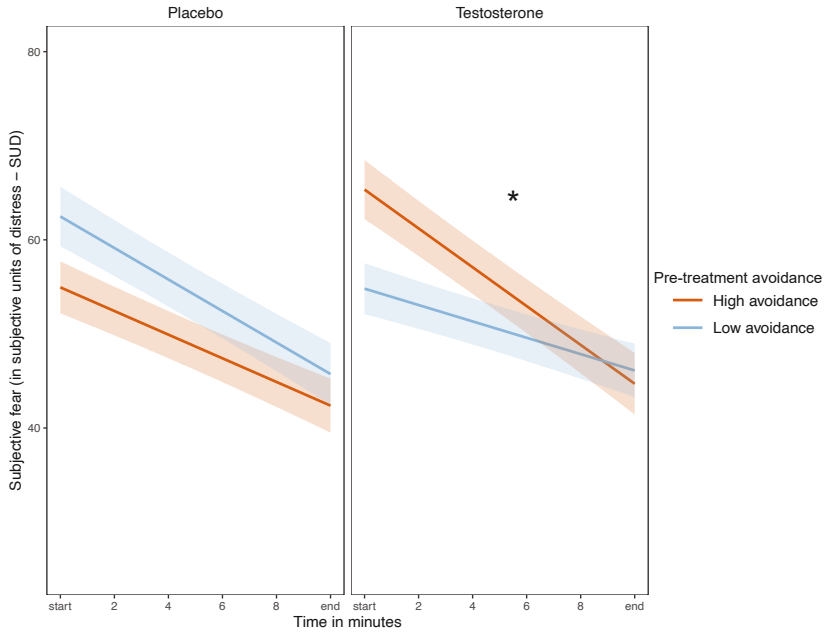

Figure 5.1 Illustration of the interaction effect between pre-treatment avoidance and testosterone (vs placebo) enhanced fear-reduction in session 1. The panels show the model based predicted values of the AAT-effect score x Time x Group (testosterone, placebo). The left panel shows the simple slopes of time for the placebo group and the right panel for the testosterone group. The separate lines reflect high (orange) and low (blue) levels of automatic avoidance (note that high and low avoidance groups were only created for display purposes). In the testosterone group we see a significant interaction of automatic avoidance and time (p = .047). Specifically, we see steeper reduction in fear levels for high levels of automatic avoidance of facial expressions compared to lower levels of avoidance. In contrast, in the placebo group relatively low and high avoidance levels are associated with similar patterns of fear reduction (p = .466). Note that there was no relation between avoidance level and the start SUD, not for the placebo group (r = .30, p = .17); nor for the testosterone group (r = .-13, p = .51).

Social anxiety symptoms

We did not observe a three-way interaction effect of AAT effect score x Time (Pre/Post/Follow-up) x Group, on social anxiety symptoms (pre-post-follow-up) or two-way interaction between AAT effect score and time (all p > .084). The inclusion of baseline testosterone as a control variable did not change the results (see Chapter 5 – Appendix for details of analysis).

Avoidance tendencies over time[3]

Results revealed a main effect of Time, *Estimate* = 13.19(3.25), 95% CI [6.10, 19.65]; $t(37) = 4.06$, $p < .001$, suggesting that reaction times on the AAT reduced from pre- to post-exposure. A significant interaction effect for picture type (social, non-social) and response direction (push, pull), *Estimate* = 8.84(3.66), 95% CI [1.30, 15.81]; $t(6) = 2.42$, $p = .050$, showed that participants were faster in pushing compared to pulling social faces (e.g. an avoidance bias), whereas they were faster in pulling compared to pushing non-social stimuli (e.g., an approach bias). This effect was present for both groups and did not change over time (pre- to post treatment). All other main effects and interactions were not significant (see Figure 5.2).

Table 5.2 *Multiple linear regression predicting exposure success (SUD reductions), without and with baseline testosterone included.*

	Exposure success			
	SUDs session 1		SUDs session 2	
	Estimate (95% CI)	**P-value**	**Estimate (95% CI)**	**P-value**
Model step 1				
AAT effect score	.004(-.08, .08)	.911	.05(-.03, .14)	.223
AAT effect score * time (linear)	.21(-.20, .65)	.346	.21(-.17, .58)	.278
AAT effect score * time (quadratic)	-.01(-.42, .43)	.963	-.08(-.43, .28)	.683
AAT effect score * group	.05(-.02, .12)	.205	.08(.001, .17)	.065
AAT effect score * group * time (l)	-.44(-.89, .02)	.051	-.08(-.44, .31)	.680
AAT effect score * group * time(q)	-.35(-.81, .07)	.099	-.02(-.39, .30)	.924
Model step 2 (with baseline testosterone)				
AAT effect score	.01(-.06, .08)	.862	.06(-.02, .14)	.153
AAT effect score * time (linear)	.19(-.28, .63)	.399	.18(-.19, .55)	.338
AAT effect score * time (quadratic)	-.06(-.05, .03)	.794	-.10(-.45, .26)	.582
AAT effect score * group	.05(-.02, .12)	.135	.09(.003, .18)	.041
AAT effect score * group * time (l)	-.41(-.87, .001)	.067	-.07(-.44, .31)	.727
AAT effect score * group * time(q)	-.34(-.74, .04)	.108	-.01(-.36, .34)	.937

Note. there was no correlation between baseline testosterone and AAT effect scores prior to exposure: r = .029, p = .837 or post exposure: r = -.079, p = .580.

3 The residuals of this model showed that the assumption of normality was violated. Therefore, a log transformation was performed. This improved the distribution of the residuals and yielded similar results compared to the model without this transformation. To improve interpretation of the estimates we reported the results of the non-transformed data. Moreover, mixed model analyses are fairly robust against violations of normality (Knief & Forstmeier, 2021; Schielzeth et al., 2020).

The inclusion of baseline testosterone as a control variable did not change the results (see Chapter 5 – Appendix 1 for details analysis).

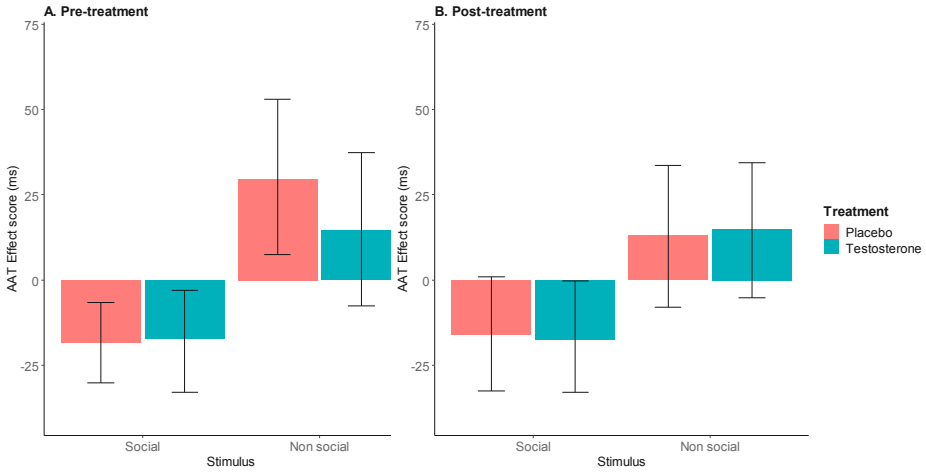

Figure 5.2 Automatic avoidance tendencies displayed in mean AAT effect scores (in ms) per picture type (social/non-social) over time separated per group (Testosterone/Placebo). The AAT effect scores are calculated for display purposes only by subtracting the individual reaction times for pull movements from the individual reaction times for push movements. Negative AAT effect scores indicate stronger avoidance and positive AAT effect scores reflect stronger approach. As shown in the left panel (A) a pre-treatment avoidance bias towards social stimuli is shown while for non-social stimuli an approach bias is shown, for both the placebo and testosterone group. This pattern stays stable over time (right panel, B).

Discussion

This study provides preliminary findings to suggest that augmenting exposure therapy for SAD with testosterone administration may be most effective when targeted to individuals who present with strong avoidance tendencies. These results are consistent with theory and replicate and extend the findings of earlier experiments (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016) and a clinical trial (Hutschemaekers et al., 2021).

It is important to note that the moderating effect of social avoidance tendencies were only observed for one outcome, namely acute changes in fear. We are left speculating as to the reasons why the observed moderator effects did not emerge for the other outcomes – fear at the second session and changes social anxiety symptom severity. The latter may not be surprising as the parent trial did not show any effects of testosterone

on social anxiety symptoms either (Hutschemaekers et al., 2021). One possibility is that single-session enhancement is not sufficient to yield longer-term effects or changes in social anxiety symptom severity. These observations call for follow-up parametric studies.

In line with previous work, individuals with SAD showed avoidance tendencies not only for angry but also neutral and (to a lesser extend) happy faces (Heuer et al., 2007; Kuckertz et al., 2017; Loijen et al., 2020; Roelofs, Putman, et al., 2010; Roelofs, van Peer, et al., 2009a). Angry faces explicitly communicate threat to the individual and may therefore automatically activate avoidance mechanisms, especially in socially anxious individuals (Heuer et al., 2007; Roelofs, Putman, et al., 2010). The same can be true for neutral faces. Indeed, neutral faces are ambiguous, activate negative bias, and have been labeled as threatening by socially anxious individuals (Heuer et al., 2007; Lange, Allart, Keijsers, Rinck, & Becker, 2012). Relative avoidance tendencies to happy faces (Heuer et al., 2007; Lange et al., 2012; Roelofs, Putman, et al., 2010) are also common, pointing perhaps to tendencies among individuals with SAD to avoid any potential social interaction partner (Roelofs, Putman, et al., 2010).

We did not observe any changes in social avoidance tendencies over time with exposure therapy. It is possible that such changes require testing social avoidance tendencies during the testosterone treatment window (Enter et al., 2014; Enter, Spinhoven, et al., 2016). The fact that social avoidance tendencies did not change from pre to post exposure may in fact suggest that avoidance tendencies in individuals with SAD are stable over time. Interestingly, Kampmann et al. (2018a) found no change in social avoidance tendencies in individuals with SAD even after 10 sessions of (successful) exposure therapy. Collectively, these observations point to the possibility that targeting social avoidance tendencies during the course of established interventions for SAD as a way to boost their efficacy and reduce relapse may require more intensive (e.g., frequency, duration) treatment with testosterone or other augmentation strategies that can directly engage this therapeutic target.

Several strengths and limitations of this study deserve comment. As far as the strengths, we only included individuals who met the diagnostic criteria of SAD (American Psychiatric Association, 2013) and we used well-established tasks and protocols. Second, the hypotheses were preregistered and were grounded in a long standing research line testing prosocial properties of testosterone and their boundary conditions in individuals with SAD (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016; Hutschemaekers et al., 2021). As far as the limitations, first, the study was underpowered to detect small effects. Second, we only included females because the administration method we used has been validated only in females (Bos, van Honk, et al., 2012; Enter, Terburg, et al., 2016; Tuiten et al., 2000). Building upon recent single-dose testosterone administration studies in men

that have documented changes in social approach and avoidance behaviors (see Carré and Robinson, 2020; Geniole and Carré, 2018), future work may focus on generalizing the findings observed here to men. Third, we did not include an additional CBT control condition and therefore cannot assess specificity. Lastly, our study was not optimized to test subtle changes in avoidance behavior *during* exposure. Therefore, we can only speculate about the effects of testosterone on avoidance behaviors, and other mechanisms of action regarding the effects of testosterone cannot be ruled out. However, the fact that testosterone facilitated in-session exposure-effects in participants with stronger automatic avoidance potentially suggests that it reduces avoidance and facilitates engagement in exposure therapy. In order to test this hypothesis, we recommend future studies to include more specific in-session approach-avoidance measures, for example body posture-, eye movement- or personal distance measures, which may help to disentangle different types of avoidance such as Pavlovian flight behaviors and more instrumental or goal directed avoidance (Cain, 2019; Lu, Kemmerer, Riecke, & de Gelder, 2023; Wagels, Radke, Goerlich, Habel, & Votinov, 2017).

Conclusion

In sum, the current study adds to a growing body of literature indicating that individuals with SAD who enter exposure treatment with strong social avoidance tendencies may benefit from additional treatment with testosterone. Specifically, probing the data from a proof-of-principle clinical trial of this augmentation strategy that included females with SAD regardless of their levels of social avoidance tendencies yielded initial evidence to support a more targeted application of this clinical strategy. We hope that these pilot findings encourage follow-up studies of testosterone-augmented exposure therapy that can aid the goal to optimize its application and efficacy.

Chapter 5 – appendix 1

Details methods as described in the main outcome paper

Participants

The participants were recruited from an outpatient clinic specializing in the treatment of anxiety disorders, from the Radboud University Nijmegen, and from the community from 2017 through 2019. Inclusion criteria were: 1) woman, 2) age: 18-45 years, 3) primary diagnosis of SAD (assessed using the Mini International Neuropsychiatric Interview (MINI; (Sheehan et al., 1998)), with a predominant fear of public speaking, and 4) score > 30 on the Liebowitz Social Anxiety Scale (LSAS; (Liebowitz, 1987)). We included women only because the pharmacodynamics of the currently applied testosterone administration method has as yet been established in women only (Tuiten et al., 2000). Our exclusion criteria were: A) prior non-response to speech exposure therapy for SAD, B) other predominant mental disorder(s), C) (current or lifetime) psychosis or delusion disorders, D) significant suicidal ideation or behavior within 6 months prior to screening, E) intellectual disability, F) substance or alcohol dependence, G) somatic illness, H) unwillingness to use an active form of birth control during the trial, I) pregnancy or lactation, J) infertility, K) antipsychotic medication, L) unstable regimen of antidepressants or benzodiazepines within 6 weeks prior to enrollment, M) insufficient proficiency in the Dutch language, N) current use of contraceptives containing cyproterone acetate. Participants received 70 Euros for their participation. Ethical approval for this study was obtained from the local Ethical Review Board (Arnhem-Nijmegen). In total, 55 women meeting the criteria for SAD (M_{age} = 23.31, SD = 5.63, range = 18-43) were included in the study. One participant dropped out prior to the first exposure session, due to illness. To ensure equal group sizes she was replaced, resulting in a total of 55 participants receiving the allocated drug (placebo/testosterone) and 54 receiving the exposure sessions: 27 per group. After study completion, one participant (testosterone group) divulged she had been on atypical antipsychotic quetiapine, something she omitted mentioning during eligibility screening. We decided not to exclude her from the analyses because she had consistently used a low, stable dose (25 mg) for the last 18 months.

Medication and randomization

The pharmacist providing the study solutions randomly assigned participants to placebo (P) or testosterone (T) in blocks of four (no stratification). Testosterone was suspended in a clear solution (0.5 ml) with 0.5 mg hydroxypropyl-beta-cyclodextrin, 0.005 ml ethanol 96%, and distilled water. Placebo contained the same ingredients, aside from Testos-

terone. Participants held the liquid under their tongues for 1 minute. This dose yields a sharp increase in plasma testosterone concentrations within 15 minutes and declines to baseline within 90 minutes in women (van Rooij et al., 2012). Pharmacodynamic effects can be assayed 4-6 hours after intake (Bos, Panksepp, et al., 2012; Tuiten et al., 2000). Until completion of the primary outcome analyses, therapists, participants and researchers were blind to the group allocation.

Exposure intervention

The participants engaged in two 90-minute public-speaking exposure sessions delivered one week apart in accordance with the protocols developed by Rodebaugh and colleagues (Rodebaugh et al., 2013; Smits, Rosenfield, Davis, et al., 2013). Sessions were standardized with respect to preparation time (max. 5 minutes), exposure length (6-8 minutes), the availability of notes and speech topic as well as reaction of the experimenter (neutral). On the morning of the first day, participants received psychoeducation about SAD and exposure, with the first session starting after four hours. In both sessions, psychoeducation was repeated and personalized goals and harm expectancies were assessed. Then, the participants presented their prepared speech in front of two confederates, a therapist and a video camera. Participants reviewed their videotaped performance afterwards with the therapist. The latter being psychology students in their last year of training (BA and MA level) trained and supervised by board-certified psychologists (authors, M.H. and M.K).

Deviations in data analytic strategy

We deviated from the pre-registered data analyses at the Open Science Framework (see https://osf.io/3cxsv) in two minor ways. First, in the pre-registration we stated that for our first preparatory analyses we would conduct a one sample t-test to check if AAT effect scores for facial expressions at baseline significantly differ from zero, followed by an ANOVA with valence (happy, angry, neutral) as factor on AAT effect scores (Push – Pull). However, we ran a two-way repeated measures ANOVA on the raw reaction times which we consider an improvement. Secondly, we did adhere to the exploration of adding baseline testosterone to our models, but only for the main effects.

Details results preparatory analyses

The two-way repeated measures ANOVA showed a main effect of picture type: $F(3, 159) = 12.95$, $p = .023$. Post-hoc comparisons with a Bonferroni adjustment showed that the Reaction times of the checkerboards were significantly slower compared to all facial stimuli (all $p < .001$). Reaction times of the facial expressions did not significantly differ

from one another (all $p > .605$). Moreover, there was a significant interaction between response direction and picture type, $F(3, 159) = 10.32$, $p = .015$. Therefore, the effect of response direction was analyzed at each type of stimulus (angry, happy, neutral, control). *P*-values were adjusted using the Bonferroni multiple testing correction method. The effect of response direction was significant for the angry ($p = .04$), neutral ($p < .001$) and control stimuli ($p = .008$), but not for happy stimuli ($p = .102$). However, the direction of the effect of control stimuli was opposite to the direction of other stimuli, indicating that participants show a significant tendency to faster push angry and neutral faces away compared to pulling them, (e.g., an avoidance tendency). The same pattern is found for happy faces, although not significant. While for checkerboards participants show a significant tendency to pulling checkerboards faster than pushing them away (e.g., an approach tendency). Based on the results of this analysis we computed a combined AAT effect score for all facial expressions (happy, angry, neutral): ([Push RTs – Pull RTs of all facial expressions combined]/3 – [Control Push – Control Pull]).

The second preparatory analyses showed that social anxiety symptoms at baseline were not related to avoidance tendencies toward facial expressions at baseline $r = .08$, $p = .556$.

Details results inclusion of baseline testosterone

Social anxiety symptom analysis

Social avoidance tendencies did not affect social anxiety symptoms over time, pre-post *Estimate* = - 0.001(.02), 95% CI [-.05, .04]; $t(92) = -.025$, $p = .980$, pre-FU: *Estimate* = -.039(.02), 95% CI [-.003, .08]; $t(92) = 1.76$, $p = .083$ nor an interaction effect of AAT effect score x Time x Group, pre-post: *Estimate* = .002(.02), 95% CI [-.04, .04]; $t(92) = -.108$, $p = .914$, pre-FU: *Estimate* = .004(.02), 95% CI [-.04, .04]; $t(98) = -.018$, $p = .985$.

The effects of exposure and testosterone on social avoidance tendencies[4]

There was a main effect of time *Estimate* = 13.24(3.33), 95% CI [6.82, 20.29]; $t(36) = 3.97$, $p < .001$. Reaction times on the AAT become less from pre-exposure to after exposure. A marginal significant interaction effect was found for picture type and response direction *Estimate* = 6.68(3.74), 95% CI [1.57, 15.33]; $t(6) = 2.32$, $p = .058$. Showing the same pattern

4 These are the results of the same model as reported in the paper without baseline testosterone. Inclusion of baseline testosterone resulted in model converging errors. Estimates of simpler random effects structures with baseline testosterone included were highly similar to the current model. Therefore, we decided to report the results of this model to stay in line with main analyses without baseline testosterone.

as the model without baseline testosterone: participants were faster in pushing facial stimuli compared to pulling (e.g., an avoidance tendency), while for non-social stimuli participants were faster in pulling compared to pushing (e.g., an approach bias). This pattern was present for both groups and did not change over time.

Chapter 6

Summary and General Discussion

Aims of this dissertation

The main aim of this dissertation was to test the potential of the steroid hormone testosterone as an enhancer for exposure treatment efficacy for social anxiety disorder (SAD). As described in the previous chapters, persistent avoidance is an important factor hindering the extinction of fear during social situations, which is why reducing avoidance behavior is the core target of exposure therapy, the treatment of choice for SAD. However around 45-55% of the individuals with SAD do not profit sufficiently from treatment (Carpenter et al., 2018; Loerinc et al., 2015). Therefore, the intervention leaves room for improvement. Based on pre-clinical research, the steroid hormone testosterone yielded promise to enhance exposure therapy effects for those suffering from SAD. From a literature review in chapter 2 we concluded that testosterone is an important regulator of social approach behavior, it alleviates social avoidance and facilitates prosocial approach and dominance-seeking behavior (Hermans & Van Honk, 2006; Maner et al., 2008; Mazur & Booth, 1998). Therefore, in our first empirical study we tested whether pre-exposure increase in endogenous testosterone is predictive of exposure efficacy in males and females with SAD. Next, we conducted a randomized controlled proof-of-concept trial in which we tested whether a single dose of exogenous testosterone could improve exposure efficacy in females with SAD. Lastly, we examined whether social avoidance tendencies moderate testosterone-enhanced exposure efficacy. This final chapter summarizes the results of these theoretical and empirical chapters, presents an integration and discussion of the findings as well as the clinical implications. Additionally, I will describe the strengths and limitations of this dissertation, followed by a conclusion.

Summary of chapters

There is a wealth of fundamental neuroendocrinological research in animals and humans pointing to the relevance of the HPG- and HPA-axis in the regulation of social motivational behavior. In **Chapter 2,** we explored the relevance of those fundamental insights for our understanding of social motivational deficiencies characterizing social motivational disorders such as SAD and psychopathy. Specifically, we presented a literature review regarding the role of the steroid hormones testosterone and cortisol and provided converging evidence that SAD as well as aggression-related psychopathologies present with an imbalance in these steroid hormones. This imbalance shows an interesting symmetry with alterations in social approach and avoidance in healthy individuals but particularly in relation to social motivational disorders, such as SAD and psychopathy. A

relevant theory explaining the role of testosterone in social behavior is the social challenge hypothesis (Wingfield et al., 1990). According to this theory, testosterone levels rise in anticipation to a socially challenging situation, thereby stimulating approach behavior and simultaneously reducing fear. I deemed this theory as particularly relevant for the current dissertation aiming to work towards an intervention to alleviate avoidance during exposure therapy in SAD.

Our literature review also indicated that individuals with SAD show (biased) action tendencies, that is, they show automatic avoidance toward socially threatening stimuli (for example angry but also happy faces). Interestingly, testosterone can reduce these tendencies. Based on these findings we presented a research agenda as a starting point for the translation of these well-established findings in the lab to a clinical application. Accordingly, in the following chapters I examined whether endogenous and exogenous testosterone challenges could improve exposure efficacy for individuals with SAD and whether the magnitude of social avoidance tendencies in SAD moderate efficacy of testosterone-enhanced exposure therapy.

The aim of **chapter 3** was to translate the social challenge hypothesis (Wingfield et al., 1990), which was described in chapter 2, to the clinical practice. Specifically, I tested whether pre-exposure endogenous testosterone levels were predictive of exposure outcome for individuals with SAD. A total of 73 participants (52 females) with SAD performed 3 speeches within one exposure session, as well as a fourth speech at a post-assessment one week later. We assessed pre-treatment testosterone levels before (baseline testosterone) and in response to a pre-exposure instruction (testosterone reactivity). Pre-treatment testosterone levels were not related to fear levels during exposure, but they were associated with reductions in social anxiety symptoms from pre-to post-exposure. Specifically, we found that the participants who displayed relatively high pre-exposure *testosterone reactivity* (e.g., rises in testosterone in anticipation of the exposure) showed larger reductions in social anxiety symptoms. This result suggest that the relative anticipatory reactivity of the HPG-axis contributes to the success of the exposure session that follows immediately after. This finding was specific for the relative reactivity (baseline-controlled testosterone increase to the pre-exposure instructions) and not the absolute reactivity (absolute increase in testosterone levels pre-exposure). This may imply that the relative reactivity of the HPG-axis may be particularly important for SAD-treatment efficacy, and perhaps more so than the absolute testosterone levels in the system during a pre-exposure instruction. In contrast to our hypotheses, low baseline testosterone was also related to larger symptom reduction. This finding might suggest there is more to gain in terms of testosterone rise in individuals with low baseline testosterone levels. Future research should replicate these findings in larger

groups to shed light on the relation between baseline and challenge-reactive testosterone levels. Taken together, these results can be interpreted as providing initial support for the social challenge hypothesis. It extends the hypothesis to a clinically relevant context and supports the relevance of further investigation into exposure-enhancing effects of testosterone in individuals with SAD. This first study on clinical effects of pre-exposure testosterone-reactivity provided promising findings, but it was correlational in nature and provided few insights into the effects of testosterone on in-session fear patterns. To ultimately test whether testosterone levels may be relevant for exposure-treatment in terms of in-session fear levels and symptom reduction, causal evidence is needed.

Therefore, following this correlational work, we conducted a pharmacological study using exogenous testosterone administration. More specifically, we conducted a randomized controlled proof-of-concept trial to test the potential of exogenous testosterone as a pharmacological enhancer for exposure for SAD in **chapter 4**. In this study 55 females meeting the criteria of SAD received two exposure therapy sessions. The first session was supplemented with either testosterone (0.50 mg) or placebo, followed by a second unenhanced exposure session. Transfer effects of testosterone administration on within-session fear and social anxiety symptoms were assessed during the second unenhanced exposure session and at 1 month follow-up. The participants having received testosterone showed a more reactive fear pattern with higher peaks and steeper reductions in fear levels in the unenhanced second session. This pattern appeared to be specific for females with high baseline (endogenous) testosterone, and it was present in the first (enhanced) as well as in the second (unenhanced) session. Participants with low baseline (endogenous) testosterone showed blunted peak fear levels in both sessions. In terms of social anxiety symptom levels we did not observe differences between the placebo and testosterone condition. The results of this study provide preliminary support for the hypothesis that testosterone may act on important fear mechanisms during exposure. However, further exploration of multi-session testosterone-enhanced exposure is needed to better understand the potential of testosterone as an enhancer for exposure treatment for SAD.

In the final empirical chapter, we explored the relationship between a core feature of SAD, namely social avoidance behavior, and testosterone-enhanced exposure. Following chapter 2, we first verified whether social avoidance tendencies characterize SAD by contrasting approach and avoidance tendencies for social and non-social stimuli in **chapter 5**. Critically, we tested whether baseline social avoidance tendencies moderate the efficacy of testosterone-enhanced exposure. For this (pilot) study we analyzed data from a social approach-avoidance task that was assessed pre- and post- treatment in the augmentation study described in chapter 4. Individuals with SAD showed stronger

avoidance for social versus non-social stimuli and this bias did not vary with testosterone enhanced exposure efficacy (no change from pre- to post exposure for both the testosterone and placebo condition). Stronger pre-treatment avoidance tendencies were associated with larger fear reduction under testosterone but not placebo. This effect was present only during the first enhanced session and not in the second non-enhanced session or at the level of symptom severity. These results may suggest that social avoidance tendencies are linked to stronger testosterone enhanced exposure efficacy. Further investigation using multiple enhancement sessions is needed to identify whether social avoidance tendencies can serve as behavioral marker for efficacy of a more extensive testosterone-enhanced exposure therapy.

Integration and overarching discussion of the chapters

Clinical translation of the challenge hypothesis

One specific aim of this dissertation was to test assumptions of the social challenge hypothesis in a clinical context, namely that strong HPG-axis reactivity prior to a social encounter (in this case a speech-exposure), would prepare the individual for successful social approach behavior during the exposure (Wingfield et al., 1990). Accordingly, individuals with high testosterone rises in response to a pre-exposure instruction showed stronger symptom reduction after exposure in chapter 3. Following the predictions of the social challenge hypothesis (Wingfield et al., 1990), we speculate that HPG-axis reactivity to the upcoming social encounter (in our case primed by the exposure instructions) may stimulate in-session approach behavior, that is stronger engagement in the exposure eventually resulting in improved learning. This interpretation was supported by the observation that high pre-exposure testosterone reactivity was related to larger reductions in social anxiety symptoms. However, it did not affect within-session fear levels. The latter may imply that endogenous testosterone reactivity is indeed important for successful exposure by promoting direct (in session) approach behavior rather than affecting fear itself. However, anxiety and approach-avoidance behavior influence each other and our design was not optimized to disentangle anxiolytic from behavioral social-approach effects. A combination of repeated objective and subjective indices for measuring approach behavior and anxiety are needed to shed light on this mechanistic question.

This pioneering endogenous testosterone reactivity study (chapter 3) demonstrated that reactivity of the HPG-axis could be a potential biomarker for the efficacy of exposure therapy of SAD. Additionally, these results raised the question whether increases in testosterone, even when administered exogenously, could aid exposure-efficacy.

This is what I tested in the subsequent testosterone administration study (chapter 4). The results from this testosterone administration study indicated that testosterone versus placebo administration resulted in a more reactive fear level pattern during exposure (i.e., steeper increase followed by a steeper decrease in fear levels). As shown in chapter 4, this effect was partly depended on endogenous baseline testosterone levels. This suggests that testosterone administration can enhance important mechanisms of exposure. In-session fear reactivity may be a relevant indicator of whether the individual is engaged in the exposure and faces the threat maximally, as this would lead to initial increase in fear, which should reduce over time. This is what we observed during exposure and what was amplified after testosterone administration over the whole group and in individuals with high baseline in particular (see below).

However, unlike the endogenous reactivity study in chapter 3, the pharmacological testosterone-activation in chapter 4 was only linked to in-session fear and did not induce significant reduction in symptom severity post-exposure. Of course, it is hard to compare those two studies for several reasons: The endogenous testosterone reactivity study (chapter 3) had a larger sample size, mixed sex, and differed in terms of the number and timing of the speeches. We cannot exclude that the samples of chapter 3 and 4 differed in terms of baseline testosterone, avoidance tendencies and individual differences in HPG-axis responsiveness (for example, due to individual differences in sensitivity of the androgen receptor (AR) (Geniole & Carré, 2018; Hogervorst et al., 2005). Unlike in chapter 3, it was not possible to assess endogenous testosterone reactivity in preparation to the exposure in the study described in chapter 4, since the exposure preparation took place after testosterone/placebo administration. Apart from the contextual differences between the endogenous and exogenous testosterone challenges, there are two additional factors that may explain these differential effects for social anxiety symptoms. First, we cannot exclude the possibility that even single-dose administration of testosterone may affect the HPG-axis feedback loop (inhibition of hypothalamus and pituitary by testosterone, see also chapter 2, page 28). Such effect has been demonstrated after repeated testosterone administration. For instance, Khodamoradi et al., (2021) describe in a review that repeated testosterone administration reduced endogenous testosterone production in testosterone replacement therapy for hypogonadal men. However, this review included different methods of testosterone administration that all required repeated doses, which brings me to the second factor: It remains the question whether the administration of a single dose testosterone is optimal to achieve effects that can be obtained by challenging the endogenous system, or whether one needs multiple session enhancement to achieve similar effects. I will address this point further in my suggestions for future research.

The role of baseline testosterone

When studying the effects of endogenous and pharmacological testosterone reactivity, it is relevant to take inter and intra-individual differences in baseline testosterone levels into account (see also Geniole & Carré, 2018 for a review). In chapter 3 low baseline testosterone seemed linked to stronger symptom reduction, an effect that we could not replicate in chapter 4. In chapter 4, we found a moderating role of baseline testosterone on testosterone enhancement. Specifically, low baseline testosterone was linked to blunted fear levels within both exposure sessions with no transfer to social anxiety symptoms. On the one hand one could argue that individuals with low baseline testosterone benefit from testosterone enhancement as they show lower fear during exposure. This could be interpreted as indicating that for individuals with relatively low endogenous baseline testosterone, administration of testosterone vs placebo works fear-reducing. However, individuals with high endogenous baseline testosterone levels showed an interesting fear level pattern as well. That is, they showed increased peaks and steeper reductions in fear levels at the end of the first session and interestingly this pattern showed transfer to the second, unenhanced exposure session (see Fig 4.2). The latter may suggest that an important mechanism of exposure is more manifested, since from theoretical models of exposure therapy (Emotional Process Theory, [EPT] and Inhibitory Learning Theory [ILT]) one can derive that higher initial fear levels during exposure are beneficial since they are important for good learning (e.g., fear activation in EPT and optimal expectancy violation in ILT), resulting in a better long term response to treatment (Craske et al., 2008; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014a; Foa & Kozak, 1986; Foa, Huppert, & Cahill, 2005). Although speculative at this point, one could imagine that follow-up research with repeated testosterone-enhanced exposure in individuals with high baseline testosterone is promising. This more reactive fear level pattern was present also when not taking baseline testosterone into account, in the second unenhanced session. Therefore, we conclude that future research using multiple enhancement sessions is needed to investigate whether the promising effects of testosterone administration on in-session fear levels transfer to symptom-level effects in the long run. The observation that individual differences in baseline testosterone modulate these effects are worth further exploration as they could provide a promising starting point towards individualized treatments that could be specified based on biomarkers such as endogenous testosterone levels.

Social avoidance tendencies: a behavioral marker of testosterone enhanced exposure?

In line with predictions from chapter 2: testosterone can alleviate avoidance in individuals with SAD (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016), we tested whether avoidance tendencies toward social stimuli moderate the efficacy of testosterone-enhanced exposure in chapter 5. The results of this study showed that individuals with SAD who showed relatively high pre-treatment social avoidance tendencies, showed larger fear decline during testosterone-enhanced exposure than individuals with relatively low avoidance. Unfortunately, our study was not optimized to test subtle changes in avoidance behavior during exposure. In order to do so we would need specific measures, for example body posture-, eye movement- or voice-trackers. Therefore, we can only speculate about the effects of testosterone on avoidance behaviors. However, the fact that testosterone facilitated in-session exposure-effects in high avoidant participants could be taken to suggest that it reduces avoidance and enables an individual with SAD to engage in exposure therapy. The moderating effect of baseline avoidance was present in the testosterone-enhanced group only and not in the placebo group. This suggests that avoidance tendencies could be a useful marker for personalizing testosterone-enhanced treatment for SAD.

It is also worth discussing some observations related to the avoidance marker itself. In regard to the specificity of avoidance tendencies observed in our study, we found faster avoidance (versus approach) of social stimuli (faces) versus non-social stimuli (checkerboards). It is interesting to discuss these effects in relation to the literature. Previous work has shown mixed effects regarding the types of facial expressions for which individuals with SAD show avoidance tendencies. Relative stronger avoidance versus approach has been typically found for angry faces and to a lesser extent for happy and neutral faces (Heuer et al., 2007; Kuckertz et al., 2017; Loijen et al., 2020; Roelofs, Putman, et al., 2010; Roelofs, van Peer, et al., 2009b). In our study we found an avoidance tendency for all facial expressions (see chapter 5). In line with others, we explain this by the fact that all types of faces could signal threat for socially anxious individuals (Heuer et al., 2007). It is important to address that there is no firm tradition yet regarding how reaction times in Approach Avoidance tasks should be analyzed and interpreted. Consequently, there are differences between the procedures in the above-mentioned studies. For example, push/pull trials have been analyzed separately (Kuckertz et al., 2017), as a differences score (Rinck et al., 2009) or with response direction as a within subjects factor (Heuer et al., 2007). Also, different control stimuli or conditions have been used, which could be an alternative explanation why mixed results are found. With regard to our own study, we followed recommendations of Rinck and Becker (2007) and used a relative differ-

ence between push/pull scores by comparing the social stimuli with a control stimulus (checkerboards) and thereby we limited the influence of factors such as participants seating position.

Our finding of relative avoidance of social vs non-social stimuli add to the rather robust findings that relative avoidance tendencies to threathening cues on AAT-tasks are found in social anxiety disorder (for review see Loijen et al., 2020). Moreover, they are in line with notions that automatic avoidance tendencies can play an important role in more complex instrumental types of avoidance (Arnaudova et al., 2017; Pittig, Treanor, LeBeau, & Craske, 2018). Specifically, during speech exposure facial expressions of the public are usually mixed (neutral, happy, angry) and therefore testing the value of avoidance tendencies toward different facial expressions as a behavioral marker of (testosterone) enhanced exposure is relevant.

Pharmacological enhancement for SAD: effects on symptom severity

It is interesting to discuss our findings on testosterone enhancement in light of other pharmacological exposure enhancement studies in SAD. Despite the promising effects of our endogenous testosterone-challenge on symptom severity in chapter 3. Single-session testosterone administration in chapter 4 only affected relevant fear-mechanisms during exposure, but it did not improve exposure efficacy more than placebo in terms of fear levels at the end of our second exposure session, neither in terms of social anxiety symptoms from baseline to follow-up. The fact that we did not find improvement at the level of symptom levels (our secondary outcome measure) contrasts our hypothesis and previous work with the pharmacological enhancer D-cycloserine (DCS). Rodebaugh and et al. (2013) found effects of a single dose of DCS on fear levels which showed transfer to social anxiety symptom levels in a sample of 34 individuals with SAD. Similarly, Hofmann et al., (2006) found reductions in symptom severity for SAD after 4 sessions DCS enhanced exposure for SAD. Another study found reductions in symptom severity after 5 sessions of DCS enhanced exposure, but not for tailored (administered after sessions marked by low fear) DCS administration (Smits et al., 2020). The scarce other pharmacological enhancement studies for SAD exposure showed mixed results regarding symptom levels. For Yohimbine (an α-2adrenoceptor antagonist) symptom level reduction was observed on self-report measures, but only for participants with low end fear (Smits, Rosenfield, Davis, et al., 2013) and for Oxytocin no effects were found for symptom severity (Guastella et al., 2009). An important factor that should be considered in this regard is that social anxiety disorder is a complex disorder in which an interplay of biological, environmental, cognitive, temperamental and cultural factors are involved (Spence & Rapee, 2016). As such, detecting reductions in a total score on a self-report measure of 'social anxiety

symptoms' may be rather challenging in a disorder that has such a heterogeneity in its manifestation. I argue that this is particularly challenging for enhancement studies, that try to show an effect that goes on and above the effect of a relatively effective treatment that exposure is (Carpenter et al., 2018). In addition, there may be differential expectations and hence placebo-effects associated with different pharmacological enhancers.

Another explanation for the mixed effects is that studies vary in the amount of enhanced sessions. Indeed for DCS-enhancement, it has been indicated that the number of enhanced sessions is related to its effects (Rosenfield et al., 2019; Smits et al., 2020). Does this mean that it is useless to conduct single-session administration studies? In the next section I will argue that as a proof of principle it may still be valuable to start with single-session enhancement, if alone to see if we can influence exposure-relevant mechanisms.

The clinical assay: a quick win, fast fail?

As mentioned above, I would like to reflect on the clinical assay design that we used in this dissertation to test the effects of testosterone as an enhancer for exposure treatment for SAD (see chapter 3, 4 and 5). This design originally described by Rodebaugh and colleagues (2013) is a brief experimental method consisting of two sessions. One session of exposure and one as an assessment session. It aims to test the potential of an intervention in a short and cost-effective manner prior to moving on to a large clinical trial. This method is referred to as a *quick win fast fail approach* as it tries to overcome the limitations of large clinical trials which are usually very expensive and time consuming, while they frequently fail to find clear effects. Although one session of exposure is insufficient as a full treatment, it does provide an elegant method for rapid assessment of the potential of different pharmacological enhancers like testosterone. Importantly, since it only consists of one dose of medication and two exposure sessions, it minimizes variance unrelated to the (pharmacological) enhancer. Rodebaugh and colleagues (2013) state that this method is an excellent complement to clinical trials. Within this dissertation we indeed experienced the advantages of this method as it allowed us to test the effects of testosterone on exposure in a restricted amount of time and we were successful in establishing reduction in social anxiety symptoms. Also, the burden to participants was as limited as possible.

However, this method raised many new questions in our studies since we did not find clear support for the effects of testosterone neither did we find a full blown "*fail.*" Namely we observed interesting effects on in-session fear patterns that may be mechanistically relevant for exposure in the long run. As described in the discussion of chapter 4 we opt for future studies with 'additional sessions plus testosterone' to fully understand

the results. Thereby we do need multiple clinical assays in a row or a full clinical design anyway. Critically, in my opinion the *fast fail* principle is rather difficult, because even in the case of a clear null-finding it is hard to decide if the intervention did actually fail, since alternative explanations are possible too (e.g., a false negative, or the drug does not work sufficient in only one session), raising a number of new questions. Taken together, the clinical assay is a valuable method to test single session enhancement, with some shortcomings. An alternative design for future studies could therefore be the single case experimental design (SCED), in which the efficacy of an intervention is tested with a small number of participants. In a SCED the intervention can be introduced in a randomized sequential order, it includes repeated measures where participants serve as their own controls and requires special data analyses. This method seems to be very promising in testing new interventions in situations where time and money is limited. Importantly, a SCED is suited to evaluate the process of an intervention (e.g., does the intervention indeed affect the target variable). Moreover, due to the repeated measures it provides researchers with the opportunity to also test interventions that are expected to show slower effects contrary to the clinical assay (see for a guide Krasny-Pacini & Evans, 2018 or a review; Smith, 2012). In sum, the SCED method seems to be a suitable method for future studies testing testosterone enhanced exposure for SAD.

Strengths, limitations & future studies

Strengths

This dissertation has several important strengths that I would like to emphasize.

First, as regards our intervention protocols: our proof-of-concept exposure intervention protocols (regardless of enhancements) were successful in establishing reduction in social anxiety symptoms, providing us with the opportunity to test our hypotheses, without applying a full treatment protocol (chapter 3 and 4). In addition, the protocol for administration of a single dose of testosterone was safe and tolerable for our participants. There were no adverse events during the clinical assay or augmentation related drop-out. Drop-out rates were low in both study samples of chapter 3 and 4. As a result, we were able to measure and analyze follow-up results for an almost complete clinical sample.

Second and more conceptually, the studies within this dissertation were built on a long fundamental research line evidencing the approach-facilitating properties of testosterone from rodents to patient studies. This groundwork, reviewed in chapter 2, provided a strong starting point for the first proof-of-concept clinical application of testosterone, within an actual treatment setting for individuals with SAD. Relatedly the aim to translate

fundamental insights to practice has value as it could improve treatment for individuals who do not profit sufficiently from current evidence based treatments (Lema, Gamo, Yang, & Ishizuka, 2018; Pratt & Hall, 2018),

Third, as regards the participants in our studies, we only included individuals who met the criteria for SAD, providing a good representation of individuals within an outpatient treatment center.

Fourth, related to the study designs, the correlational studies were complemented with a more causal pharmacological intervention using a well-established testosterone administration method (Tuiten et al., 2000) tested in a randomized triple blind design. Participants, research assistants who administered the drug and researchers who analyzed the effects were blind to the allocated treatment condition. Therefore, we minimized the influence of expectations from participants as well as the researchers on the results of the study.

Fifth, the studies were preregistered and the results of all studies were analyzed with linear mixed models, which take into account random effects, are better suited to handle missing data (Baayen, 2008) and critically, the chance of a type I error is much lower compared to repeated measures ANOVA, because they take into account non-independence of the repeated measures within the studies (Barr, Levy, Scheepers, & Tily, 2013; Matuschek, Kliegl, Vasishth, Baayen, & Bates, 2017).

Limitations

In addition to the strengths of this dissertation there are also several limitations that should be addressed.

First, our randomized proof-of-concept trial only included females due to the fact that the administration method we used has as yet only been applied in females (Tuiten et al., 2000). Therefore, we cannot say whether our findings will generalize to males. However, recent single dose administration studies in males do show that testosterone administration affects different social approach and avoidance behaviors (see Carré & Robinson, 2020; Geniole & Carré, 2018 for reviews). Based on these findings similar results for males may be expected. Relatedly, due to inclusion restrictions such as hormonal birth control types and pregnancy, it remains to be tested whether findings generalize to a broader group of individuals with SAD. This limitation is especially important since there are large intra and inter-individual differences in testosterone levels (e.g., depending on time of the day, menstrual cycle and age) and these differences should be taken into account in future studies by including a larger and more varied sample to improve the ecological validity of the current findings.

Second, there are limitations regarding the reliability of baseline testosterone assessments. Endogenous testosterone levels fluctuate during the day and menstrual cycle and are affected by for example levels of activity, contraceptive use and sex. Although we did try to control for these variables (e.g., assessing all participants at the same time of the day and within their menstrual cycle), it is impossible to control for all the variance within the natural context of an exposure session. Some have even argued that the use of salivary testosterone samples is not (yet) justified (Wood, 2009). In previous work, our lab took an average of six samples in the morning and evening to achieve an estimate of baseline testosterone (Giltay et al., 2012). This was not possible in the studies in this dissertation, due to practical and financial constraints. The reliability of our baseline testosterone estimates (based on two samples) may therefore be flawed. On the other hand, more recent work sheds a slightly more positive picture indicating that saliva samples of testosterone form a moderately stable measure over time (good test-retest reliability), and contraceptive use did not affect testosterone levels (Dabbs, 1990; Liening, Stanton, Saini, & Schultheiss, 2010; Sellers, Mehl, & Josephs, 2007). Therefore, salivary testosterone can be regarded as a suitable marker for individual differences, but multiple samples (at least two) are recommended. More research on its reliability and the relationship between testosterone and exposure outcomes is encouraged.

Third, the proposed mechanism of action described in this dissertation stated that testosterone reactivity reduces subtle avoidance and safety behaviors in individuals with SAD. In other words, it stimulates within-session approach behavior and engagement, resulting in better learning during exposure as assessed by retention of learning in the following session. In our design we used fear levels as a marker for learning and retention effects and we assessed automatic avoidance tendencies prior and post exposure. However, we did not directly assess differences in within session approach/avoidance behaviors between the two treatment groups. Therefore, it remains unclear whether testosterone indeed affects within session approach behavior. Due to time constrains, we did not analyze the videotaped speeches of the participants as a measure of avoidance-approach behavior. It would be worthwhile to analyze these in the future. Although, these video ratings could be a valuable addition to the current results, they are not the answer to all our remaining questions. For example, it is questionable if video ratings are a reliable measure of avoidance and safety behavior since safety behaviors are a complex construct. For example, they frequently take a cognitive form (e.g., rehearsing text in your mind over and over) and therefore cannot be observed (Cuming et al., 2009). Moreover, the individual may not even be aware that their cognitive strategies are considered a safety behavior. As such, using videos or self-report questionnaires may not be a reliable measure of subtle avoidance and safety behavior during exposure.

This discussion has parallels with the upcoming line of literature discussing the often overlooked role of avoidance in in fear-conditioning studies that provide an experimental model for the maintenance and treatment of anxiety disorders (Arnaudova et al., 2017; Krypotos, Beckers, Kindt, & Wagenmakers, 2015; LeDoux, Moscarello, Sears, & Campese, 2017; Pittig et al., 2018). Important within this line of research is the notion that avoidance is a complex construct fueled by several processes. As such, there is no one-size-fits- all solution in deciding which measure of avoidance is most suitable, and I would advise different type of measures (e.g., video ratings, eye-tracking, self-report questionnaires).

Fourth, power analyses indicated that our number of participants was sufficient to detect effects of testosterone enhancement on changes in fear levels during exposure therapy and symptom severity, but it was not optimized for individual differences analyses regarding baseline testosterone and social avoidance.

Future studies

In chapter 2 we presented an agenda for future research that was too ambitious to cover within the scope of the present dissertation. However, we made a start with a few points related to SAD and based on those, I will complement the agenda with additional suggestions for future research.

First, there is need to replicate the current findings as well as to extend them, ideally using a more extended protocol including multiple sessions of exposure treatment rather than two sessions, combined with additional doses of testosterone. An example could be the previously mentioned SCED design with a full treatment protocol (12 sessions) in which some sessions would be supplemented with testosterone or placebo. The use of an outcome measure close to the hypothesized mechanism of action (enhanced approach behavior), for example eye-tracking, could be used to gain more insight if testosterone indeed affects within session approach behavior. Repeated administration throughout the complete treatment is not recommended as it could lead to minor hair growth and acne (Islam, Bell, Green, Page, & Davis, 2019). Moreover, individuals may misattribute their exposure success to the use of testosterone, and thereby it could hamper treatment effects rather than boosting it. A more extended protocol is needed to better understand the effects of exogenous testosterone on fear activation and reduction within and across multiple exposure sessions. The current studies did not include a long follow-up period and therefore it would be interesting to follow participants for a longer period (for example six months) to gain insight in the long-term effects of testosterone enhancement on symptom reduction.

Next, in all of the empirical chapters we used subjective fear levels as our main outcome measure. Although this is in line with many other proof-of-concept studies (Davis

et al., 2017; Rodebaugh et al., 2013; Van Der Flier et al., 2019) and the earlier discussed EPT (Foa & Kozak, 1986; Foa et al., 2005), the more recent theoretical model of exposure therapy: ILT (see also introduction) suggests that inhibitory learning and maximizing expectancy violation are crucial for exposure success. Fear activation may be useful for expectancy violation, but according to ILT it is not a necessity (Craske et al., 2008, 2014a, 2022). As such, a suggestion for a future study would be to use an alternative outcome for exposure success, for example an index of expectancy violation. Currently, I would not argue that an index of expectancy violation would be a better outcome compared to fear reduction, since studies testing the value of expectancy violation as a marker for exposure success find mixed results (Buchholz et al., 2022; de Kleine, Hendriks, Becker, Broekman, & van Minnen, 2017; Elsner, Jacobi, Kischkel, Schulze, & Reuter, 2022; Guzick, Reid, Balkhi, Geffken, & McNamara, 2020; Pittig et al., 2022; Scheveneels, Boddez, Van Daele, & Hermans, 2019), and there is not yet consensus on how to assess expectancy violation.

Furthermore, as described in the introduction and chapter 2, the HPG-axis and its end-product testosterone do not work in isolation. For example, there are complex interactions between the HPG and HPA-axis. Although the HPG and HPA axis work in conjunction during high acute stress-exposure, the HPG-axis can also work in antagonism with the hypothalamus-pituitary-adrenal (HPA) axis in such a way that cortisol, the end product of the HPA-axis, may disrupt the production and inhibits actions of testosterone which in turn inhibits the stress induced activation of the HPA-axis (Viau, 2002). Moreover, testosterone also interacts with neuropeptides such as oxytocin. Therefore, it is worthwhile to take into account the assessment of other hormones, peptides and -if possible- neurotransmitters in future studies to further understand the interplay between different hormones in social anxiety disorder and its treatment. For example, there is some support for a dual process hypothesis that states that the HPA and HPG-axis interact to regulate several behavioral domains including dominance and social status (Mehta & Josephs, 2010; Mehta & Prasad, 2015). Specifically, the dual process hypothesis states that testosterone is positively related to dominance and social status related behaviors, but only in individuals with low cortisol levels, which may be of relevance in an exposure context too.

To conclude, the current dissertation is part of a novel line of research in which different pharmacological enhancers are being tested to improve exposure efficacy for SAD as well as other (anxiety) disorders. Different enhancers appeared to be promising candidates such as DCS (Hofmann et al., 2019; Rodebaugh et al., 2013; Rosenfield et al., 2019), but replication is often difficult and therefore these augmentation strategies are not (yet) considered effective for SAD (De Cagna et al., 2019; Pelissolo, Abou Kassm, &

Delhay, 2019; Steenen et al., 2016). Moreover, research groups seem to struggle with similar questions regarding doses and timing of the drug as within this dissertation (Rosenfield et al., 2019). As such, it may be of interest to strive for collaborations between research groups that examine pharmacological enhancers. By doing so, research groups could use larger sample sizes, which are especially relevant to study individual differences and relevant moderators (see also Geniole & Carré, 2018, for a review regarding testosterone administration), aiding more personalized enhancement strategies in the future.

Conclusion

This dissertation aimed to test the potential of testosterone as an enhancer for exposure treatment for SAD. Endogenous and exogenous testosterone challenges were therefore combined with short exposure therapy protocols in individuals with SAD, resulting in three main conclusions: First, relatively high endogenous pre-exposure testosterone-reactivity was linked to better exposure-efficacy in terms of symptom-reduction. Second, exogenous testosterone administration did not affect exposure-efficacy at the level of symptom change but amplified exposure-relevant mechanisms in terms of in-session fear patterns. Third, those individuals with SAD who showed relatively high avoidance tendencies at pre-treatment, benefited more from the exogenous testosterone-enhanced exposure compared to their low avoidance counterparts in terms of stronger reductions in fear levels. Taken together, the results provide a promising starting point for the future investigation of the longer-term effects of repeated testosterone-enhancements in SAD.

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Research data management

All the studies within this dissertation were part of an external PhD studentship of the mental healthcare institution Pro Persona and the Radboud University. Research Data Management was conducted according to the data management policy of the Radboud University (http://www.ru.nl/rdm/) and the Behavioural Science Institute (BSI) and in accordance with the General Data protection regulations (GDPR: https://www.ru.nl/privacy/english/protection-personal-data/general-data-protection-regulation-gdpr/). The paragraphs below specify in detail how this was achieved, following the FAIR principles (Findable, Accessible, Interoperable, Reusable).

Ethics

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The parent trial of the study described in chapter 3 was registered at clinicaltrials.gov/c2/show/NCT02482805. The local ethical committee (Commissie Mensgebonden Onderzoek region Arnhem-Nijmegen) approved the studies described in chapter 4 and 5 and the trial was registered in the Dutch Trial Register (Trial 6238) and at EudraCT (2014-004475-23). This work was supported by a VICI grant (#453-12-001) from the Netherlands Organization for Scientific Research (NWO) and a consolidator grant from the European Research Council (ERC_CoG-2017_772337) awarded to K. Roelofs.

Findable, Accessible

The Netherlands Code of Conduct for Research Integrity requires researchers to make data as open as possible after publication. For an overview of the accessibility of the data per chapter see table 6.1. The data of chapter 3 is owned by the Department of Psychology and Institute for Mental Health Research, The University of Texas at Austin, United States and was shared with the Radboud University (collaborators). In a Memorandum of Understanding it was stated that the collaborators would not share the data with others and therefore the data of this project is not shared in an online accessible Repository. A request for accessing the data of this project could be sent to Prof. Jasper Smits (University of Texas at Austin). The anonymized data and analyses scripts of chapter 4 are available upon request at the Radboud Data Repository (https://doi.org/10.34973/z5pf-p041). We preregistered the study presented in chapter 5 at Open Science Framework (OSF): https://osf.io/3cxsv. This article is still under review. The data are currently shared

with the reviewers in a Data Sharing Collection (DSC: https://doi.org/10.34973/ebv1-4h69) and will be made publicly available, once the article has been published.

For chapters 3, 4 and 5 the final research data and data analyses scripts have also been stored on the network drive of the Radboud University. Informed consent was obtained on paper. The forms are archived in the archive of Pro Persona for 10 years after termination of the studies.

Table 6.1. *Overview of the accessibility of the data per chapter*

Chapter	Collection	DOI or contact
2	NA (no research data was collected)	NA
3	Data Acquisition collection	Prof. Jasper Smits
4	Data sharing collection (Radboud Repository) Raw data is stored on a secured drive at Pro Persona (only accessible for members involved in the project)	https://doi.org/10.34973/z5pf-p041
5	Data sharing collection (Radboud Repository) Raw data is stored on a secured file at Pro Persona (only accessible for members involved in the project)	https://doi.org/10.34973/ebv1-4h69

Interoperable, Reusable

The raw data of the randomized controlled trial of chapter 4 and 5 was stored on the network drive of Pro Persona. These data were only accessible to members involved in the project. For chapter 4 and 5 we used long-lived file formats (e.g. .sav, .csv) to ensure that data remains usable in the future. We added readme files explaining the structure and content of the shared documents.

Privacy

The privacy of the participants in this thesis has been warranted using random individual subject codes. A pseudonymization key linked this random code with the personal data. This pseudonymization key was stored on a network drive that was only accessible to members of the project who needed access to it because of their role within the project. For chapter 3 the collaborators did not have access to this key. The pseudonymization key for chapter 4 and 5 was stored separately from the research data. Data in chapters

4 and 5 are not identifiable since we used new random individual subject codes that are not linked to any personal data.

Nederlandse Samenvatting

Voorwoord

De sociale angststoornis (SAS) is een van de meest voorkomende angststoornissen en wordt gekenmerkt door angst en vermijding in sociale situaties. Mensen met SAS hebben tevens verlaagde testosteron niveaus. Experimenteel onderzoek heeft laten zien dat testosteron toediening sociale vermijding kan doorbreken. De bewezen effectieve behandeling voor SAS bestaat onder andere uit exposure therapie. Echter, sterke vermijding vermindert de kans van slagen van deze therapie. Daarom onderzochten we of toediening van testosteron de effectiviteit van exposure therapie kan verbeteren. Na een theoretische verkenning onderzochten we eerst de effecten van endogene (lichaamseigen) testosteron verhoging voorafgaand aan de behandeling van SAS. Daarna onderzochten we de invloed van testosteron toediening in SAS. Tenslotte onderzochten we of testosteron toediening het beste werkt in individuen met SAS die sterk vermijdend zijn. We vonden aanwijzingen dat testosteron invloed heeft op subjectieve angstniveaus tijdens exposure sessies en dat met name mensen met hoge vermijding baat lijken te hebben bij toediening met testosteron. Echter we vonden geen effecten van testosteron toediening op het niveau van sociale angstklachten. Deze eerste bevindingen zijn veelbelovend en geven aanleiding om vervolgonderzoek te doen met herhaalde testosterontoediening om de lange termijneffecten van testosteron op exposure therapie te onderzoeken.

Inleiding

Sociale angststoornis en vermijding

De sociale angststoornis (SAS) is een veel voorkomende angststoornis onder volwassenen en jongeren. Deze stoornis belemmert mensen die hier last van hebben sterk in hun dagelijks leven. Zo lukt het hen bijvoorbeeld niet meer goed om een opleiding te volgen of sociale relaties te onderhouden. SAS wordt gekenmerkt door een intense angst voor sociale situaties waarbij iemand afgewezen kan worden zoals feestjes of het geven van een presentatie. Deze sociale situaties worden meestal vermeden of met veel angst doorstaan. Theoretische modellen over het ontstaan en de instandhouding van SAS stellen dat vermijdings- en veiligheidsgedragingen een belangrijke rol spelen bij de instandhouding van SAS. Vermijdingsgedrag is een strategie die erop gericht is dat een individu

niet blootgesteld wordt aan de sociale situatie (bijvoorbeeld niet naar een feestje gaan). Veiligheidsgedrag is erop gericht om in de sociale situatie de angst zo laag mogelijk te houden (bijvoorbeeld oogcontact vermijden). Beide gedragingen zorgen ervoor dat angst op de korte termijn zakt, maar op de lange termijn houden ze de angst juist in stand. Op deze manier kan iemand met SAS namelijk niet leren dat zijn/haar angstige verwachting over een sociale situatie (bijvoorbeeld afgewezen of uitgelachen worden) niet uit hoeft te komen. Immers, als je nooit naar een feestje gaat kun je ook niet ervaren dat mensen je daar niet zullen uitlachen of afwijzen. Naast deze duidelijke, openlijke vormen van vermijding hebben mensen met SAS ook meer subtiele, impliciete vormen van vermijding. Een voorbeeld hiervan is dat zij in het algemeen moeite hebben om bij experimentele computertaken in het lab plaatjes van boze gezichten, die zij als dreigend ervaren, door middel van een joystick naar zich toe te trekken (te benaderen). Deze meer impliciete vormen van vermijding liggen mogelijk ten grondslag aan meer openlijke vormen van vermijding en het wordt verondersteld dat deze vormen van vermijding effectieve therapie in de weg staan. Het doorbreken van vermijdings- en veiligheidsgedrag is dan ook een belangrijke focus in therapie voor SAS.

Cognitieve gedragstherapie

Cognitieve gedragstherapie (CGT) is de meest effectieve behandeling voor SAS, maar ondanks dat CGT effectief is, knapt slechts 45-55% van de mensen met SAS op van deze behandeling. Het doel van CGT is om disfunctionele overtuigingen ten aanzien van sociale dreiging (bijvoorbeeld "ik zal afgewezen worden in een sociale situatie") te beïnvloeden door middel van cognitieve (bijvoorbeeld gedachtenschema's) en gedragsmatige technieken (bijvoorbeeld exposure). Exposure wordt gezien als de meest cruciale interventie in CGT. Bij exposure wordt iemand met SAS herhaaldelijk blootgesteld aan sociale situaties zonder dat de verwachte negatieve uitkomst (sociale afwijzing) optreedt. Het proces van extinctie, waar in het lab veel onderzoek naar is gedaan, helpt het werkingsmechanisme van exposure beter te begrijpen. In dit proces wordt een stimulus (CS) die voorheen gekoppeld was aan een aversieve uitkomst (US) herhaaldelijk aangeboden zonder gevolgd te worden door deze aversieve uitkomst (geen US). Dit zorgt ervoor dat de angst afneemt. Bijvoorbeeld wanneer iemand met SAS in exposuretherapie herhaaldelijk wordt blootgesteld aan het geven van een presentatie aan een klein publiek (CS) zonder dat hij/zij wordt uitgelachen (geen US), zal diegene in de toekomst in mindere mate verwachten dat het geven van een presentatie leidt tot sociale afwijzing. Vermijdings- en veiligheidsgedragingen worden in exposuretherapie ontmoedigd, omdat ze het effect van de therapie negatief beïnvloeden.

Testosteron

Testosteron is een hormoon dat in het lichaam geproduceerd wordt door de Hypothalamic–Pituitary–Gonadal (HPG)-as. Het hormoon is een belangrijke regulator van sociaal motiverend gedrag, inclusief toenaderings- en vermijdingsgedrag. Testosteron kan gemeten worden in het lijf (endogene testosteron genoemd), maar ook toegediend worden (exogene testosteron genoemd). Endogene testosteronwaarden zijn gemiddeld 4-5 keer hoger bij mannen dan bij vrouwen. Vanuit de literatuur is het bekend dat endogene testosteronwaarden in het lijf stijgen voorafgaand aan een sociale situatie. De *Social challenge* hypothese stelt dat deze reactiviteit van testosteron ter voorbereiding op een sociaal uitdagende situatie (bijvoorbeeld het geven van een presentatie) leidt tot toenaderingsgedrag en de angst verlaagt. Een stijging in testosteron voorafgaand aan het geven van een presentatie stimuleert iemand om de presentatie volledig aan te gaan en minder of geen veiligheidsgedrag te vertonen. In lijn met deze hypothese is in studies regelmatig gevonden dat hoge niveaus van testosteron samenhangen met toenaderingsgedrag. Daarnaast komt uit een andere studie naar voren dat vrouwen met SAS lagere testosteronwaarden hebben vergeleken met vrouwen zonder SAS.

Er zijn ook studies gedaan waarbij mensen, met en zonder sociale angststoornis, eenmalig een lage dosering testosteron of placebo toegediend kregen voorafgaand aan een computertaak. In deze taak moesten deelnemers boze, neutrale en blije gezichten naar zich toe trekken of van zich afduwen met behulp van een joystick. Hieruit bleek dat het toedienen van testosteron leidt tot verminderde vermijding en juist toenadering van gezichten die als dreigend ervaren worden (bijvoorbeeld de boze gezichten). Op basis van deze bevindingen die laten zien dat het toedienen van testosteron vermijding ten aanzien van sociale dreiging kan doorbreken, zou testosteron een potentiële kandidaat kunnen zijn om de effecten van exposuretherapie te verbeteren door toenadering binnen een exposuresessie te versterken. Dit is dan ook wat we in dit proefschrift hebben onderzocht.

Het beoogde werkingsmechanisme van testosteron versterkte exposure

Zoals hierboven beschreven is een van de werkingsmechanismen van exposuretherapie dat mensen met SAS leren dat blootstelling aan sociale situaties niet leidt tot de gevreesde uitkomst. Om dit goed te leren moet een individu exposuretherapie volledig aangaan in plaats van (subtiel) bepaalde aspecten van sociale interacties te vermijden (bijvoorbeeld het maken van oogcontact). De *Social challenge* hypothese geeft aan dat reactiviteit van endogeen testosteron leidt tot sociale toenadering en uit eerder onderzoek is gebleken dat toediening van testosteron leidt tot sociale toenadering bij mensen met SAS. Samenvattend verwachtten wij voorafgaand aan de studies in dit proefschrift daarom het volgende: reactiviteit van endogene testosteron en ook de toediening van

testosteron leidt tot meer toenaderingsgedrag tijdens een exposuresessie en daarmee zou het de effecten van exposure kunnen verbeteren.

Methoden en bevindingen

Het doel van dit proefschrift was dan ook om te onderzoeken of het toedienen van testosteron het effect van exposuretherapie kan verbeteren. In **hoofdstuk 2** gaven we een theoretisch overzicht van de hormonen testosteron en cortisol en hun relatie met psychopathologie, in het bijzonder SAS. In deze literatuursamenvatting vonden we onderbouwing voor een disbalans van deze hormonen bij mensen die lijden aan SAS. Ook lieten we in deze literatuursamenvatting zien dat de *Social challenge* hypothese wordt ondersteund door veel empirische (experimentele) studies. Op basis van de beschreven bevindingen presenteerden we een onderzoeksagenda gericht op de vertaling van deze fundamentele bevindingen naar de klinische praktijk.

In **hoofdstuk 3** beoogden we de *Social challenge hypothese* te vertalen naar de klinische praktijk. Hierbij onderzochten we in het bijzonder of reactiviteit van endogene testosteron samenhing met de effecten van een exposuresessie voor sociale angst. In totaal namen er 73 deelnemers met SAS deel aan deze studie. Voorafgaand aan een exposuresessie waarin deelnemers driemaal dezelfde presentatie moesten geven, werden basale testosteronwaarden en testosteron reactiviteit (in reactie op de exposure instructie) gemeten. We vonden dat hoge testosteron reactiviteit en lage basale testosteronwaarden gerelateerd waren aan afname in sociale angstklachten. Deze studie bood daarmee de eerste steun voor de *Social challenge hypothese* in de klinische praktijk. Bovendien liet deze studie zien dat het relevant was om verder onderzoek te doen naar de mogelijkheden van testosteron om exposuretherapie te verbeteren.

De studie in hoofdstuk 3 liet een samenhang zien tussen testosteron en effecten van exposuretherapie. Om meer inzicht te krijgen in de potentie van testosteron als een therapieverbeteraar was het nodig om causaal bewijs te verzamelen. Daarom onderzochten we in de studie in **hoofdstuk 4** of het toedienen van een enkele dosering testosteron voorafgaand aan een exposuresessie het effect van de exposure kon verbeteren. In deze studie ondergingen 55 vrouwen met SAS twee exposure sessies. We onderzochten in deze studie alleen vrouwen, omdat de methode die we gebruikten voor het toedienen van testosteron goed gevalideerd is bij vrouwen maar (nog) niet bij mannen. Voorafgaand aan de eerste sessie kregen zij een dosering testosteron of een placebo toegediend. Tijdens de exposure werden subjectieve angstniveaus gemeten. Voorafgaand, direct na de twee sessies en een maand na de tweede exposuresessie werden daarnaast sociale

angstklachten gemeten. In deze studie vonden we dat de groep die testosteron kreeg een reactiever patroon van angstniveaus liet zien met hogere pieken gevolgd door sterkere daling van angst in de tweede sessie. Dit patroon bleek voornamelijk zichtbaar te zijn bij vrouwen met hoge endogene testosteron waarden. Vrouwen met lagere endogene testosteron waarden lieten een afgevlakt patroon van angstniveaus zien. Ten aanzien van de sociale angstklachten vonden we geen verschillen tussen de testosteron- en placebogroep. Deze studie gaf daarmee voorzichtige steun voor de hypothese dat testosteron inwerkt op belangrijke processen tijdens exposuretherapie voor SAS. Angstniveaus tijdens exposure geven namelijk een indicatie van het (leer)proces tijdens de sessie. Maar, meer onderzoek met meerdere exposuresessies en testosteron toediening zijn nodig om de effecten van testosteron op exposure beter te begrijpen.

In de laatste empirische studie van dit proefschrift (**hoofdstuk 5**) onderzochten we de relatie tussen sociale vermijding en het effect van toediening van testosteron voorafgaand aan exposure. Sociale vermijding is immers een belangrijk kenmerk van SAS. In dezelfde groep deelnemers als de studie uit hoofdstuk 4 onderzochten we de aanwezigheid van impliciete vermijdingstendenties ten opzichte van sociale stimuli (boze, blije en neutrale gezichten) bij vrouwen met SAS. Deze tendenties werden voor en na de exposure gemeten door middel van een joysticktaak. In deze taak moesten deelnemers sociale stimuli (plaatjes van boze, blije en neutrale gezichten), die op een computerscherm gepresenteerd werden, met behulp van de joystick naar zich toe trekken of van zich afduwen. Wanneer deelnemers een plaatje relatief sneller van zich afduwen dan naar zich toe trekken spreken we van een vermijdingstendentie. Vervolgens toetsten we of deze vermijdingstendenties voorafgaand aan exposure het effect van de exposure met testosteron modereerde. We vonden dat vrouwen met SAS een vermijdingstendentie lieten zien ten opzichte van sociale stimuli. Deze tendenties veranderden niet tussen de voor- en nameting. Daarnaast vonden we dat vermijdingstendenties voorafgaand aan de exposure gerelateerd waren aan sterkere afname van angst tijdens de eerste exposuresessie wanneer deelnemers testosteron hadden gekregen. Dit effect was afwezig voor de tweede sessie en ook voor de sociale angstklachten. Deze resultaten suggereren dat vermijdingstendenties gerelateerd zijn aan sterkere effecten van met testosteron toegevoegde exposure. Echter, het is belangrijk om de huidige resultaten te repliceren, zodat we meer zicht krijgen of vermijdingstendenties een voorspeller kunnen zijn voor de effecten van testosteron toediening bij exposure.

Discussie

Het effect van testosteron op exposuretherapie

De bevindingen van dit proefschrift bieden de eerste voorzichtige ondersteuning voor de relatie tussen testosteron en exposure-effecten binnen de klinische praktijk. In hoofdstuk 3 vonden we dat de reactiviteit van endogene testosteron samenhing met de afname van sociale angstklachten na een exposuresessie. Bij de studie in hoofdstuk 4 vonden we daarentegen dat exogene testosteron wel van invloed was op de subjectieve angst van deelnemers binnen een sessie, maar niet op de sociale angstklachten. Deze studies zijn moeilijk direct met elkaar te vergelijken omdat er verschillen waren in deelnemers (bijvoorbeeld sekse) en het onderzoeksdesign (bijvoorbeeld aantal presentaties). Het kan daarom zo zijn dat de verschillende uitkomsten van de studies het best begrepen kunnen worden door de verschillen in onderzoeksopzet. Ook blijft het de vraag of een enkele dosering testosteron (zoals in hoofdstuk 4 beschreven) dezelfde effecten teweegbrengt als wanneer het endogene HPG-systeem geactiveerd wordt. Mogelijk zijn er meer exposuresessies met toediening van testosteron nodig om dit systeem te beïnvloeden. Vervolgonderzoek met meerdere exposuresessies gecombineerd met toediening van testosteron zijn aan te raden om het effect van testosteron op exposure beter te begrijpen en te toetsen of toediening van testosteron op de langere termijn ook invloed kan uitoefenen op de afname van sociale angstklachten.

Individuele verschillen

Een interessante bevinding uit dit proefschrift was de samenhang tussen endogeen testosteron en exogeen testosteron. Wij vonden namelijk dat de effecten in de exogene testosteronstudie (hoofdstuk 4) gedeeltelijk afhankelijk waren van endogene testosteronwaarden, die verschillend zijn per individu. Deelnemers met lage endogene testosteronwaarden lieten een afgevlakt patroon van angstniveaus zien. Daarentegen lieten deelnemers met hoge endogene testosteron een meer reactief angstpatroon zien met hogere pieken en een steilere afname tegen het eind van de sessie, zowel voor de eerste als de tweede exposuresessie. Het lijkt daarmee van belang om individuele verschillen in endogene testosteron in acht te nemen bij vervolgonderzoek naar de effecten van toediening van testosteron voorafgaand aan exposure. Mogelijk geeft dit in de toekomst meer inzicht in voor wie toediening van testosteron werkt en kunnen behandelingen meer gespecificeerd worden per individu.

Naast verschillen in endogene testosteron zien we in dit proefschrift ook aanwijzingen dat vermijdingstendenties voorafgaand aan exposuretherapie relevant kunnen zijn voor de effecten van met testosteron toegevoegde exposure: mensen met sterke-

re vermijdingstendenties ten opzichte van sociale stimuli lieten een steilere afname in angst zien tijdens de eerste exposuresessie. Mogelijk betekent dit dat testosteron van invloed is op vermijdingsgedrag en dat het deelnemers stimuleert om hun vermijding te doorbreken. Ondanks dat dit een interessante bevinding is kunnen we op basis van dit proefschrift geen uitspraken doen over subtiele vermijding binnen een sessie (zoals het vermijden van oogcontact). Daarom hebben we in de toekomst andere instrumenten nodig om vermijding te meten en deze hypothese te toetsen.

Testosteron en angstniveaus binnen een exposuresessie

Zoals genoemd vonden we in dit proefschrift verschillende patronen van angst binnen een exposuresessie tussen de testosteron- en placebogroep, deels afhankelijk van endogene testosteron. Zo zagen we een afgevlakt patroon van angst bij vrouwen met lage endogene testosteron, wat zou kunnen betekenen dat testosteron bij deze groep zorgt voor een verlaging van angst. Het meer reactieve patroon bij vrouwen met hoge endogene testosteronwaarden lijkt in eerste opzicht misschien negatief, echter dit patroon is ook interessant omdat we van theorieën over het werkingsmechanisme van exposure kunnen afleiden dat hoge angstniveaus tijdens een exposuresessie belangrijk zijn om een goed exposure-effect te genereren. Het lijkt daarmee de moeite waard om verder te onderzoeken of dit patroon ook op de langere termijn positieve effecten kan hebben.

Sterke punten, beperkingen en toekomstige studies

Dit proefschrift heeft meerdere sterkere kanten. Zo vonden we dat het twee-sessie protocol effectief was in het verminderen van sociale angstklachten waardoor we in staat waren om onze hypothesen te toetsen. Ook vonden we dat de toediening van testosteron veilig was voor deelnemers en dat er vrijwel geen enkele deelnemer stopte met deelname aan deze studie. Daarnaast was het design van deze studie sterk. We gebruikten een triple blind design voor de toedieningsstudie waarbij deelnemers, onderzoekassistenten en de onderzoekers die de analyses uitvoerden blind waren voor de toegewezen conditie. Hierdoor hebben we de invloed van verwachtingen op de onderzoeksresultaten geminimaliseerd. Tot slot waren de studies vooraf geregistreerd en werden de resultaten geanalyseerd met Linear Mixed Models waardoor we de kans op het vinden van een vals-positieve bevinding (een zogenaamde type 1 fout) verkleind hebben.
Naast de sterke kanten zijn er ook een aantal beperkingen aan dit proefschrift. Zo onderzochten we in de studies beschreven in hoofdstuk 4 en 5 enkel vrouwen. Daarom kunnen we niet zeggen of onze resultaten ook te generaliseren zijn naar mannen. Echter, andere onderzoeken waarbij testosteron wordt toegediend laten wel zien dat toediening van testosteron bij mannen ook leidt tot meer toenaderingsgedrag. Een andere beperking

is dat het beoogde werkingsmechanisme van testosteron is dat dit toenaderingsgedrag binnen een exposuresessie stimuleert waardoor deelnemers beter kunnen leren en de exposure meer effect heeft. In onze studies hebben we subjectieve angstniveaus gemeten tijdens de exposuresessies als uitkomstmaat voor het leereffect, maar we hebben geen directe maat gebruikt om toenaderingsgedrag tijdens de exposure sessie te meten. Daarom blijft het nog onduidelijk of testosteron daadwerkelijk toenaderingsgedrag heeft gestimuleerd tijdens de exposure. In de toekomst zou het interessant zijn om dit te onderzoeken, bijvoorbeeld door middel van een eye-tracking instrument.

Op basis van de bevindingen in dit proefschrift wil ik nog een aantal andere aanbevelingen doen voor toekomstig onderzoek. De belangrijkste aanbeveling is het uitvoeren van een onderzoek met een uitgebreider exposureprotocol (bijvoorbeeld 12 sessies exposure) waarbij er meerdere keren testosteron toegevoegd wordt. Daarbij is het van belang te noemen dat toediening van testosteron tijdens elke exposuresessie niet wenselijk is omdat dit mogelijk kan leiden tot bijwerkingen (acné of haargroei). Daarnaast kan het tot gevolg hebben dat deelnemers hun exposuresucces onterecht toeschrijven aan het gebruik van testosteron in plaats van aan hun veranderde gedrag (het toenaderen van sociale situaties) wat juist de effecten van therapie op de lange termijn negatief zou kunnen beïnvloeden. Een andere aanbeveling is om naast testosteron ook andere hormonen te onderzoeken, omdat de HPG-as niet in isolatie werkt, maar in samenhang met andere systemen in het lichaam. Zo interacteert testosteron ook met het stresshormoon cortisol. Het is van toegevoegde waarde om de interactie tussen deze hormonen en de relatie met exposuretherapie verder te onderzoeken.

Conclusie

Het doel van dit proefschrift was het onderzoeken van testosteron als een potentiële verbeteraar van exposuretherapie voor SAS. Om dit doel te bereiken werden endogene en exogene testosteronstijgingen bewerkstelligd voorafgaand aan korte exposure therapiesessies voor mensen met SAS. Uit de studies binnen dit proefschrift kunnen drie belangrijke conclusies worden getrokken: ten eerste, relatief hoge endogene testosteron reactiviteit voorafgaand aan exposure hing samen met betere exposure-effecten in termen van afname van sociale angstklachten. Ten tweede: toediening van testosteron had geen invloed op het effect van exposuretherapie in termen van afname van sociale angst symptomen. Echter, het versterkte wel een belangrijk exposuremechanisme, namelijk het patroon van angstniveaus binnen de exposuresessies. Ten derde: mensen met SAS die bij aanvang van exposuretherapie sterke sociale vermijdingstendenties hadden, lieten

een sterkere afname van angst zien tijdens een met testosteron versterkte exposure in vergelijking met degenen die weinig vermijdingstendenties lieten zien. Samenvattend zijn de resultaten uit dit proefschrift een veelbelovend startpunt om in de toekomst meer onderzoek te doen naar de langetermijneffecten van de toevoeging van testosteron aan exposure bij mensen met een sociale angststoornis.

Curriculum Vitae

Moniek Hutschemaekers (1991) was born in Tilburg and raised in Houten near Utrecht, the Netherlands. After she obtained her VWO diploma in 2009 she moved with her parents to Beuningen (Gelderland). Here, she started studying Psychology at the Radboud University Nijmegen and moved to Nijmegen in 2010. After she received her Bachelor degree *Cum Laude* in 2012, she was admitted to the Research Master program Behavioural Science and she obtained her master degree *Cum Laude* in 2014. During the Research Master she worked as a Research Assistant at the Korsakov Clinic (Vincent van Gogh GGZ) and at the Radboud University. During her Master Thesis she studied interpretation biases in hazardous drinkers at the Department of Experimental Research and Psychopathology. Subsequently, she obtained another Master Degree in Healthcare Psychology at Radboud University in 2015. During her second Master she completed an internship at Overwaal Centre of Expertise for Anxiety Disorders, OCD and PTSD (part of Pro Persona) and she continued to work there as a psychologist and junior researcher. At Overwaal she worked on research projects with a focus on fear extinction and avoidance in patients with anxiety disorders and cognitive bias modification in patients with PTSD. In 2017 she was accepted to the National Topklas Program, a trajectory in which a PhD project is combined with the Post-Master's Healthcare training program (GZ-psychologist) as well as the post-license program for Specialists (Clinical Psychologist). She was the first candidate working at Pro Persona to participate in this program. In 2019 she registered as a Healthcare (GZ)-psychologist and currently she is in her final year of the program for specialists. During her training she worked with different patient groups and she registered as a licensed Dialectical Behavioral Therapist. Eager to translate fundamental research findings to the clinical practice, her PhD-project focused on enhancing exposure therapy for Social Anxiety Disorder by means of Testosterone. For the future Moniek hopes to work as a clinical psychologist, as a collaborator on research projects and as a teacher for the post-Master program for Healthcare psychologist.

List of publications

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