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Anxiolytic Effects of Exogenous Intranasal Testosterone in Humans

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Anxiolytic Effects of Exogenous Intranasal Testosterone in Humans

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Dedication

For my family and friends. I would not have traversed so far without you.

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Abstract

Anxiolytic Effects of Exogenous Intranasal Testosterone in Humans

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Higher levels of testosterone have been associated with fewer anxiety symptoms and greater psychological wellbeing. However, additional research is needed to advance the clinical use of testosterone in mental healthcare. This dissertation aims to investigate the anxiolytic effects of testosterone in men and women through three studies. The first study examines the pharmacokinetic profile of a novel testosterone nasal spray, designed for the safe and rapid delivery of exogenous testosterone in men and women. The second study leverages the utility of this novel spray preparation, and investigates the effect of intranasal testosterone on subjective anxiety during a psychosocial challenge. The final study extends the anxiolytic effects of exogenous testosterone in women, and explores the effect of intranasal testosterone on test anxiety and cognitive performance. Collectively, these three studies aim to contribute to a broader understanding of the anxiolytic effects of testosterone, and with it, the potential for testosterone to act as a novel pharmaceutical in the treatment of anxiety.

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Chapter 1: Introduction

There's no denying it. She is next. Her heart pounding, and breaths quickening with looming anticipation. The room is reduced to a blur as she is called to give her presentation. She steadies herself against the podium with her sweaty palms as she scans the audience for looks of disapproval. Her eyes sunken with dark circles from rehearsing her speech repeatedly into the early morning. As she opens her mouth, a new rush of panic spills over her as she reads the introductory sentences of her thesis out loud. Suddenly, the room is punctured by the sound of applause, signaling the end of her presentation. She walks away from the podium in a state of dissociation. She is convinced that she was stuttering and making a fool out of herself in front of her esteemed colleagues. She makes a mental note to submit an abstract for a poster presentation instead at the next conference.

Anxiety is a stranger to almost no one. However, for some, feelings of anxiety may become so frequent and intense that it is debilitating. The experience of excessive anxiety in situations that pose no real threat is the hallmark of a class of mental disorders called anxiety disorders.

DEFINING FEATURES OF ANXIETY DISORDERS

Nature and Epidemiology

According to the DSM-5 (American Psychiatric Association, 2013), anxiety disorders are a cluster of psychiatric conditions characterized by excessive fear and behavioral avoidance. *Fear* is the emotional response to real or perceived imminent threat, whereas *anxiety* is the anticipation of future threat. Although related, fear is associated with surges of autonomic arousal necessary for fight or flight, thoughts of immediate danger,

and escape behaviors; whereas anxiety is more often associated with muscle tension and vigilance in preparation for future danger and cautious or avoidant behaviors. Although recognizing the fear is unrealistic and disproportionate to the actual stimuli (e.g., public speaking), anxiety sufferers avoid fear-eliciting situations, or endure them with significant distress. Moreover, a DSM-5 diagnosis of anxiety disorder, irrespective of subtype, warrants that the anxiety associated with the fear-eliciting situation and related avoidance cause significant interference with the normal routine, occupational functioning, or social activities, and relationships (American Psychiatric Association, 2013).

Anxiety disorders have the highest overall prevalence of all psychiatric diagnoses, with an estimated 12-month prevalence of 18.1% and a lifetime prevalence of 28.8% (Kessler, Berglund, et al., 2005; Kessler, Chiu, Demler, & Walters, 2005). Globally, 273 million (4.5% of the world's population) has a diagnosis of anxiety disorder (Vos et al., 2012), with prevalence and severity twice as high in women than men (McLean, Asnaani, Litz, & Hofmann, 2011).

According to the National Comorbidity Survey Replication, a nationally representative face-to-face household survey conducted between 2001 and 2003, the median onset for anxiety disorder is approximately 11 years of age (Kessler, Berglund, et al., 2005). In a 12-year prospective study, survival analyses revealed an overall chronic course for the majority of anxiety disorders (Bruce et al., 2005). Of the different subtypes of anxiety disorders, social phobia had the smallest probability of recovery after 12 years of follow-up (Bruce et al., 2005). When left untreated, anxiety disorders are unremitting illnesses that substantially worsen the quality of life (Olatunji, Cisler, & Tolin, 2007).

Psychiatric comorbidity is often observed in those with anxiety disorders. The co-occurrence of anxiety disorders with other mental, addictive, or physical disorders has important implications for treatment and clinical course. According to the Epidemiologic Catchment Area study, nearly half (47.2%) of those meeting lifetime criteria for major depression also met criteria for a comorbid anxiety disorder (Regier, Rae, Narrow, Kaelber, & Schatzberg, 1998). Moreover, individuals with social and simple phobias typically have an earlier illness onset, and are more vulnerable to major depression and addictive disorders (Regier et al., 1998). The presence of comorbid psychiatric disorders significantly lowered the likelihood of recovery from anxiety disorders and increased the probability of their recurrence (Bruce et al., 2005).

Indirect and Direct Costs of Illness

Anxiety disorders are associated with significant impairment. Even after controlling for comorbidity and sociodemographic factors, baseline presence of any anxiety disorder is significantly associated with suicidal ideation and suicide attempts in both cross sectional and longitudinal analysis (Khan, Leventhal, Khan, & Brown, 2002; Nepon, Belik, Bolton, & Sareen, 2010; Sareen et al., 2005). Moreover, compared to non-anxious controls, individual with anxiety disorders report greater impairments in general health (Gater et al., 1998; Ormel et al., 1994; Simon, Ormel, VonKorff, & Barlow, 1995), family relationships (Lochner et al., 2003; Rapaport, Clary, Fayyad, & Endicott, 2005), marriage or romantic relationships (Aderka et al., 2012; Davila & Beck, 2002; Eng, Heimberg, Hart, Schneier, & Liebowitz, 2001), social network (Barrera & Norton, 2009; Lochner et al., 2003; Rapaport et al., 2005; Wittchen, 2002), and occupational functioning

(Goetzel et al., 2004; Lim, Sanderson, & Andrews, 2000; Turner, Beidel, Dancu, & Keys, 1986).

Given the adverse effects associated with anxiety disorders, it is not surprising that they are linked to increased use of medical services. Compared with individuals diagnosed with other psychiatric disorders, patients with anxiety disorders are high care utilizers who present to general practitioners more frequently than to psychiatric professionals (Marciniak et al., 2005).

The staggering costs associated with anxiety disorders have been well documented. In 1990, the estimated annual cost of anxiety disorders was approximately $42.3 billion in the United States alone (Greenberg et al., 1999), a figure which accounted for 31.5% of all mental health expenditures that year (DuPont et al., 1996). Although a more recent cost estimate of anxiety disorders in the United States has yet to be published, the World Health Organization approximated the global cost of all mental health expenditures as $2.5 trillion in 2010, and projected this value to increase to $6.0 trillion by 2030 (Bloom et al., 2011).

Using retrospective, multivariate analyses while controlling for demographic characteristics, medical comorbidities, anxiety diagnosis, and prior resource utilization, a smearing estimate was used to calculate the total medical costs for patients with any anxiety disorder (Marciniak et al., 2005). Findings show that the mean estimated total medical cost for individuals diagnosed with any anxiety disorder was $6,475 in 2005 (Marciniak et al., 2005); taking into account inflation, this value increases to $8,427.84 in 2019.

In summary, anxiety disorders are associated with impaired qualify of life, increased risk for comorbidity, and significant financial cost.

ETIOLOGY OF ANXIETY DISORDERS

Learning Theory

The modern learning theory of anxiety disorders stems from the pioneering work of Watson and Rayner (1920). In particular, their research on the acquisition of fears and phobias with Little Albert. Watson and Rayner (1920) argued that phobias are simply intense classically conditioned fears that develop when a neutral stimulus is paired with a traumatic event, such as when their Little Albert acquired an intense fear of rats (and likely Watson himself), after hearing a frightening gong paired with the presence of a rat. Indeed, across numerous studies using retrospective recall, research has found that people with specific phobias can recall a traumatic conditioning event when their phobia began (Öst & Hugdahl, 1981).

Aside from concerns associated with recall bias in research on early learning histories, another criticism of early conditioning theory centered on the observation that many people with phobias do not appear to have had any relevant history of classical conditioning (Rachman, 1984). One explanation for this apparent discrepancy is the speculation that simply observing others experiencing a trauma or behaving fearfully can be sufficient for fears or phobias to develop. The strongest experimental evidence in support of vicarious conditioning stems from a series of primate studies showing that strong and persistent fears can be learned through observation alone (Cook, Mineka, Wolkenstein, & Laitsch, 1985). When laboratory-reared young adult rhesus monkeys, who, initially, were not afraid of snakes, watched unrelated, wild-reared monkeys reacting very fearfully in the presence of live and toy snakes, they showed a rapid acquisition of an intense fear

of snakes that did not diminish over a three-month period (Cook et al., 1985). Extending beyond simple phobias, vicarious conditioning may also develop in social anxiety (e.g., observing another being ridiculed or humiliated in a social situation; Mineka & Zinbarg, 2006). Öst and Hugdahl (1981) found that 13% of individuals with social anxiety recalled vicarious learning experiences as having played a role in the origins of their anxiety.

Taken together, research suggests that in addition to direct conditioning, fears may also be acquired through vicarious conditioning. It should be noted, however, that theories of early direct-conditioning and early vicarious-conditioning are susceptible to bias associated with retrospective recall, thus weakening subsequent causal inferences.

Cognitive Theory

Contemporary cognitive theory of anxiety disorders has its origins in three lines of theory and research. The first is Albert Ellis's (1973) influential work on rational psychotherapy, later called rational-emotive behavior therapy. This theory posited that emotional dysfunction is the consequence of irrational beliefs. Similarly, Beck, Emery, and Greenberg (1985) suggested that anxiety disorders develop in response to faulty threat appraisals, which are the result of threat-related schemas. Lastly, cognitive theories of anxiety disorders have also been heavily influenced by basic cognitive psychology investigations into information processing in healthy individuals.

From a cognitive perspective, excessive fear and anxiety are consequences of exaggerated threat appraisals to neutral or innocuous situations. According to Beck et al. (1985), these appraisals are experienced as negative automatic thoughts in response to situations that are of relevance to the individual with an anxiety disorder. For example,

someone with social anxiety may have an automatic thought of "they think I'm stupid" in a public speaking scenario. According to cognitive theories, individuals with anxiety disorders overestimate the probability and cost of harm. In addition, they show biased safety estimates and underestimate their own ability to cope with harm (Beck et al., 1985; Salkovskis, 1996).

It is noteworthy that threat appraisals in anxiety disorders are not only related to external stimuli, but may also concern symptoms of anxiety, such as in individuals with generalized anxiety disorder, who perceive their own worrying as threatening (Wells & Carter, 2001). Threat appraisals may also extend to bodily sensations, such as in individuals with panic disorder, in which the catastrophic appraisal of bodily sensations lies at the core of the disorder (Clark, 1986).

According to Beck's cognitive model, the threat appraisals of individuals with anxiety disorder(s) are thought to be influenced by maladaptive cognitive schemas, or underlying cognitive structures that have developed in response to earlier experiences (Beck et al., 1985). It is thought that these negative schemas lead to biased automatic processing when they are activated (Beck et al., 1985), resulting in greater salience of threat-related information relative to safety-related information. It is believed that through this process, maladaptive schemas are maintained over time.

Behavioral Genetics

Family and twin studies have found that panic disorder, generalized anxiety disorder, and phobias aggregate in families and demonstrate significant heritability (Hettema, Neale, & Kendler, 2001). This is supported by large-scale twin studies of panic

disorder (Kendler, Neale, Kessler, Heath, & Eaves, 1993; Scherrer et al., 2000), generalized anxiety disorder (Hettema, Prescott, & Kendler, 2001; Scherrer et al., 2000), and specific phobias (Carey & Gottesman, 1981). Estimated heritabilities across the disorders are modest, ranging from 30% - 40%, which are significantly lower compared to disorders such as schizophrenia and bipolar disorder (Hettema, Neale, et al., 2001). These findings suggest that a significant portion of the variance in liability is explained by environmental factors.

Because most large scale family and twin studies limit analyses to anxiety *disorders*, the association between anxiety *symptomatology* seen in individuals with subclinical symptoms compared to those diagnosed with an anxiety disorder is unclear (Hettema, Neale, et al., 2001). However, if symptoms and disorders are considered on the same continuum of increased severity and duration, twin studies have indeed found genetic contributions to nonspecific anxiety symptoms (Jardine, Martin, Henderson, & Rao, 1984), fears (Kendler, Karkowski, & Prescott, 1999; Kendler, Neale, Kessler, Heath, & Eaves, 1992; Neale et al., 1994; Torgersen, 1979), and phobia (Kendler et al., 1995).

Finally, despite nearly two times greater prevalence rate in women compared to men, the same underlying liability structure broadly account for patterns of comorbidity among anxiety disorders, suggesting that risk factors are independent of sex (Hettema, Prescott, Myers, Neale, & Kendler, 2005).

Neurocircuitry

Progress in the development of neurocircuitry models of anxiety disorders was made possible by major developments in the field of neuroimaging. Many of the early

neuroimaging studies were based on preclinical models of stress and conditioned fear (Davis, 1992; LeDoux, Iwata, Cicchetti, & Reis, 1988). Through the elegant work of these and other researchers, a basic model of normal fear responding was developed and refined, leading to the description of a "fear neurocircuitry" centered around the amygdala (LeDoux, Cicchetti, Xagoraris, & Romanski, 1990). Since then, the structure, function, and activity of the amygdala have served as the basis for most contemporary human neurocircuitry models of anxiety.

Located in the anterior part of the medial temporal lobe, the amygdala has been established as the central node for the coordination of autonomic and behavioral fear responding (LeDoux et al., 1990). Sensory information critical to threat assessment is processed via pathways running through the anterior thalamus to the amygdala (LeDoux et al., 1988). In preparation for action in response to threat, the amygdala activates ascending projections to motor areas and descending projections to brain stem nuclei that control autonomic responses and arousal (Kent & Rauch, 2003). The amygdala also facilitates the acquisition of additional information regarding specific threats via reciprocal projections to subcortical and limbic cortical regions (LeDoux, 1996). Critical feedback to the amygdala is provided by specific brain regions, including the medial frontal cortex and cortico-striato-thalamic circuits, which mediate "gating" at the level of the thalamus, thereby regulating the flow of incoming information that reaches the amygdala (Kent & Rauch, 2003). The medial frontal cortex, including anterior cingulate and medial and orbitofrontal cortex, provides critical "top-down" governance over the amygdala, curbing

the fear response once danger has passed or when a potentially threatening stimulus has changed (Kent & Rauch, 2003).

Convergent neuroimaging data are consistent with the notion of exaggerated amygdala activation in response to emotionally-provocative stimuli, in a number of anxiety disorders (Kent & Rauch, 2003; Shin & Liberzon, 2010). Facial expressions have been especially effective probes of amygdala responses in social anxiety, consistent with hypersensitivity in a specialized system for assessing a specific class of potentially threating stimuli (Birbaumer et al., 1998; Schneider et al., 1999). Data regarding amygdala function in panic disorder and generalized anxiety disorder are limited, and thus overall are inconclusive (Shin & Liberzon, 2010). One potential conceptualization of these findings is that amygdala hyperactivation is a common pathway for exaggerated fear in response to specific emotional stimuli (Shin & Liberzon, 2010). Thus, anxiety disorders characterized by specific identifiable stimuli (e.g., social anxiety and specific phobia) will result in exaggerated amygdala reactivity following exposure to the feared stimulus. Panic attacks, on the other hand, can occur in the absence of such stimuli (i.e., "out of the blue") and may involve activation of other structures within the fear/anxiety neurocircuitry. In line with this hypothesis, neuroimaging research has raised a wide range of possible neural substrates underlying panic disorder. Specifically, regional abnormalities within the temporal lobe may reflect fundamental deficits in threat assessment in panic disorder (Fontaine, Breton, Déry, Fontaine, & Elie, 1990; Vythilingam et al., 2000). In particular, abnormalities in the parahippocampal gyrus have been identified in panic disorder (Massana et al., 2003).

In addition to the amygdala, research has also reported exaggerated insular cortex activation in specific phobia versus healthy controls in response to phobia- or fear-related pictures, videos, and words (Dilger et al., 2003; Goossens, Schruers, Peeters, Griez, & Sunaert, 2007; Schienle, Schäfer, Walter, Stark, & Vaitl, 2005; Straube, Mentzel, Glauer, & Miltner, 2004). In addition, treatment studies have reported decreased insula activation following cognitive behavioral treatment (Goossens, Sunaert, Peeters, Griez, & Schruers, 2007; Straube, Glauer, Dilger, Mentzel, & Miltner, 2006).

Although the literature presently is small, it appears that the medial prefrontal cortex may be elevated in generalized anxiety disorder. Activations in the dorsal anterior cingulate cortex (dACC) and rostral anterior cingulate cortex (rACC) appear to be elevated in response to fearful facial expressions in adolescents with generalized anxiety disorder (McClure et al., 2007). Moreover, in a mixed cohort of subjects with generalized anxiety and social anxiety, those with higher intolerance for uncertainty had elevated rACC and subgenual ACC activation during a decision-making task (Krain et al., 2008).

Taken together, anxiety disorders are associated with hyperactivation in emotion-generating regions, and hypoactivation in prefrontal/regulatory regions. Interestingly, evidence of differential patterns is also emerging, such that greater involvement of emotion-generating regions is reported in panic disorder and specific phobia, and greater involvement of prefrontal regions is reported in generalized anxiety disorder (Duval, Javanbakht, & Liberzon, 2015; Sylvester et al., 2012).

Psychoneuroendocrinology

Advances in diagnosis and treatment have led to renewed interest in the pathophysiology of anxiety symptoms and disorders, particularly in understanding pervasive sex differences in prevalence rates among men and women. A growing body of literature has focused on the role of gonadal hormones such as testosterone in the development and maintenance of anxiety (e.g., Fernández-Guasti, Fiedler, Herrera, & Handa, 2012; Goel & Bale, 2008, 2009; Maeng & Milad, 2015; McHenry, Carrier, Hull, & Kabbaj, 2014; Mueller, Grissom, & Dohanich, 2014; Palanza, 2001). Testosterone, the end product of the hypothalamic-pituitary-gonadal axis, is primarily secreted in the testes of males, and the ovaries of females. On average, testosterone levels are about 7 to 8 times greater in men as they are in women (Torjesen & Sandnes, 2004).

Women are more likely to experience symptoms of anxiety and depression during periods of marked hormonal fluctuations, such as puberty, postpartum, and perimenopause (Douma, Husband, O'donnell, Barwin, & Woodend, 2005). Beginning in puberty, gonadal hormones estrogen and progesterone in females, and testosterone in males, begin to surge. In male rats, prepubertal castration is associated with anxiogenic effect of a novel environment, as evidenced by reduced activity in open field tests (Brand & Slob, 1988), and behavioral inhibition among interaction with other male rats (Primus & Kellogg, 1990). Relatedly, proestrous (i.e., high levels of 17-β estradiol) female rats and mice show decreased activity and behavioral inhibition, indicating increased anxiety-like behaviors (Díaz-Véliz, Butrón, Benavides, Dussaubat, & Mora, 2000; Diaz-Veliz, Soto, Dussaubat, & Mora, 1989; Mora, Dussaubat, & Díaz-Véliz, 1996; Morgan & Pfaff, 2002; Paré &

Redei, 1993). In humans, earlier pubertal timing is linked to higher reported anxiety symptoms in girls, but not boys (Deardorff et al., 2007; Reardon, Leen-Feldner, & Hayward, 2009). One explanation for this set of findings is the interaction between gonadal and stress hormones predisposing females to stress-related diseases such as anxiety and depression (Solomon & Herman, 2009). Depression and anxiety are both associated with aberrant secretion of glucocorticoids (Burke, Davis, Otte, & Mohr, 2005; Vreeburg et al., 2010). The hypothalamic-pituitary-gonadal axis has been shown to modulate the deleterious effects of glucocorticoids by suppressing the hypothalamic-pituitary-adrenal axis (Solomon & Herman, 2009). In fact, the anxiolytic effects of testosterone have been shown in both animals and humans in response to stress.

Anxiolytic Effects of Testosterone in Animals

In animals, anxiety-like behaviors are assessed based on the animal's willingness to explore a novel environment as opposed to avoiding open exposure (reviewed in Nestler & Hyman, 2010). Several effects of testosterone have been observed in measures of anxiety-like behavior in adult male rodents. Gonadectomy in adult male rodents results in increased anxiety-like behaviors in a battery of behavioral tests, such as the elevated plus maze, open field test, and defensive probe-burying, compared to sham-operated controls (Adler, Vescovo, Robinson, & Kritzer, 1999; Fernández-Guasti & Martínez-Mota, 2003; Frye & Seliga, 2001; Morsink et al., 2007; Slob, Bogers, & van Stolk, 1981). These effects were reversed by testosterone replacement (Adler et al., 1999; Fernández-Guasti & Martínez-Mota, 2005; Frye & Seliga, 2001; Slob et al., 1981). Moreover, testosterone replacements were equally as effective as the administration of typical tricyclic

antidepressant imipramine in alleviating anxiety-like behaviors induced by two-weeks of chronic social isolation (Carrier & Kabbaj, 2012). Similarly, testosterone administration in intact adult male rodents reduced anxiety-like behavior in the elevated plus maze, open field test, and light-dark box test, compared to vehicle-treated controls (Bitran, Kellogg, & Hilvers, 1993; Frye, Edinger, & Sumida, 2008).

In intact female rodents, injections of testosterone reduced anxiety-like behavior in the open field test and elevated plus maze, compared to vehicle treated controls (Frye & Lacey, 2001). Similarly, testosterone administration in intact female rodents also decreased anxiety-like behavior in a defensive burying task through an androgen-mediated effect, compared to vehicle treated controls (Gutiérrez-García, Contreras, Vásquez-Hernández, Molina-Jiménez, & Jacome-Jacome, 2009).

Anxiolytic Effects of Testosterone in Humans

The association between testosterone levels and anxiety symptoms in humans is evident in males with hypogonadism, a condition in which reduced functional activity of the gonads results in decreased levels of testosterone. Hypogonadal men exhibit significantly higher prevalence of anxiety disorders compared to those with androgens within the normative range (Aydogan et al., 2012; Lašaitė, Čeponis, Preikša, & Žilaitienė, 2014). Similarly, men treated with androgen-depleting drugs exhibit lower mood (Schmidt et al., 2004), and greater likelihood of developing an anxiety disorder (DiBlasio et al., 2008). In men with social anxiety, defeat following competition is associated with a significant drop in testosterone, an effect not observed in non-anxious men (Maner, Miller, Schmidt, & Eckel, 2008). In healthy adolescent males, declines in salivary testosterone due

to circadian flux is correlated with an increase in anxiety-like measures (Granger et al., 2003).

Although studies of testosterone administration in women are limited, existing evidence supports an anxiolytic effect of testosterone. Compared to placebo-treated controls, administration of testosterone resulted in diminished preconscious selective attention to threatening stimuli (van Honk, Peper, & Schutter, 2005), reduced gaze avoidance in individuals with social anxiety disorder (Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2016), reduced fear-potentiated startle response (Hermans, Putman, Baas, Koppeschaar, & van Honk, 2006), and increased behavioral approach (Enter, Spinhoven, & Roelofs, 2014, 2016). Further, transdermal application of testosterone in women experiencing age-related declines in androgens resulted in substantially improved mood and psychological well-being, compared to placebo treated individuals (Goldstat, Briganti, Tran, Wolfe, & Davis, 2003). Compared to healthy controls, women with anxiety disorders, including generalized anxiety, social phobia, and agoraphobia express lower levels of salivary testosterone (Giltay et al., 2012).

Effects of Testosterone on Neurocircuitry Implicated in Anxiety

In humans, anxiety disorders are associated with both structural and functional abnormalities within the amygdala, hippocampus, and prefrontal cortex (Shin & Liberzon, 2010), three brain regions which appear to be strongly influenced by testosterone (McHenry et al., 2014). In animal models, more discrete sub-regions of these general brain sites have been implicated in anxiety-like behaviors (reviewed in Singewald, 2007). Collectively, testosterone exerts its anxiolytic effects in both humans and animals through

the down regulation of the amygdala (Bingham & Viau, 2008; Hermans et al., 2007; Linfoot et al., 2009; Radke et al., 2015; Stanton, Wirth, Waugh, & Schultheiss, 2009; van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010), up regulation of the prefrontal cortex (Schutter & van Honk, 2004; Sladky et al., 2015; van Wingen et al., 2010; Volman, Toni, Verhagen, & Roelofs, 2011), and increased cell proliferation in the hippocampus by dampening the neurotoxic effects of glucocorticoids (Hermans et al., 2007; Rubinow et al., 2005; Viau, 2002).

Amygdala. The amygdala plays a major role in anxiety and fear responses in humans and rodents (reviewed in Ressler, 2010). Although human imaging studies examining potential brain regions involved in the effects of testosterone on anxiety are extremely limited, existing findings demonstrate sexually divergent associations between testosterone and amygdala activation. Specifically, greater levels of testosterone is associated with increased amygdala activation in men (Derntl et al., 2009), but not in women (Stanton et al., 2009).

In male neonatal rodents, gonadectomy is associated with fewer androgen-receptor-containing cells in the medial amygdala in adulthood, compared with sham operated controls; an effect that was reversed by testosterone administration 1-5 days during the postnasal period, but not in adulthood (Bingham & Viau, 2008). Further, defensive burying was associated with expression of androgen receptors in the medial amygdala (Linfoot et al., 2009).

Across a series of studies, injection of a 5α-reductase inhibitor (a class of drugs with anti-androgen effects) into the amygdala of intact female rodents increased anxiety-

like behavior in the open field test, elevated plus maze, and defensive freezing tests, compared to vehicle-treated controls (Walf, Sumida, & Frye, 2006). Aromatization of testosterone to estradiol may also have anxiolytic effects within the amygdala. Injection of estradiol into the amygdala of ovariectomized female rats decreased anxiety-like behavior in the open field, elevated plus maze, and hot plate tasks, compared to sham-operated controls (Frye & Walf, 2004).

Hippocampus. The hippocampus has been extensively documented as a critical site in anxiety disorders (McHenry et al., 2014). Preclinical evidence suggests that testosterone acting on the hippocampus has a number of anxiolytic and protective cellular actions. Some of the protective effects of testosterone in the hippocampus may be due to its ability to reduce the aversive effects of glucocorticoids in response to stress (Hermans et al., 2007; Rubinow et al., 2005; Viau, 2002), and facilitate molecular mechanisms that favor cell proliferation, growth, and survival (McHenry et al., 2014).

The protective effects of testosterone on the hippocampus can be seen during both organizational and activational periods in rodent models. Perinatal androgen treatment increases neuronal soma size, dendritic length and branching, and also the volume of the CA3 pyramidal cell layer and the entire CA3 region of the hippocampus (Isgor & Sengelaub, 1998, 2003). Further, neonatal gonadectomy decreased hippocampal spine density in adulthood, an effect reversed with testosterone treatment (J. L. Dawson, Cheung, & Lau, 1975). Similarly, gonadectomy in adulthood is associated with cellular oxidative damage and morphological alterations in the hippocampus, an effect that was improved following testosterone administration, compared to vehicle treatment (Meydan et al.,

2010). In intact female rodents, testosterone administration following adrenalectomy decreased the number of pyknotic cells undergoing cell death in the hippocampus, compared to control-treated females (Frye & McCormick, 2000).

Prefrontal cortex. Neuroimaging studies in humans suggest the anxiolytic effects of testosterone may be the result of its influence on the cortical connectivity between the prefrontal cortex and subcortical regions (e.g., amygdala), particularly in the modulation of approach and avoidance behaviors (Volman et al., 2011). In a sample of age matched male participants, a negative feedback loop is observed between the orbitofrontal cortex and amygdala in healthy controls, compared to a positive, excitatory connection in patients with social anxiety (Sladky et al., 2015).

Exogenous studies in healthy females show that testosterone administration rapidly reduced functional coupling of the amygdala with the orbitofrontal cortex, and enhanced amygdala coupling with the thalamus (van Wingen et al., 2010). Similarly, testosterone administration is associated with significant increase in delta power in the midfrontal region of the brain (Schutter & van Honk, 2004). These findings suggest that the anxiolytic effects of testosterone may be the result of increased top-down, regulatory control of the amygdala from the prefrontal cortex.

Taken together, clinical and preclinical evidence in humans and rodents suggests an anxiolytic effect of testosterone. Considering pervasive sex differences in the prevalence of anxiety disorders (Kessler, Chiu, et al., 2005), investigation of testosterone's therapeutic effects has the potential to improve mental health in both men and women worldwide.

Overview of Proposed Studies

This dissertation includes three experiments, that, taken together, describe a multipronged investigation of the anxiolytic effects of testosterone in men and women. The first study examines the pharmacokinetic profile of a novel testosterone nasal spray, designed for the safe and rapid delivery of exogenous testosterone in men and women. The second study leverages the utility of this novel spray preparation, and investigates the effect of intranasal testosterone on subjective anxiety during a psychosocial challenge. The final study extends the anxiolytic effects of exogenous testosterone in women, and explores the effect of intranasal testosterone on cognitive performance. Collectively, these three studies aim to contribute to a broader understanding of the anxiolytic effects of testosterone, and with it, the potential for testosterone to act as a novel pharmaceutical in the treatment of anxiety.

Chapter 2: Pharmacokinetics of Intranasal Testosterone Spray

ABSTRACT

Despite various existing methods of exogenous testosterone administration, currently none are ideal for psychological research purposes. The aim of the present study is to examine the pharmacokinetic profile of a novel intranasal testosterone spray in both healthy men and women. Twenty participants (35% female) contributed pharmacokinetic data by self-administering 14 mg of testosterone via intranasal spray, and providing blood serum samples at baseline (pre-administration), 15, 30, and 60 minutes after spray administration. Results indicate that total and free testosterone concentrations increased in a monotonic fashion at 15- and 30-minutes post testosterone administration, peaking at 30 minutes (242.0% and 2653.6% increases in total testosterone from baseline for men and women, respectively), and declining 60 minutes following administration. This study establishes the pharmacokinetic profile of a novel intranasal testosterone delivery system and provides evidence for the effectiveness and utility of intranasally-delivered testosterone in both healthy men and women.

BACKGROUND

Limitations of Existing Exogenous Testosterone Administration Modalities

Despite numerous established modes of exogenous testosterone administration, currently none are ideal for psychological research purposes. Testosterone injections are invasive and results in wide fluctuations in circulating levels, making rigorous experimental control difficult to establish (Nieschlag, 2006). Testosterone patches are associated with high rate of skin irritation at the site of administration (Tenover, 2003),

thus possibly confounding subsequent behavioral outcomes. Oral administration of testosterone must be coupled with a high fat meal, thereby making experimental designs unnecessarily complex; and even then, they typically yield low absorption rates (Haren, Chapman, Coates, Morley, & Wittert, 2005; Wittert et al., 2003). Testosterone gels, while one of the most widely accepted modes of exogenous testosterone administration, poses significant likelihood of skin-to-skin transfer, which drastically increases risk of unintended contamination (Kunz, Klein, Clemons, Gottschalk, & Jones, 2004; Swerdloff et al., 2000). Following standard topical gel application, up to 60% of testosterone remains on unwashed skin eight hours after use (de Ronde, 2008). Despite this and similar findings (e.g., Kathiresan, Carr, & Attia, 2011; Mazer et al., 2005; Merhi & Santoro, 2007), initial studies with gel applications minimized concerns that meaningful transfer could occur even after intense skin-to-skin contact (Rolf, Knie, Lemmnitz, & Nieschlag, 2002). Instead, they suggested that the rapid evaporation of alcohols left the residual testosterone crystallized and inert (Cabrera & Rogol, 2013). These studies likely underestimated the heightened androgen sensitivity of pre-pubertal children and women. More recent studies in untreated women showed significant increases in total serum testosterone from baseline following contact with a man two hours after application of a 1.62% testosterone gel (Stahlman et al., 2012b, 2012a).

Although rare, the consequences of unintended contamination in children and women can be devastating. Potential consequences include virilization of young girls and boys (the development of male physical characteristics in a female or precociously in a boy), heightened sexuality, and aggressive behavior (Bhowmick, Ricke, & Rettig, 2007;

Brachet, Vermeulen, & Heinrichs, 2005; Cavender & Fairall, 2011; Kathiresan et al., 2011; Kunz et al., 2004; Merhi & Santoro, 2007; Yu, Punyasavatsu, Elder, & D'Ercole, 1999). Moreover, premature virilization carries negative psychosocial effects that extend beyond childhood; and long-term exposure of androgens accelerates skeletal maturation, an irreversible outcome that can truncate adult height (Cabrera & Rogol, 2013). Additionally, clitoromegaly and penile enlargement may not be completely reversible once exposure to the androgen ceases (Kathiresan et al., 2011; Merhi & Santoro, 2007). In May 2009, the United States Food and Drug Administration (FDA) imposed a black-box warning on two testosterone gels after investigating eight reports of children nine months to five years old who showed signs of virilization after being exposed to adults who used the products, AndroGel 1% and Testim 1% (Brachet et al., 2005; Kunz et al., 2004; Lakshman & Basaria, 2009; Miller, Rogol, & ZumBrunnen, 2012). Despite these black-box warnings, which are the highest of their kind, instances of unintentional secondary exposure continue to be reported (Cabrera & Rogol, 2013). In fact, more than a dozen additional cases of children who developed complications due to unintended contamination are currently under FDA review.

Taken together, these data point to a critical need for safer and more efficacious route of testosterone administration in men and women that minimizes potential risk to others.

Intranasal Drug Administration

Intranasal administration represents a viable option for local and systemic delivery of diverse therapeutic compounds (Behl, Pimplaskar, Sileno, & Romeo, 1998; Costantino,

Sileno, & Johnson, 2005; Illum, 2000, 2003; Song, Wang, Thakur, Meidan, & Michniak, 2004), and has been reported to effectively bypass the blood-brain barrier in mice, rats, primates, and humans (De et al., 2005; Deadwyler, Porrino, Siegel, & Hampson, 2007; Reger et al., 2006; Ross et al., 2004; Thorne, Hanson, Ross, Tung, & Frey, 2008). Intranasal targeting of drugs to the central nervous system avoids hepatic first pass metabolism, which allows for a lower therapeutic dose and fewer systematic side effects (Johnson, Hanson, & Frey, 2010); all of which may maximize convenience, comfort, and compliance associated with drug administration (Banks, Morley, Niehoff, & Mattern, 2009; Costantino, Illum, Brandt, Johnson, & Quay, 2007; Mattern, Hoffmann, Morley, & Badiu, 2008). Compared to other administration methods, intranasal delivery is non-invasive, painless, does not require sterile preparation, minimizes possible contamination, and is easily and readily administered by the individual (Costantino et al., 2007).

There are three routes of delivery from the nasal cavity to the central nervous system (Ross et al., 2004; Thorne et al., 2008; Thorne, Pronk, Padmanabhan, & Frey, 2004):

Olfactory Pathway

Once therapeutic compounds are administered through the nasal cavity, they travel to the olfactory mucosa, which contains olfactory receptor neurons that are responsible for transduction (Selvaraj, Gowthamarajan, & Karri, 2017). Molecules reach the olfactory receptor neurons by paracellular or transcellular mechanism, and cross the cribriform plate to reach the olfactory bulb located on the surface of the brain (Thorne et al., 2004). From the olfactory nerves, the therapeutic compounds then enter the cerebrospinal fluid and are

distributed throughout the brain. Following nasal administration, only few minutes is required for compounds to reach specific brain regions (i.e., cortex, cerebrum, and cerebellum) via olfactory transport.

Trigeminal Pathway

Testosterone molecules may also enter the brain through the trigeminal pathway. Once compounds diffuse through the mucosa of the nasal cavity, it reaches the ophthalmic branch of the trigeminal nerve, which innervates the dorsal part of the nasal mucosa. Therapeutic compounds transport through the trigeminal nerve pathway via intracellular transport (axonal transport) or endocytosis (Johnson et al., 2010), passing through the cribriform plate to reach the forebrain.

Systemic Pathway

Drug uptake into the brain from nasal cavity also occurs through blood circulation. Due to the rich vasculature of the respiratory epithelium, both small and large molecules are absorbed into blood circulation and are transported to the brain by bypassing the blood brain barrier (Banks, 2012; Banks et al., 2009). Lipophilic molecules such as testosterone easily crosses the blood brain barrier, and is then transferred to the carotid arterial blood supply to the brain and spinal cord, through a processed called counter current exchange (Selvaraj et al., 2017). Compared to olfactory and trigeminal pathways, the systemic pathway of drug delivery may take upwards of 20-30 minutes (Upadhyay, 2014).

Pharmacokinetic Profiles of Intranasal Testosterone Gel

Several studies have established the pharmacokinetic profiles of intranasal testosterone gel administration (Mattern et al., 2008; Rogol, Tkachenko, & Bryson, 2016).

In a study with eight hypogonadal men, a single dose of 7.6 mg of intranasal testosterone gel led to rapid absorption and peak total testosterone concentrations 1.08 hours (*SD* 0.71) following administration, resulting in a 341.9% increase of total testosterone from mean baseline concentration of 130.8 ng/dL (*SD* 87.4 ng/dL) to mean peak concentration of 578 ng/dL (*SD* 234 ng/dL) (Mattern et al., 2008).

When the dose is doubled to 15.2 mg, mean total testosterone concentration peaked at 804 ng/dL (*SD* 315 ng/dL) 1.40 hours (*SD* 0.94) following administration, resulting in 514.6% overall increase from baseline (Mattern et al., 2008). When the dose is tripled to 22.8 mg, it did not yield significantly larger total testosterone concentrations compared to dose of 15.2 mg. Here, the mean total testosterone concentration peaked at 842 ng/dL (*SD* 431 ng/dL) 1.02 hours (*SD* 0.68) following administration, resulting in 543.7% overall increase from baseline (Mattern et al., 2008).

Similarly, Acerus Pharmaceuticals Corporation (Mississauga, ON Canada), developed another intranasal testosterone gel (Natesto™) for the treatment of male hypogonadism (Rogol et al., 2016). A single pump of Natesto™ from a non-pressurized, manual pump dispenser administers 5.5 mg testosterone through a nasal applicator directly into the mucosa of the nasal vestibule of each nostril, resulting in 11 mg of testosterone in total to be absorbed into the blood stream (Rogol et al., 2016).

In a sample of 306 hypogonadal men (M_{age} = 54.4, SD_{age} =10.9), a single dose of 5.5 mg of Natesto™ intranasal gel led to peak total testosterone concentrations 1.4 hours (*SD* 2.5) following administration, resulting in a 461.3% increase in total testosterone from

mean baseline concentration of 186.3 ng/dL (*SD* 92.6) to mean peak concentration of 1045.7 ng/dL (*SD* 467.1) (Rogol et al., 2016).

Present Study

Although existing intranasal testosterone gel applications are promising, time to peak concentrations and the speed of uptake might be improved by using a more aqueous form of testosterone, such as in the form of an intranasal spray. Moreover, existing studies typically employ hypogonadal men as research participants, making it difficult to generalize pharmacokinetic data to healthy young men and women typically employed in psychological research studies. Thus, the aim of the present study is to examine the pharmacokinetic profile of an intranasal testosterone spray in both healthy, eugonadal men and women.

METHODS

Testosterone Spray Formulation

An intranasal testosterone spray was developed for the purpose of this study in collaboration with a local compounding pharmacy. The testosterone nasal spray used in this study contains 1% synthetic ester of testosterone (testosterone propionate), combined with non-active ingredients including purified water, polyoxyethylated castor oil, medium chain triglycerides, and polycarbophil. Based on values obtained in previous studies using intranasal testosterone (Mattern et al., 2008; Rogol et al., 2016), where doses of 11 mg and 15.2 mg yielded peak total testosterone concentrations (and greater doses did not yield significantly higher total testosterone concentrations), 14 mg of testosterone is administered via intranasal spray. Individuals familiar with AndroGel® (Unimed

Pharmaceuticals, Inc., Deerfield, IL) may find this dose to be extremely low given that the average dose is 150 mg for transdermal testosterone (Eisenegger, von Eckardstein, Fehr, & von Eckardstein, 2013; Marbury, Hamill, Bachand, Sebree, & Smith, 2003). However, the typical AndroGel® application protocol uses 1 gm of a 100 mg/gm product, with absorption around 9-10%. Thus, the goal of 14% via intranasal administration is slightly higher than the amount delivered transdermally.

There are no known side effects associated with single dose intranasal testosterone. The locally compounded spray poses no additional risks or side effects, and should minimize the potential for side effects due to being biologically identical to naturally occurring testosterone, as opposed to AndroGel's synthetic testosterone (Wang et al., 2004).

Participants

Twenty-five participants (40% female) responded to flyers and online postings for the pharmacokinetics study. Two female participants were excluded prior to enrollment due to medical reasons related to either venipuncture procedures or testosterone administration. Three additional participants (one female) were excluded from hormonal analysis due to inability to successfully obtain blood samples within four minutes of the targeted venipuncture time, resulting in 20 participants who completed the study. Male participants reported a mean age of 20.92 (*SD* 3.04) and BMI of 22.90 kg/m^2 (*SD* 3.65). Female participants reported a mean age of 21.14 (*SD* 2.41) and body-mass index (BMI) of 23.50 kg/m^2 (*SD* 3.73). See Table 1 for descriptive statistics.

Table 1. Descriptive Statistics of Participants from Study 1 by Sex

	Male (*n* = 13)		**Female (*n* = 7)**	
	M / %	(SD) / (n)	M / %	(SD) / (n)
Demographics				
Age (years)	20.9	(3.0)	21.1	(2.4)
BMI (kg/m^2)	22.9	(3.7)	23.5	(3.7)
Ethnicity (Hispanic or Latino)	23.0%	(3)	14.3%	(1)
Race				
White	30.1%	(4)	57.1%	(4)
Black or African American	7.7%	(1)	0.0%	(0)
Asian	38.5%	(5)	28.6%	(2)
Other	0.0 %	(0)	0.0%	(0)
Serum Testosterone Levels				
Free				
Baseline	1.4	(0.6)	0.4	(0.3)
15 mins post administration	3.2	(2.0)	1.9	(1.4)
30 mins post administration	4.4	(2.4)	3.4	(2.0)
60 mins post administration	3.7	(2.1)	2.7	(1.8)
Total				
Baseline	500.6	(169.7)	50.3	(23.9)
15 mins post administration	1255.6	(762.1)	748.9	(617.4)
30 mins post administration	1712.1	(912.3)	1386.2	(744.1)
60 mins post administration	1334.6	(548.8)	1152.7	(603.8)

Note. M = mean, SD = standard deviation. Where appropriate, percentage (%) and number of participants (n) are provided instead of mean (M) and standard deviation (SD). All testosterone levels are represented as ng/dL.

Procedures

Study participation was completed in a single laboratory visit. Upon arrival to the laboratory, experimenters obtained informed consent after detailing study procedures, risks associated with testosterone administration and venipuncture, and exclusionary criteria. Participants were informed that they could withdraw from the study at any time without

penalty. Female participants conducted a mandatory urinary pregnancy test in order to ensure safety associated with drug administration due to testosterone's potential adverse effects on the fetus. Negative pregnancy status was verified prior to proceeding with drug administration. A staff medical doctor then conducted the first of four 2.5 ml blood draws using standard venipuncture protocol. All blood samples were centrifuged prior to blood serum isolation and stored at -60 to -80 °C until hormone analysis.

After providing a baseline blood sample, participants self-administered 14 mg of testosterone via intranasal spray (Figure 1). Three further blood draws were taken at 15, 30, and 60 minutes after spray administration, using independent venipuncture sites for each draw. Participants were then debriefed about the purpose of the pharmacokinetic study, and compensated $25 USD for study participation. All procedures involving human participants were approved and in accordance with the ethical standards of The University of Texas at Austin Institutional Review Board (IRB# 2014-12-0029).

Figure 1. Testosterone nasal spray

Note. A sterile applicator is applied to individual spray containers immediately prior to use for each participant.

Hormone Analysis

All serum samples were assayed in-house using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits from DRG International (Springfield, NJ). All samples were thawed prior to centrifuge at 3,000 rpm for 15 minutes. Serum sample reagents were brought to room temperature (22 °C to 28 °C) prior to beginning analyses. Consistent with prior research (Feldman et al., 2002; Vermeulen, Verdonck, & Kaufman, 1999), intra-assay coefficients of variation (CV) and inter-assays CVs for total and free testosterone were each below 8%, values which fit within the acceptable range.

Statistical Analysis

Descriptive statistics, independent samples *t*-tests comparing total and free testosterone mean concentrations between males and females, and paired samples *t*-tests comparing post-administration time points (i.e., 15, 30, and 60) to baseline, were conducted using SPSS version 21.0 (Armonk, NY).

In addition, area under the curve with respect to increase (AUC_I) and area under the curve with respect to ground (AUC_G) were separately calculated for males and females to determine total and free testosterone increase, and total and free testosterone output, respectively (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). AUC values were then compared between males and females using independent samples *t*-tests.

RESULTS

Overall, total and free testosterone increased in a linear fashion at 15- and 30-minutes post testosterone administration, peaking at 30 minutes, and declining 60 minutes following administration (see Figures 11 and 12 in the Appendix for individual participant plots).

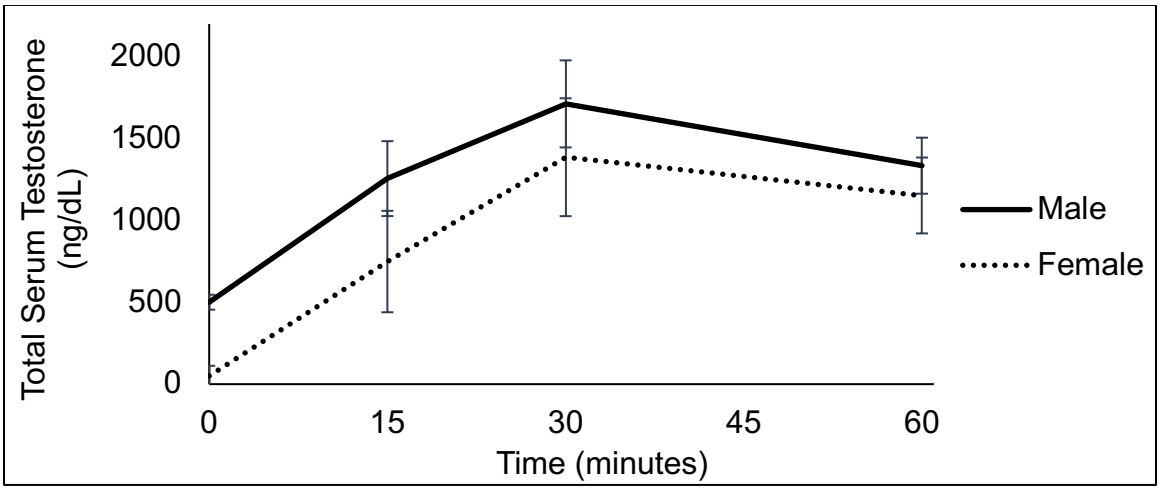
As expected, male and female participants differed significantly in baseline total ($t(18) = -6.898$, $p < .000$) and free ($t(18) = -4.013$, $p = .001$) testosterone levels. Mean total and free concentrations did not differ significantly between male and female participants for other time points (all p's > .05).

Further, male and female participants did not differ significantly from each other in terms of increase (AUC_I) in total or free testosterone from baseline, nor total or free testosterone output (AUC_G) (all p's > .05).

Males

Male participants' total testosterone concentrations increased from baseline values of 500.57 ng/dL (*SD* 169.67) to 1255.57 ng/dL (*SD* 762.09) 15 minutes post-administration, peaking at 1712.10 ng/dL (*SD* 912.32) 30 minutes post-administration, and declining to 1334.62 ng/dL (*SD* 548.80) 60 minutes post-administration. This corresponded to increases in total testosterone concentrations by 150.8% ($t(12) = -3.948$, $p = .002$, $d = 1.368$), 242.0% ($t(11) = -5.014$, $p < .001$, $d = 1.846$), and 166.6% ($t(10) = -5.989$, $p < .001$, $d = 2.053$) over baseline levels at the 15, 30, and 60 minute time points, respectively (see Figure 2 for a plot of this data).

Figure 2. Total serum testosterone concentrations by sex

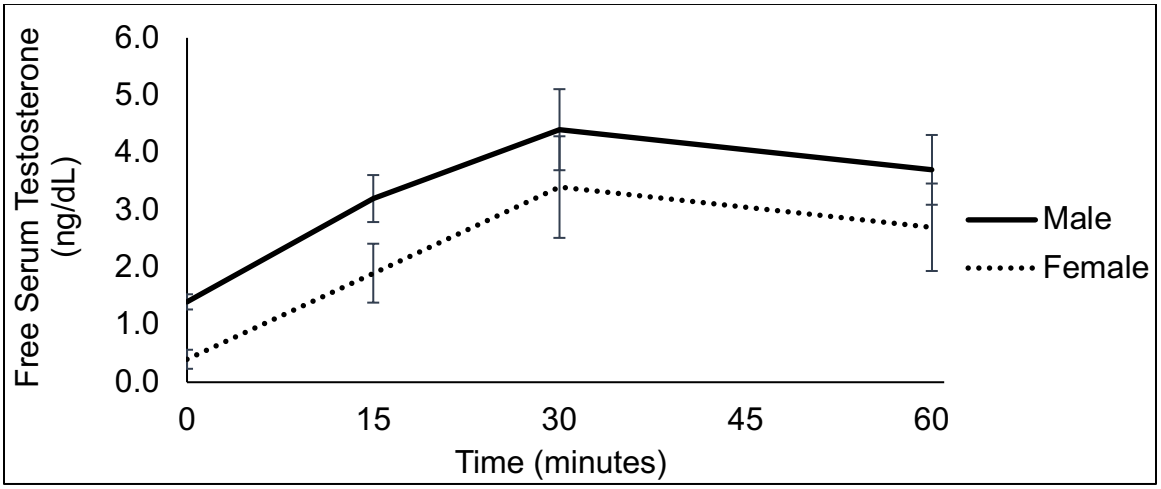


Note. Error bars indicate individual standard error for each time point separately for males and females.

Male participants' free testosterone concentrations increased from baseline values of 1.39 ng/dL (*SD* 0.62) to 3.24 ng/dL (*SD* 1.98) 15 minutes post-administration, peaking at 4.43 ng/dL (*SD* 2.41) 30 minutes post-administration, and declining to 3.70 ng/dL (SD

2.15) 60 minutes post-administration. This corresponded to increases in total testosterone concentrations by 133.1% ($t(12) = -4.324$, $p = .001$, $d = 1.261$), 218.7% ($t(11) = -4.873$, $p < .001$, $d = 1.728$), and 166.2% ($t(10) = -3.982$, $p = .003$, $d = 1.460$) over baseline levels at the 15, 30, and 60 minute time points, respectively (see Figure 3 for a plot of this data).

Figure 3. Free serum testosterone concentrations by sex



Note. Error bars indicate individual standard error for each time point separately for males and females.

Females

Female participants' total testosterone concentrations increased from baseline values of 50.34 ng/dL (*SD* 23.88) to 748.93 ng/dL (*SD* 617.40) 15 minutes post-administration, peaking at 1386.25 ng/dL (*SD* 744.11) 30 minutes post-administration, and declining to 1152.72 ng/dL (SD 603.76) 60 minutes post-administration. This corresponded to increases in total testosterone concentrations by 1387.7% ($t(5) = -2.692$, $p = .043$, $d = 1.599$), 2653.6% ($t(5) = -4.301$, $p = .008$, $d = 2.538$), and 2189.7% ($t(5) = -$

4.397, p = .007, d = 2.580) over baseline levels at the 15, 30, and 60 minute time points, respectively (see Figure 2 for a plot of this data).

Female participants' free testosterone concentrations increased from baseline values of 0.36 ng/dL (*SD* 0.34) to 1.89 ng/dL (*SD* 1.44) 15 minutes post-administration, peaking at 3.35 ng/dL (*SD* 2.01) 30 minutes post-administration, and declining to 2.69 ng/dL (SD 1.77) 60 minutes post-administration. This corresponded to increases in free testosterone concentrations by 425.0% (t(6) = -2.797, p = .031, d = 1.462), 830.6% (t(6) = -3794, p = .009, d = 2.074), and 647.2% (t(6) = -3.644, p = .011, d = 1.828) over baseline levels at the 15, 30, and 60 minute time points, respectively (see Figure 3 for a plot of this data).

DISCUSSION

The present study established the pharmacokinetic profile of a novel intranasal testosterone delivery system in both healthy men and women. In contrast to existing testosterone pharmacokinetic studies sampling mostly hypogonadal men, the present study fills a critical gap in the literature by employing a healthy, eugonadal sample of both men and premenopausal women.

Due to paucity of exogenous testosterone pharmacokinetic data from eugonadal men, direct comparisons of time to peak concentrations and percent increase from baseline are limited. In the present study, 13 eugonadal men reached mean peak total serum concentrations (1712.10 ng/dL) 0.5 hours following administration of 14 mg testosterone via intranasal spray, resulting in 242.0% increase from baseline (500.57 ng/dL). In comparison, five eugonadal men reached mean peak total serum concentrations (1956

ng/dL) 0.5 hours following administration of 10 mg testosterone via transbuccal tablet, resulting in 531.0% increase from baseline (approximately 310 ng/dL) (Kim, Snipes, Hodgen, & Anderson, 1995). It is worth noting that baseline total serum concentrations were significantly lower in the transbuccal study (approximately 310 ng/dL, Kim et al., 1995), than in the present study (500.57 ng/dL), which may have resulted in ceiling effects in the present study.

Although a direct comparison to the present study is not appropriate due to differences in gonadal status, in a sample of eight hypogonadal men, participants reached mean peak total serum concentrations 1.4 hours following 15.2 mg intranasal gel administration, resulting in approximately 514.6% increase from baseline (Mattern et al., 2008). In another study with 29 hypogonadal men, participants reached mean peak total serum concentrations 18 hours following 50 mg transdermal gel administration, resulting in approximately 140.0% increase from baseline (Marbury et al., 2003).

Few exogenous testosterone pharmacokinetic studies are available in healthy, premenopausal women. In the present study, 6 eugonadal, premenopausal women reached mean peak total serum concentrations (1386.25 ng/dL) 0.5 hours following administration of 14 mg testosterone via intranasal spray, resulting in 2653.6% increase from baseline (50.34 ng/dL). In comparison, 16 eugonadal, premenopausal women reached mean peak total serum concentrations (673 ng/dL) 0.23 hours following administration of 0.75 mg testosterone via sublingual solution, resulting in 3265% increase from baseline (20 ng/dL) (van Rooij et al., 2012).

One other comparison comes from an unpublished report in which 0.5 mg of sublingual testosterone administration in healthy premenopausal women resulted in "a tenfold increase in total testosterone" 15 minutes after intake, or approximately a 1000% increase from baseline (Tuiten & van Honk, unpublished data; Tuiten et al., 2000). Time to peak concentration, and peak percent increase from baseline were not reported.

Overall, time to peak concentrations and percent testosterone increase from baseline to peak concentrations obtained from the present study were comparable to transbuccal and sublingual administration studies with eugonadal men and women. However, unlike the direct-to-central-nervous-system delivery associated with intranasal testosterone (via direct transmission through trigeminal and olfactory nerve pathways), oral administration of testosterone (e.g., transbuccal and sublingual) is subject to metabolic degradation, and associated with increased risk of liver damage (Gluud et al., 1983; Werner, Hanger, & Kritzler, 1950). Compared to studies using similar administrative route (i.e., intranasal), the testosterone spray used in the present study resulted in shorter time to peak concentration, likely due to the usage of a more readily absorbed aqueous form of testosterone, compared to a viscous intranasal gel. Moreover, unlike studies using transdermal administration, the testosterone spray in the present study resulted in larger testosterone percent increase from baseline and few, if any, risks associated with unintended, secondary contamination.

As with any study, the present findings must be interpreted in light of limitations. First, the study was conducted utilizing a convenience sample consisting of participants relatively similar in terms of age, body mass, and gonadal status. Replication of findings

using heterogeneous samples is needed. Second, since pharmacokinetic analysis was the primary outcome of interest, the present study was not blinded and did not include an active comparator or a placebo control. Third, the pharmacokinetic data does not extend beyond 60 minutes following the baseline sample. Relative to the 30-minute time point, both free and total testosterone levels for men and women are lower, suggesting the beginning of the descending limb. Nonetheless, an extension of the present study with serum samples throughout a 24-hour observational period is needed. Fourth, the present study had a relatively small sample of 20 participants; and individual pharmacokinetic profiles indicate heterogeneity among participants' responses (Figures 11 and 12). Replication with a larger sample would be helpful in bolstering the present findings, particularly with regard to timing of peak testosterone concentrations. Fifth, testosterone administration may have impacted endogenous testosterone production. Levels of gonadal hormones in the brain are typically modulated via a negative feedback loop. In a healthy brain, gonadotropin-releasing hormone (GnRH) from the hypothalamus signals the production and secretion of the gonadotropins, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Once the gonadotropins are secreted into the blood stream, LH acts on its receptor in the gonads, which in turn stimulates the release of the sex steroids, such as testosterone. Testosterone then complete the negative feedback loop by inhibiting the release of GnRH (Blair, McGee, Bhatta, Palm, & Casadesus, 2015). It is possible that exogenous testosterone may have inhibited the production of endogenous testosterone through the negative feedback loop. Sixth, the time of day for testosterone administration was not controlled. Levels of testosterone follow a diurnal pattern, with levels peaking between

0530 and 0800 h, and trough levels occurring approximately 12 hours later (Brambilla, Matsumoto, Araujo, & McKinlay, 2009; Bremner, Vitiello, & Prinz, 1983; Cooke, McIntosh, & McIntosh, 1993; de la Torre, Sjöberg, Hedman, Bártfai, & Diczfalusy, 1981; Diver, Imtiaz, Ahmad, Vora, & Fraser, 2003). It would be important to account for differences in endogenous testosterone levels based on time of day in future studies. Lastly, the intranasal administration of testosterone resulted in post-nasal drip, which prohibited salivary testosterone samples from being effectively assayed due to contamination, and therefore no salivary data is available.

Despite these limitations, this study provides essential evidence for the effectiveness and utility of intranasally-delivered testosterone in both healthy men and women, and demonstrates a novel mode of exogenous testosterone administration for psychological research purposes.

Chapter 3: Effects of Exogenous Testosterone on Subjective Anxiety

ABSTRACT

Can a single-dose of a testosterone-containing nasal spray reduce anxiety? Although both exogenous and endogenous testosterone have been associated with reductions in implicitly measured fear responses, it remains unknown whether exogenous testosterone can reduce the explicit, subjective experience of anxiety in humans. In the present study, participants (N = 104, 48.1% female) were randomly assigned to receive either testosterone or placebo via intranasal spray before taking part in an acute psychosocial stressor. Participants used visual analogue scales to rate their subjective anxiety before, during, and after the stressor. Results revealed a statistically significant drug by sex interaction, in which women—as expected—experienced significantly higher levels of subjective anxiety in the placebo condition compared to men; a sex difference that was eliminated in the drug condition. Further, women randomized to the testosterone condition experienced significantly lower levels of anxiety during recovery from the acute stressor relatively to women in the placebo condition. Taken together, these results have important implications for the etiology of anxiety and treatment.

BACKGROUND

Due to the vital role that fear plays in the survival of the organism, the distinction between normal and pathological anxiety is one of degree rather than kind (Beck & Clark, 1997). Anxiety disorders are characterized by excessive fear and behavioral avoidance in response innocuous stimuli (American Psychiatric Association, 2013), and has the highest prevalence of all psychiatric diagnoses (Kessler, Berglund, et al., 2005; Kessler, Chiu, et

al., 2005). Globally, 1 in every 3 people will be diagnosed with an anxiety disorder in their lifetime (Baxter, Scott, Vos, & Whiteford, 2013), with prevalence and severity twice as high in women than men (Baxter et al., 2013; Gater et al., 1998; McLean et al., 2011). Testosterone has been linked to fear reduction and reductions in anxiety-related behaviors (Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016; Giltay et al., 2012), prompting researchers to point to sex differences in testosterone concentrations as a plausible explanation for the greater prevalence of anxiety disorders in women (Giltay et al., 2012; McHenry et al., 2014; Toufexis, Myers, & Davis, 2006).

Anxiolytic effects of testosterone have been observed in measures of anxiety-like behavior in rodents. Gonadectomy in adult male rodents results in increased anxiety-like behaviors in a battery of behavioral tests, including the elevated plus maze, open field test, and defensive probe-burying, compared to sham-operated controls (Adler et al., 1999; Fernández-Guasti & Martínez-Mota, 2003; Frye & Seliga, 2001; Morsink et al., 2007; Slob et al., 1981), effects that were reversed by testosterone replacement (Adler et al., 1999; Fernández-Guasti & Martínez-Mota, 2005; Frye & Seliga, 2001; Slob et al., 1981). Moreover, testosterone replacements were equally as effective as the administration of the typical tricyclic antidepressant imipramine in alleviating anxiety-like behaviors induced by two-weeks of chronic social isolation (Carrier & Kabbaj, 2012). In intact adult female rodents, injections of testosterone or its metabolites reduced anxiety-like behaviors in the open field test, elevated plus maze (Frye & Lacey, 2001), and defensive burying (Gutiérrez-García et al., 2009), compared to vehicle-treated controls.

To date, only a handful of experimental studies have directly tested the anxiolytic effect of testosterone in humans. Consistent with animal research, compared to placebo-treated controls, administration of testosterone resulted in diminished preconscious selective attention to threatening stimuli (van Honk et al., 2005), reduced gaze avoidance in individuals with social anxiety disorder (Enter, Terburg, et al., 2016), reduced fear-potentiated startle response (Hermans et al., 2006), and increased behavioral approach (Enter et al., 2014; Enter, Spinhoven, et al., 2016).

Although quite promising, there are several limitations associated with existing testosterone administration studies in humans. First, no male subjects were tested in conjunction with females, raising several important questions. Second, despite the fact that the majority of clinical assessments of anxiety require patients to report on their subjective experiences of anxiety, limited research has examined the effect of exogenous testosterone on self-reported anxiety. Of the few existing studies, all have reported null effect of drug administration (e.g., Hermans et al., 2007; van Honk et al., 2005). In addition to the possibility that testosterone has no effect on subjective anxiety, it is plausible that these null effects were the result of an insufficiently emotionally provocative task. Specifically, van Honk et al. (2005) used an emotional Stroop task, which has not been consistently associated with self-reported measures of anxiety (e.g., Amir et al., 1996; Becker, Rinck, Margraf, & Roth, 2001; de Ruiter & Brosschot, 1994; Egloff & Hock, 2001; Richards, French, Johnson, Naparstek, & Williams, 1992). Third, considering that clinical assessment and diagnosis of anxiety via emotional Stroop or startle response in clinical settings is not feasible nor appropriate, but is rather accomplished via a patient's subjective

assessment of his or her anxiety, a more clinically valid test of the putative anxiolytic effects of exogenous testosterone in response to ecologically valid stimuli is warranted.

The Trier Social Stress Test (TSST), originally developed as a tool to elicit psychosocial stress in the laboratory, incorporates public speaking as a part of its protocol (Kirschbaum, Pirke, & Hellhammer, 1993). Considering that public speaking is one of the most common fears in the general population (Stein, Torgrud, & Walker, 2000; Stein, Walker, & Forde, 1994, 1996), it is not surprising that the induction of the TSST is consistently associated with significant increases in self-reported stress and anxiety (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014), making it a robust, anxiety-eliciting task for laboratory research.

Moreover, unlike observations of behavioral outcomes (e.g., magnitude of startle response), which do not reliably distinguish between different emotional states (e.g., Cornwell, Johnson, Berardi, & Grillon, 2006; Elsesser, Sartory, & Tackenberg, 2004; Kaviani et al., 2004; Vrana, Spence, & Lang, 1988), self-report scales are easy to administer and provide a better assessment of subjective experiences (Flynn, van Schaik, & van Wersch, 2004; Hasson & Arnetz, 2005). Of various self-report measures, visual analogue scales offer superior measurement characteristics by allowing for greater variances in scores compared to traditional Likert scales (Flynn et al., 2004; Hasson & Arnetz, 2005). In particular, visual analogues are less susceptible to memory effects (Hasson & Arnetz, 2005), and thus can be effectively administered repeatedly throughout a study (e.g., Ali, Nitschke, Cooperman, & Pruessner, 2017). As a result, rather than using a single measure to capture the subjective experience of anxiety in response to a stimulus,

repeated measures allows for more nuanced assessment of changes over time, and also the computation of metrics such as stress-evoked subjective anxiety (AUC_I) (Ali et al., 2017; Pruessner et al., 2003).

Further, repeated measures of subjective anxiety over time allows for the comparison of intervals of interest across the course of reactivity. One period of interest is recovery following exposure to the TSST. Slower and poorer recovery following acute stress has been associated with greater anxiety symptoms (reviewed in Chida & Hamer, 2008). Presently, it is not clear whether exogenous testosterone has any effect on psychological recovery following acute stress.

Taken together, existing research point to several gaps in the current literature. The present study aims to fill these gaps by investigating possible effects of testosterone administration on subjective anxiety in both men and women, in response to a robust, emotionally-evocative stimulus.

METHODS

Design

A mixed-sex, repeated measures research design was used to examine the effects of single-dose, intranasal testosterone administration in response to the TSST. Participants were randomized to receive placebo or testosterone administration via intranasal spray (see Study 1). In addition to baseline trait measures, acute self-reported anxiety was assessed across multiple time points corresponding to pre-, during-, and post-stressor using visual analogue scales. All procedures involving human participants were approved and in

accordance with the ethical standards of The University of Texas at Austin Institutional Review Board (IRB# 2015-07-0024).

Participants

Power Analysis

Based on medium to large effect sizes seen in previous testosterone administration studies in humans (e.g., Bos, van Honk, Ramsey, Stein, & Hermans, 2013; Hermans, Putman, Baas, et al., 2006; Hermans, Ramsey, & van Honk, 2008; van Honk et al., 2004), the present study was powered to identify a medium effect size. Power analyses using G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007), with alpha set to .05, indicated that a sample size of 110 participants would have greater than .80 power to detect a medium effect ($f = .3$) between conditions.

Descriptive Statistics

One hundred and ten participants registered in an introductory psychology class from The University of Texas at Austin enrolled in the study for course credit. Eligible participants were English-speaking adults between ages 18-30 with body mass index (BMI) between 17 kg/m^2 and 35 kg/m^2. Participants were excluded if they had history of any psychological or physical illness, or medication use that would impact endocrine functioning or preclude testosterone administration. In addition, participants with daily recreational drug use (including, but not limited to, nicotine and alcohol), were excluded from participation. Female participants currently using hormonal birth control, or experiencing irregular periods were also excluded from participation.

Of the 110 participants enrolled in the study, two participants did not complete the study due to adverse effects associated with the TSST. Three participants were not able to complete the study due to discomfort or difficulties associated with nasal spray administration. And one participant was excluded from data analyses due to incomplete data.

The final sample of 104 participants (48.1% female) was approximately one-third Hispanic or Latino (28.8%), one-third Asian (31.7%), and one-third White (29.8%), with mean age of 19.05 ± 1.05 years and BMI of 23.06 ± 3.18 kg/m^2. Most participants were born in the United States (78.8%), and nearly half (56.7%) spoke language(s) other than English (see Table 2 for demographic characteristics by sex).

Table 2. Descriptive Statistics of Participants from Study 2 by Group and Sex

	Placebo (*n* = 52)				**Testosterone (*n* = 52)**			
	Male (*n* = 27)		**Female (*n* = 25)**		**Male (*n* = 27)**		**Female (*n* = 25)**	
	M / %	(SD) / (n)	M / %	(SD) / (n)	M / %	(SD) / (n)	M / %	(SD) / (n)
Demographics								
Age (years)	19.2	(1.3)	19.1	(0.9)	19.0	(0.9)	18.8	(1.0)
BMI (kg/m^2)	22.6	(2.5)	23.4	(3.9)	23.3	(2.6)	23.0	(3.7)
Ethnicity (Hispanic or Latino)	29.6%	(8)	32.0%	(8)	25.9%	(7)		(6)
Race								
White	33.3%	(9)	40.0%	(10)	33.3%	(9)	16.0%	(4)
Black or African American	3.7%	(1)	12.0%	(3)	3.7%	(1)	20.0%	(5)
Asian	33.3%	(9)	16.0%	(4)	37.0%	(10)	36.0%	(9)
Other	0.0%	(0)	0.0%	(0)	0.0%	(0)	4.0%	(1)
Born in the US (Yes)	70.3%	(19)	92.0%	(23)	85.2%	(23)	68.0%	(17)
Speak language(s) other than English (Yes)	70.0%	(19)	48.0%	(12)	51.9%	(14)	56.0%	(14)
Baseline Measures								
Depression (CESD)	11.6	(6.6)	13.1	(7.6)	11.9	(7.0)	11.7	(7.3)
Anxiety (BAI)	7.6	(5.9)	10.1	(7.3)	6.3	(5.7)	7.2	(7.4)
Anxiety Sensitivity (ASI-3)	14.9	(9.9)	16.6	(9.8)	13.7	(8.2)	12.4	(11.8)
Social Anxiety (FNES)	13.6	(7.7)	16.6	(6.5)	13.7	(8.2)	13.5	(7.7)
Baseline Subjective Anxiety (VAS)	1.7	(1.8)	1.9	(1.8)	1.8	(1.6)	1.7	(1.7)

Note. M = mean, SD = standard deviation. Where appropriate, percentage (%) and number of participants (n) are provided instead of mean (M) and standard deviation (SD).

Procedures

Scheduling

All female participants were scheduled during the estimated mid-luteal phase of their menstrual cycles (between 17th and 24th day following the most recent onset of menstruation) in order to minimize the possible effects of cycling gonadal hormones on endocrine response (Kirschbaum et al., 1993; Viau, 2002). To assess cycle phase, female participants were asked to recall the first day of their most recent menstruation during a telephone screening. Male participants were scheduled according to the availability of laboratory facilities and the experimenter.

Pregnancy Test

Following informed consent, all female participants conducted a mandatory urinary pregnancy test (Wondfo Pregnancy Strips, Willowbrook, IL) due to testosterone's potential adverse effects on the fetus. Negative pregnancy status was verified prior to proceeding with drug administration.

Baseline Questionnaires

All participants completed baseline measures of depression, anxiety, anxiety sensitivity, and fear of negative evaluation (social anxiety) prior to testosterone administration.

Testosterone Administration

Participants were randomized to either placebo or testosterone (stratified by sex) using a random sequence generated at randomizer.org. Experimenters and participants were blind to the drug condition. All participants self-administered an aqueous solution

containing either 14 mg testosterone propionate or placebo, via intranasal spray (Figure 1). Participants were instructed to insert a sterile applicator into one nostril and to pump until they felt a full strong spray. Once they felt a full spray, participants were instructed to inhale and alternate between their nostrils until the container is empty. After the self-administration of the nasal spray, participants were asked to relax for 30-minutes to allow for absorption of the hormone. The protocol for intranasal testosterone administration is based on the pharmacokinetic data from Study 1. In Study 1, using the same protocol, dosage, and formulation as used here in Study 2, blood samples obtained via venipuncture showed peak levels for both free (bioavailable) and sex hormone-binding globulin (SHBG)-bound testosterone 30 minutes post-administration in both men and women, which coincide with the start time of the public speaking challenge in the present study (see below).

Public Speaking Challenge

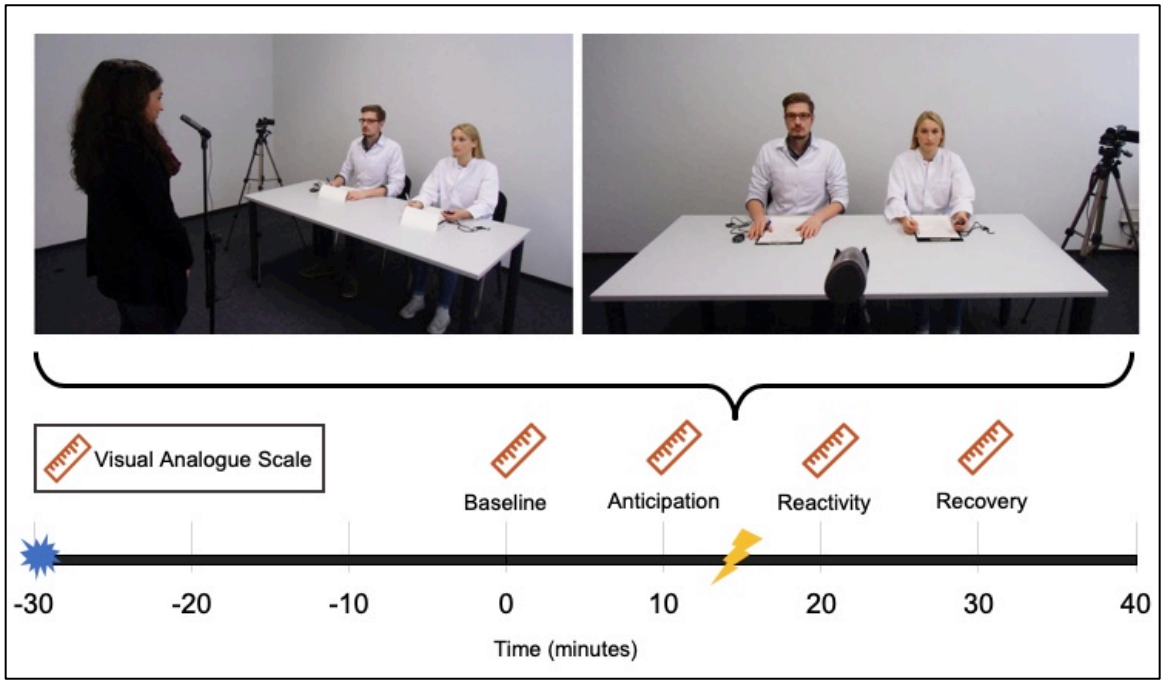
Thirty minutes following testosterone administration, all participants began the Trier Social Stress Test (Birkett, 2011; Kirschbaum et al., 1993). Participants first provided a baseline assessment of subjective anxiety using a visual analogue scale. Immediately after, participants were brought in front of one male and one female confederate and told via a pre-recorded message that they will give a speech of their qualifications for their ideal job to the panel of behavioral experts in front of them. Participants were informed that the behavioral experts will assess their verbal and non-verbal skills, and that their performance will be video recorded for subsequent analyses. Participants were then escorted to a quiet preparatory room, where they were told they had ten minutes to prepare their oral

presentations. In reality, each participant was timed for eight minutes before they were interrupted.

Immediately prior to their speeches, participants were asked to complete a second visual analogue scale corresponding to anticipatory anxiety. When the five minutes allotted for their speeches elapsed, participants were asked to count down from 1,022 in decrements of 13. If an incorrect response was given at any point in the countdown sequence, participants were interrupted and instructed to begin again at 1,022. Participants were given five minutes to complete the countdown and asked to "please continue" if they did not produce an answer after 10 seconds. The confederates on the panel were trained not to provide any verbal or behavioral reinforcement, and were instructed to remain impassive throughout the course of the study.

Following their speeches and mental arithmetic, participants were escorted back to the preparatory room to complete a third visual analogue scale assessing subjective anxiety. Once 10 minutes elapsed, a fourth visual analogue scale was collected. After the collection of the last visual analogue scale, participants were debriefed, awarded five credits for study participation, and dismissed. See Figure 4 for study timeline.

Figure 4. Trier Social Stress Test data collection timeline



Note. Visual analogue scales are collected every 10 minutes throughout the study.

Measures

Baseline Clinical Measures

The Center for Epidemiologic Studies Depression Scale (CESD; Radloff, 1977) is a 20-item, multiple choice (ranging from "rarely or none of the time" to "most or all of the time") self-report questionnaire designed to measure levels of depressive symptomatology in the past week. The CESD assesses symptoms such as inattentiveness and irritability; cognitions such as hopelessness; and physical symptoms such as a lack of appetite and restless sleep.

The Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) is a 21-item multiple choice (ranging from 0 to 3) self-report questionnaire designed to measure

levels of anxious symptomatology. The BAI assesses symptoms such as numbness and tingling, fear of losing control, and catastrophizing.

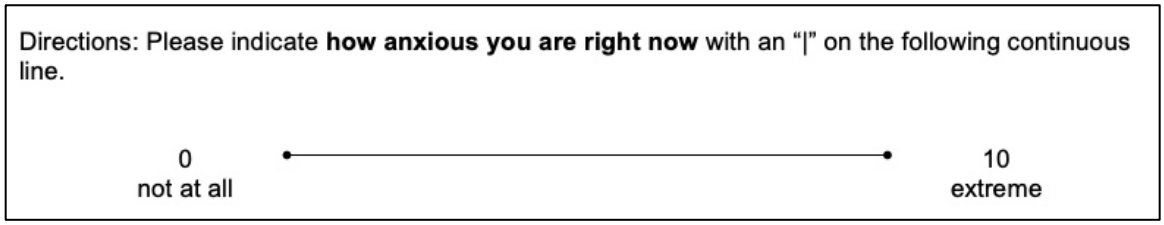
The Anxiety Sensitivity Index-3 (ASI-3; Taylor et al., 2007) is an 18-item self-report questionnaire designed to assesses physical, cognitive, and social concerns associated with the fear of arousal-related sensations. Respondents are asked to rate the extent to which the 18 items describe themselves using five-point Likert scales (ranging from 0 or "very little" to 4 or "very much").

The Fear of Negative Evaluation Scale (FNES; D. Watson & Friend, 1969) is a 30-item, self-report questionnaire designed to measure social anxiety. Respondents are asked to indicate whether each statement is true or false of their personality (e.g., "I rarely worry about seeming foolish to others."). If the choice is difficult, respondents are asked to select the response that is most applicable at the moment.

Acute Subjective Anxiety

A visual analogue scale assessing acute state anxiety was developed for the purpose of this study. Participants were asked to self-report their anxiousness by indicating a position along a continuous line between two end-points: 0 for "not at all anxious" and 10 for "extremely anxious" in response to the prompt "please indicate how anxious you are right now." An example of the visual analogue scale is included as Figure 5.

Figure 5. Visual analogue scale for subjective anxiety



Note. Responses for each time point were measured using a ruler and recorded to the 10th decimal place.

Data Analytic Plan

Pre-Drug Administration Group Comparisons

Multiple one-way between-subjects analysis of variance (ANOVA) were conducted to test whether there were mean group differences in age, BMI, baseline subjective anxiety, depression, anxiety, anxiety sensitivity, and fear of negative evaluation across the four groups (male placebo, female placebo, male testosterone, female testosterone).

Group Differences in Subjective Anxiety Across Sexes and Drug Conditions

A two-way univariate ANOVA was conducted to test group differences in subjective anxiety across sexes and drug conditions. A single value of area under the curve with respect to increase (AUC_I) was calculated for each participant to represent stress-evoked subjective anxiety (Ali et al., 2017; Pruessner et al., 2003). Simple main effects were analyzed following significant interaction between sex and drug condition.

Recovery Comparisons

Independent samples *t*-tests were conducted to test within-sex differences in psychological recovery following the TSST between placebo and the drug condition. A single delta value between time point 2 (peak, anticipatory anxiety) and time point 3 (first recovery time point following the stressor) was calculated for each participant to represent psychological recovery.

Moderation Analyses

Multiple linear regressions were conducted to test moderating effects of trait measures for depression (CESD; Radloff, 1977), anxiety (BAI; Beck, Epstein, Brown, & Steer, 1988), anxiety sensitivity (ASI-3; Taylor et al., 2007), and fear of negative evaluation (FNES; D. Watson & Friend, 1969) on drug condition in predicting subjective anxiety. Analyses were conducted separately for males and females for ease of interpretation (i.e., two-way versus three-way interactions).

RESULTS

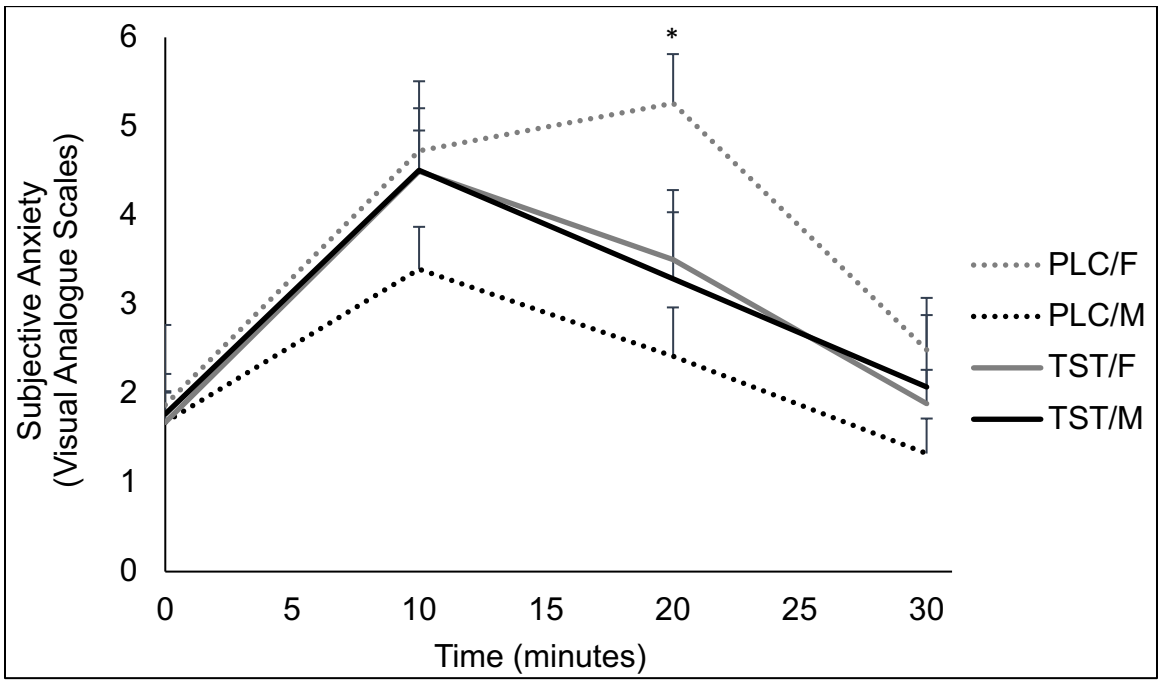
Pre-Drug Administration Group Comparisons

Across four groups (male placebo, female placebo, male testosterone, female testosterone), participants did not differ significantly in age ($F(3,100) = .753$, $p = .523$), BMI ($F(3,100) = .338$, $p = .798$), baseline subjective anxiety ($F(3,100) = .073$, $p = .974$), depression symptomatology ($F(3,100) = .238$, $p = .869$), anxiety symptomatology ($F(3,100) = 1.555$, $p = .205$), anxiety sensitivity ($F(3,100) = .806$, $p = .493$), or fear of negative evaluation ($F(3,100) = 1.01$, $p = .392$). See Table 2 for baseline descriptive statistics.

Group Differences in Subjective Anxiety Across Sexes and Drug Conditions

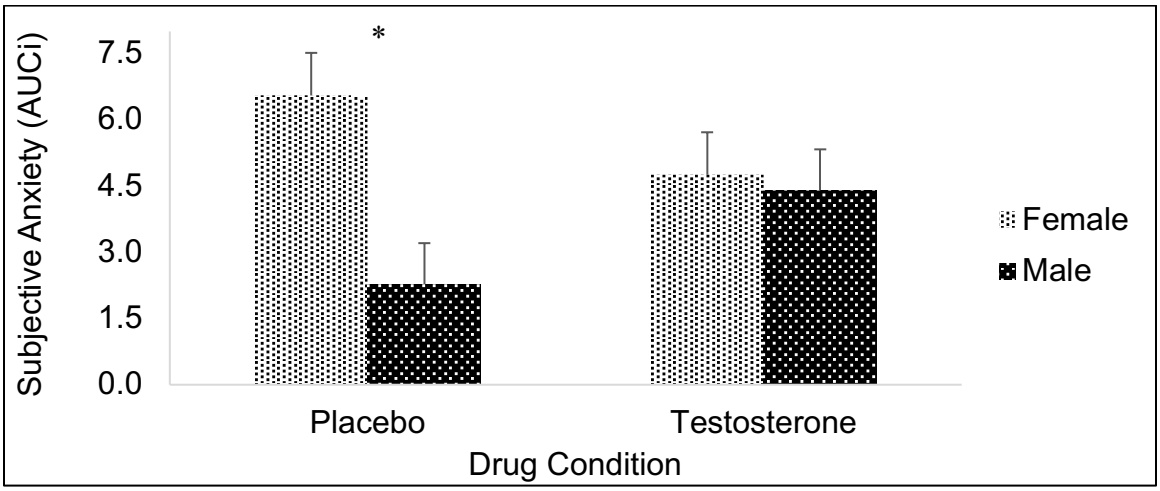
There was a significant two-way interaction between sex and drug condition for stress-evoked subjective anxiety (AUC_I: $F(1,100) = 4.309$, $p = .04$). Additional simple main effects are reported below. See Figure 6 for changes in subjective anxiety across time, Figure 7 for a plot of the AUC_I interaction, and Figure 13 in the Appendix for individual participant plots of changes in subjective anxiety across time.

Figure 6. Subjective anxiety by sex, drug condition, and time



Note. Error bars indicate individual standard error for each time point separately for males (M) and females (F) by drug condition (PLC = Placebo; TST = Testosterone).

Figure 7. Sex by drug condition interaction in subjective anxiety



Note. Error bars indicate standard error.

Across Drug Conditions

A statistically significant difference in subjective anxiety was observed between men and women in the placebo condition ($t(50) = 2.856$, $p = .006$, $d = .788$), such that women reported significantly higher levels of subjective anxiety than men. No difference in subjective anxiety was observed between men and women in the drug condition ($t(50) = .108$, $p = .915$, $d = 0.030$); in other words, testosterone administration reduced the sex difference observed in the placebo condition to non-significance.

Across Sexes

No difference in subjective anxiety was observed between women randomized to the placebo condition versus the drug condition ($t(48) = 1.213$, $p = .231$, $d = 0.343$), nor between men randomized to the placebo condition versus the drug condition ($t(52) = -1.915$, $p = .061$, $d = 0.521$).

Recovery Comparisons

There was a significant difference in post-stressor recovery between the placebo and drug condition in women ($t(48) = 2.154$, $p = .036$, $d = 0.609$), such that there was an increase in subjective anxiety in the placebo condition, but a decrease in anxiety in the testosterone condition from peak to recovery. No difference in post-stressor recovery was observed between men randomized to the placebo condition versus the drug condition ($t(52) = .417$, $p = .678$, $d = 0.113$).

Moderation Analyses

Males

Changes in subjective anxiety across drug conditions was not moderated by measures of depression ($\beta = 0.179$, $p = 0.576$), anxiety ($\beta = 0.366$, $p = 0.128$), anxiety sensitivity ($\beta = 0.404$, $p = 0.146$), or fear of negative evaluation ($\beta = 0.224$, $p = 0.480$).

Females

Changes in subjective anxiety across drug conditions was not moderated by measures of depression ($\beta = 0.470$, $p = 0.121$), anxiety ($\beta = 0.113$, $p = 0.653$), anxiety sensitivity ($\beta = -0.233$, $p = 0.378$), or fear of negative evaluation ($\beta = -0.070$, $p = 0.845$).

DISCUSSION

The present study examined the joint influence of sex and 14 mg testosterone administration on self-reported anxiety, in response to a psychosocial challenge. To date, this is the first study to test healthy male and female subjects in parallel using the same exogenous testosterone administration protocol. Results revealed a statistically significant sex by drug interaction, in which women experienced significantly higher levels of stress-

evoked subjective anxiety in the placebo condition compared to men, a sex difference that was eliminated in the drug condition. These differences were not moderated by individual differences in depression, anxiety, anxiety sensitivity, or fear of negative evaluation. Considering the substantial, pervasive sex differences in the prevalence of anxiety disorders worldwide (Gater et al., 1998; McLean et al., 2011), this finding is particularly noteworthy, and suggests that sex differences in health disparity may be mediated by differences in levels of testosterone. Further, results demonstrate that women reported significantly less subjective anxiety during recovery to acute stress following testosterone administration versus placebo administration. A significant body of research suggest that slower recovery from acute stressors is a significant risk factor for the development and maintenance of both psychological and physical illnesses (reviewed in Brosschot, Pieper, & Thayer, 2005; Burke, Davis, Otte, & Mohr, 2005; Chida & Hamer, 2008). Specifically, the failure to shut down the stress response results in greater exposure to hormones such as cortisol, which has significant deleterious effects on health following prolonged exposure (McEwen, 1998; McEwen & Stellar, 1993; Sapolsky, Romero, & Munck, 2000). This finding suggests that the anxiolytic effects of testosterone may be exerted through its protective effects during acute stress recovery.

Anxiety disorders are associated with both structural and functional abnormalities within the amygdala and hippocampus (Shin & Liberzon, 2010), two brain regions that appear to be strongly influenced by testosterone (McHenry et al., 2014). In both humans and rodents, the amygdala plays a major role in modulating anxiety and fear responses (reviewed in Rauch, Shin, & Wright, 2003; Ressler, 2010; Rodrigues, LeDoux, &

Sapolsky, 2009). Hyperactivity of the amygdala has been associated with increased anxiety symptoms in humans (Shin & Liberzon, 2010), and in rodents models (Singewald, 2007). Consistent with findings of the present study, neuroimaging studies examining the anxiolytic effects of testosterone found sexually divergent associations between testosterone and amygdala activation. Specifically, greater levels of testosterone are associated with increased amygdala activation in men (Derntl et al., 2009), but not in women (Stanton et al., 2009).

The hippocampus has also been extensively documented as a critical site involved in anxiety disorders. Structural abnormalities and reduced activity in the hippocampus are observed in individuals with anxiety disorders, compared to healthy controls (Ferrari, Busatto, McGuire, & Crippa, 2008). Chronic stress is associated with hippocampi atrophy and impaired neurogenesis (McEwen & Magarinos, 2001). These effects can impair memory, impact hypothalamic-pituitary-adrenal axis negative feedback, and contribute to the development of anxiety symptoms (Revest et al., 2009). With regard to present findings, there is evidence suggesting that adult hippocampal neurogenesis is sexually dimorphic, with females exhibiting higher cell proliferation than males (Galea & McEwen, 1999). Preclinical evidence suggests that testosterone exerts anxiolytic and protective cellular effects in the hippocampus. Although its underlying mechanisms are not clearly understood, some of the protective effects of testosterone in the hippocampus may be due to testosterone's ability to lessen the aversive effects of stress and facilitate molecular mechanisms that favor cell proliferation, growth and/or survival (Galea & McEwen, 1999; McEwen, 1998; McEwen & Stellar, 1993).

In addition to sexual dimorphism in the amygdala and the hippocampus, sex differences in subjective anxiety following testosterone administration may also be explained in part by sex differences in the salience of instrumentally oriented tasks that speak directly to one's competence, such as public speaking challenges (Stroud, Salovey, & Epel, 2002). Instrumental traits (e.g., dominance, competitiveness, and self-confidence) tend to be more central to men's self-construal than to women's (Cross & Madson, 1997), which may lead to greater desire to maintain social status (Josephs, Newman, Brown, & Beer, 2003; Josephs, Sellers, Newman, & Mehta, 2006; Lee, Gino, Jin, Rice, & Josephs, 2015; Mehta & Josephs, 2010; Mehta, Lawless DesJardins, van Vugt, & Josephs, 2017; Sherman, Lerner, Josephs, Renshon, & Gross, 2016). Testosterone administration in men may further highlight the salience associated with status, and thus increase anxiety due to the enhanced sense of failure created by the Trier Social Stress Test, regardless of one's objective performance. Additional research using stressors that more salient to women (e.g., a social rejection stressor) with a mix-sex sample is warranted to better understand the underlying mechanism associated with sex differences in the present study.

Differences in subjective anxiety during recovery between women randomized to placebo versus testosterone may be explained in part by differences in rumination due to perceived failure. In particular, administration studies in healthy females show that exogenous testosterone rapidly reduces functional coupling of the amygdala with the orbitofrontal cortex, and enhanced amygdala coupling with the thalamus (van Wingen et al., 2010). Specifically, this decoupling decreases emotional rumination by either inhibiting signals from the amygdala to the orbitofrontal cortex, or redirecting those signals away

from the orbitofrontal cortex (van Wingen, Ossewaarde, Bäckström, Hermans, & Fernández, 2011). It is possible that one of the mechanisms for reduced subjective anxiety is reductions in rumination following perceived failure.

The finding that exogenous testosterone is associated with lower subjective anxiety and faster recovery in response to stress has significant clinical implications. Extensive research suggests that slower psychological recovery following acute stress (i.e., rumination), is associated with greater anxiety symptoms (McLaughlin & Nolen-Hoeksema, 2011; Mellings & Alden, 2000). Moreover, current evidence suggest that a large subset of individuals diagnosed with an anxiety disorder do not recover following evidence based psychological or pharmacological treatments (Blanco et al., 2003; Gould, Ott, & Pollack, 1995; Gould, Otto, Pollack, & Yap, 1997; Hofmann & Bögels, 2006), highlighting the need for new treatment strategies that enhance remission rates. A novel line of research has shown that treatment effects are augmented by pairing exposure therapy with a pharmacological agent (Hofmann, Fang, & Gutner, 2014; Otto, Behar, Smits, & Hofmann, 2009; Otto, Smits, & Reese, 2005; Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015; Smits, Reese, Powers, & Otto, 2010). Given the present findings in women, combined with previous research showing that exogenous testosterone promotes social approach behavior in individuals with social anxiety disorder (Enter, Spinhoven, et al., 2016), it would be interesting for future investigations to explore whether the addition of testosterone as a pharmacological enhancer may augment the efficacy of exposure therapy in anxiety disorders.

In conclusion, the present findings demonstrate sex-dependent anxiolytic effects of testosterone in response to a psychological stressor. Taken together, these findings contribute to a better understanding of potential mechanisms associated with the development and maintenance of anxiety symptoms, and may help inform more efficacious treatments.

Chapter 4: Effects of Exogenous Testosterone on Cognitive Performance

ABSTRACT

Can a single-dose of exogenous testosterone improve cognitive performance by reducing subjective anxiety? The present study extended findings from Study 2, and examined the effect of 14 mg intranasal testosterone on acute subjective anxiety and performance on the quantitative section of the Graduate Record Exam (GRE-Q) in a large, randomized, placebo-controlled trial (N = 150, 100% female). Participants completed pre-drug administration trait anxiety measures, and rated their acute subjective anxiety using visual analogue scales before and after the GRE-Q. Results revealed a statistically significant anxiolytic effect of exogenous testosterone relative to placebo. Moreover, this drug effect was moderated by math anxiety, such that exogenous testosterone exerted the greatest anxiolytic effects for participants with highest levels of pre-drug administration math anxiety. No difference in GRE-Q performance was found between drug conditions. Instead, GRE-Q performance was predicted by correlates of general intelligence, including quantitative SAT performance, and verbal intelligence.

BACKGROUND

Test anxiety is one of the most pervasive academic impediments among students worldwide (Lowe & Ang, 2012). It describes a cluster of symptoms characterized by heightened anxiety before and/or during the taking of a test (Sarason, 1980) and has been associated with significant barriers to learning and performance (Andrews & Wilding,

2004). Compared with their low anxiety peers, highly test-anxious students score about 12 percentiles below on standardized testing (Cassady & Johnson, 2002; R. Hembree, 1988; McDonald, 2001), and are significantly more likely to drop out of school (Andrews & Wilding, 2004; Pritchard & Wilson, 2003; Vaez & Laflamme, 2008). Not surprisingly, test anxiety can have broad negative impacts on general well-being and life satisfaction. A greater understanding of the etiology of test anxiety is warranted, and may lead to the development of better and more efficacious treatment for what is currently seen as an insidious and difficult-to-treat anxiety disorder.

Research on the neuroendocrine basis of anxiety disorders in humans has linked testosterone to reductions in subjective anxiety (Study 2), and anxiety related behaviors (Enter et al., 2014; Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016; Hermans et al., 2006; van Honk et al., 2005). Presently, it is unknown whether testosterone administration will affect test performance. The present study aims to replicate and extend the findings from Study 2 by investigating the effect of testosterone administration on subjective anxiety and standardized test performance (using a quantitative section of the Graduate Record Examination, GRE-Q). It is hypothesized that testosterone administration will reduce subjective anxiety, and in turn, mediate improvements in GRE-Q performance. A secondary hypothesis is that trait test anxiety will moderate the effect of testosterone administration, such that the largest effects of testosterone administration will appear in individuals highest in test anxiety.

METHODS

Design

A between-subjects, randomized, placebo-controlled research design was used to test the effect of single-dose, intranasal testosterone administration on GRE-Q performance. In addition to baseline trait measures (i.e., test anxiety, math anxiety, trait anxiety, and anxiety sensitivity), acute subjective anxiety was assessed before and after the GRE-Q using visual analogue scales. All procedures involving human participants have been approved and are in accordance with the ethical standards of The University of Texas at Austin Institutional Review Board (#IRB 2016-11-0049).

Participants

Power Analysis

Based on medium to large effect sizes seen in previous testosterone administration studies in humans (e.g., Bos et al., 2013; Hermans et al., 2006, 2008; van Honk et al., 2004), the present study was conservatively powered to detect a medium effect size. Power analyses using G*Power 3 (Faul et al., 2007), with alpha set to .05 indicated that a sample size of 110 participants will have greater than .80 power to detect a medium effect ($f = .3$) between conditions.

Descriptive Statistics

One hundred and fifty female participants registered in an introductory psychology class from The University of Texas at Austin enrolled in the study for course credit. Eligible participants were English-speaking adults between ages 18 and 30, with body mass index (BMI) between 17 kg/m^2 and 35 kg/m^2. Only female participants were recruited for this

study due to prior research (Study 2) demonstrating an anxiolytic effect of exogenous testosterone in women, but not in men. Participants were excluded if they had history of psychological or physical illness, or medication use that would impact endocrine functioning or preclude testosterone administration (e.g., breast/ovarian cancer, diabetes, thyroid disease). In addition, participants with daily recreational drug use (including, but not limited to, nicotine and alcohol), were excluded from participation. Participants currently using hormonal birth control, or experiencing irregular periods were also excluded from participation.

The sample was approximately one-quarter Hispanic or Latino (25.7%), one-quarter Asian (26.3%), and one-third White (34.9%), with mean age of 18.93 ± .99 years and BMI of 22.52 ± 2.96 kg/m^2. Most participants were born in the United States (82.2%) and nearly half (46.7%) spoke language(s) other than English (see Table 3 for demographic characteristics by group). All participants provided written informed consent in accordance with The University of Texas at Austin Institutional Review Board prior to taking part in the study.

Table 3. Descriptive Statistics of Participants from Study 3 by Group

	Placebo (*n* = 75)		**Testosterone (*n* = 75)**	
	M / %	(SD) / (n)	M / %	(SD) / (n)
Demographics				
Age (years)	19.0	(1.1)	18.9	(0.9)
BMI (kg/m^2)	22.4	(3.1)	22.6	(2.8)
Ethnicity (Hispanic or Latino)	25.3%	(19)	25.3%	(19)
Race				
White	37.3%	(28)	33.3%	(25)
Black or African American	8.0%	(6)	14.7%	(11)
Asian	22.7%	(17)	25.3%	(19)
Other	6.7%	(5)	1.3%	(1)
Born in the US (Yes)	80.0%	(60)	86.6%	(65)
Speak language(s) other than English (Yes)	49.3%	(37)	58.7%	(44)
Baseline Measures				
Test Anxiety (CTAS)	45.3	(13.8)	41.1	(12.8)
Math Anxiety (AMAS)	23.0	(6.4)	21.5	(7.3)
Trait Anxiety (BAI)	9.4	(9.0)	7.8	(7.9)
Anxiety Sensitivity (ASI-3)	15.0	(10.8)	13.3	(10.2)
Baseline Subjective Anxiety (VAS)	2.2	(2.2)	2.4	(2.1)

Note. M = mean, SD = standard deviation. Where appropriate, percentage (%) and number of participants (n) are provided instead of mean (M) and standard deviation (SD).

Procedures

Baseline Questionnaires

All participants completed a demographic profile consisting of age, gender, ethnicity, high school grade point average (GPA), and SAT score, in addition to a series of study-specific questionnaires (test anxiety, math anxiety, trait anxiety, and anxiety sensitivity) prior to enrollment. Both the demographic profile and pre-enrollment

questionnaires were included as a part of the institution-approved pre-screen process associated with the online recruitment system used for research participation and entirely voluntary on the part of the participant.

Scheduling

Eligible participants were scheduled during the estimated mid-luteal phase of their menstrual cycles (between 17th and 24th day following the most recent onset of menstruation) in order to minimize the possible effects of cycling gonadal hormones on endocrine response (Kirschbaum et al., 1993; Viau, 2002). To assess cycle phase, participants were asked to recall the first day of their most recent menstruation during a telephone screening with a female research assistant.

Intelligence Assessment

To control for individual differences in intelligence, a brief assessment of intellectual functioning was administered prior to drug administration. The Wechsler Test of Adult Reading (WTAR) is a neuropsychological assessment tool used to provide a measure of intelligence by using vocabulary level as a correlate of general intelligence (Wechsler, 2001). All participants were presented with irregularly spelled words and prompted to pronounce each. The irregular grapheme-to-phoneme translations (such as the "gh" in the word tough) in the prompts make the word difficult to pronounce without having previously learned the word. Because participants cannot apply standard pronunciation rules to complete the task, the experimenter can assess participants' vocabulary via their ability to pronounce the irregularly spelled words, and by extension, estimate their general intelligence (Wechsler, 2001). In standardized samples, WTAR

scores were shown to correlate highly with measures of verbal IQ (r = .75), verbal comprehension (r = .74), and full scale IQ (r = .73) (Strauss, Sherman, & Spreen, 2006).

Pregnancy Test

Following informed consent, all participants self-administered a urinary pregnancy test (Wondfo Pregnancy Strips, Willowbrook, IL) due to testosterone's mutagenic effects on the fetus. Negative pregnancy status was verified prior to proceeding with drug administration.

Testosterone Administration

Participants were randomized to placebo or testosterone using a random sequence generated at randomizer.org. Only the study coordinator—who was neither involved in participant screening nor study administration—had access to the electronic list identifying each spray as containing testosterone or placebo, and its assigned participant. Both experimenters and participants were blind to the drug condition. All participants self-administered an aqueous solution containing either 14 mg testosterone propionate or placebo, via intranasal spray (Figure 1). After self-administration of the nasal spray, participants were asked to relax for 30-minutes to allow testosterone levels to attain peak concentrations, based on the pharmacokinetic data from Study 1. Using the same protocol, dosage, and formulation as used here in Study 3, blood samples obtained via venipuncture in Study 1 showed peak levels for both free (bioavailable) and sex hormone-binding globulin (SHBG)-bound testosterone 30 minutes post-administration in women, which coincide with the start time of the GRE-Q (see below).

Graduate Record Examination Quantitative (GRE-Q) Battery

30 minutes post drug administration, participants were instructed to complete 15 quantitative questions from the Graduate Record Examination (GRE-Q) under time pressure (Clawson, Firment, & Trower, 1981; Reteguiz, 2006). Visual analogue scales (described below) assessing subjective anxiety were administered before and after the GRE-Q task.

Measures

Baseline Trait Measures

The Cognitive Test Anxiety Scale (CTAS; Cassady & Johnson, 2002) is a 27-item self-report questionnaire designed to assess cognitive concerns associated with test taking. Respondents are asked to rate the extent to which the 27 items describe themselves using Likert scales (ranging from 0 or "not at all typical of me" to 4 or "very typical of me"). Example items include, "My mind goes blank when I am pressured for an answer on a test" and "I am a poor test taker in the sense that my performance on a test does not show how much I really know about a topic."

The Abbreviated Math Anxiety Scale (AMAS; Hopko, Mahadevan, Bare, & Hunt, 2003) is a 9-item, self-report questionnaire designed to assess mathematics-related anxiety. Respondents are asked to rate the degree of anxiousness they experience in response to various situations (e.g., "Being given a 'pop' quiz in math class") on a scale from 1 ("low anxiety") to 5 ("high anxiety").

The Beck Anxiety Inventory (BAI; Beck et al., 1988) is a 21-item multiple choice (ranging from 0 to 3) self-report questionnaire designed to measure levels of anxious

symptomatology. The BAI assesses symptoms such as numbness and tingling, fear of losing control, and catastrophizing.

The Anxiety Sensitivity Index-3 (ASI-3; Taylor et al., 2007) is an 18-item self-report questionnaire designed to assess physical, cognitive, and social concerns associated with fear of arousal-related sensations. Respondents rate the extent to which the 18 items describe themselves using five-point Likert scales (ranging from 0 or "very little" to 4 or "very much").

Acute Subjective Anxiety

A visual analogue scale was developed for the repeated assessment of acute state anxiety (Study 2). Participants were asked to report their anxiousness by indicating a position along a continuous, 10 cm line between two end-points: 0 for "not at all anxious" and 10 for "extremely anxious" in response to the prompt "Please indicate how anxious you are right now." Data from each visual analogue scale was manually extracted using a metric ruler by a research assistant blind to drug condition. An example of the visual analogue scale is included as Figure 5.

Data Analytic Plan

Pre-Drug Administration Group Comparisons

Independent samples *t*-tests were conducted to test for differences between the placebo and testosterone group in: intelligence (i.e., WTAR), prior academic performance (i.e., GPA, SAT-V, SAT-Q scores), baseline subjective anxiety (visual analogue scale), test-taking anxiety (CTAS), mathematics anxiety (AMAS), trait anxiety (BAI), and anxiety sensitivity (ASI-3).

Predictors and Moderators of Subjective Anxiety and GRE-Q Performance

Multiple linear regression models were used to test for main effect of drug condition, and moderators of drug effect including trait anxiety (BAI), test anxiety (CTAS), math anxiety (AMAS), and anxiety sensitivity (ASI-3) on subjective anxiety and GRE-Q performance, respectively. Significant two-way interactions between drug condition and moderators were further analyzed with simple slope analysis (Aiken, West, & Reno, 1991; J. F. Dawson & Richter, 2006).

Condition Comparisons by Measurement Period

Independent samples *t*-tests were conducted to test for drug condition differences in subjective anxiety at each of the three time points.

Mediation of GRE-Q Performance

A test of mediation (Baron & Kenny, 1986) was conducted to examine whether changes in subjective anxiety mediates any purported association between testosterone administration and GRE-Q performance. A series of simple regression analyses were conducted, including: (1) drug condition predicting GRE-Q performance, (2) drug condition predicting subjective anxiety, and (3) both drug condition and subjective anxiety predicting GRE-Q performance.

RESULTS

Pre-Drug Administration Group Comparisons

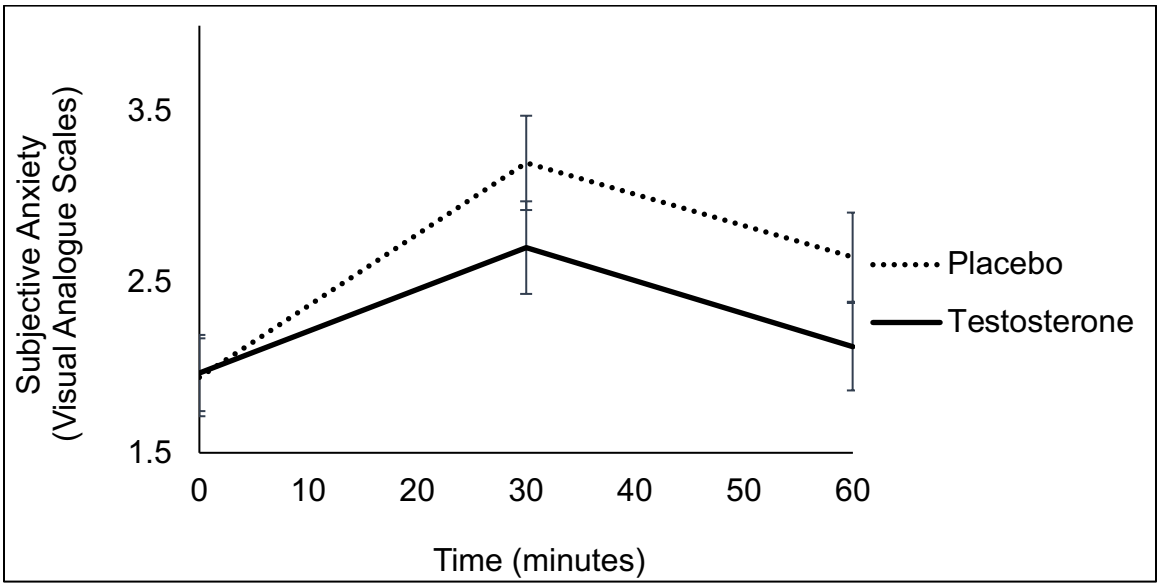
No significant drug condition differences were found in any of the baseline, pre-drug administration measures. Drug condition did not predict: general intelligence as measured by the Weschler Test of Adult Reading ($t(149) = 0.861$, $p = 0.391$, $d = 0.140$);

prior academic performance as indicated by high school GPA ($t(144) = -0.048$, $p = 0.962$, $d = 0.008$), verbal SAT score ($t(150) = 0.665$, $p = 0.507$, $d = 0.108$), or quantitative SAT score ($t(150) = -0.024$, $p = 0.981$, $d = 0.004$); baseline subjective anxiety as measured by visual analogue scale ($t(141) = -0.353$, $p = 0.725$, $d = 0.059$); trait anxiety as measured by the Cognitive Test Anxiety Scale ($t(128) = 1.805$, $p = 0.073$, $d = 0.317$), the Abbreviated Math Anxiety Scale ($t(137) = 1.286$, $p = 0.201$, $d = 0.218$), the Beck Anxiety Inventory ($t(142) = 1.121$, $p = 0.264$, $d = 0.186$), or the Anxiety Sensitivity Index ($t(142) = 1.021$, $p = 0.309$, $d = 0.170$).

Predictors and Moderators of Subjective Anxiety and GRE-Q Performance

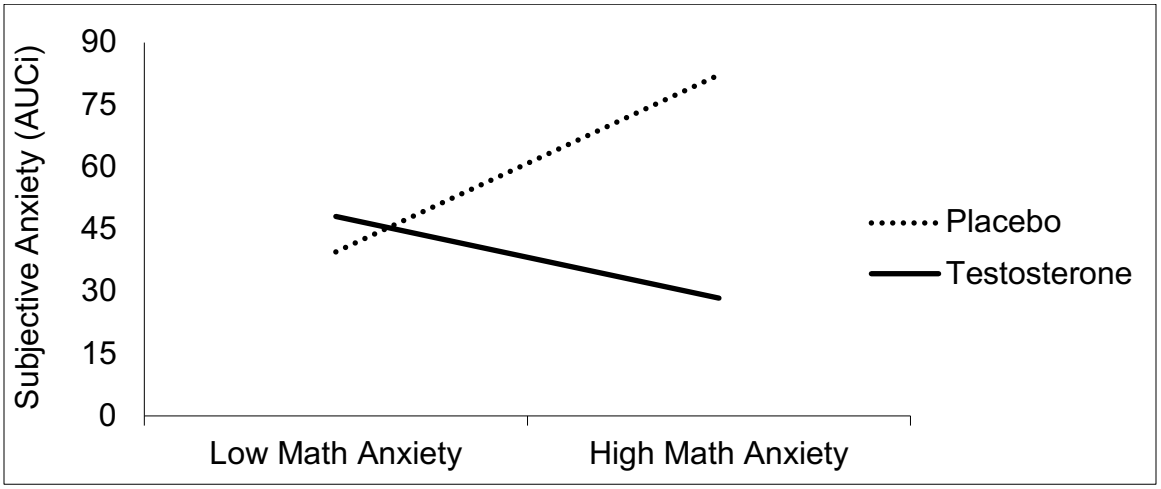
Multiple linear regressions revealed a significant drug effect on changes in self-reported anxiety (AUC_I) as measured by repeated visual analogue scales ($M_{placebo} = 63.21$, $SD = 70.58$; $M_{testosterone} = 38.37$, $SD = 52.52$; $\beta = -24.847$, $p = 0.018$, $d = 0.399$; see Figure 8 for summary data, and Figure 14 in the Appendix for individual participant plots). Moderation analyses further indicated that math anxiety (Abbreviated Math Anxiety Scale) significantly interacted with drug condition ($\beta = -0.872$, $p = 0.004$). Simple slope analysis of this significant interaction (Aiken et al., 1991; J. F. Dawson & Richter, 2006) suggested that exogenous testosterone had the largest effects on self-reported anxiety (AUC_I) for participants with greatest trait math anxiety assessed pre drug-administration ($\beta = -1.870$, $p = 0.002$; Figure 9).

Figure 8. Subjective anxiety by drug condition across time



Note. Error bars indicate standard error for each time point separately drug condition.

Figure 9. Moderation of drug effect on subjective anxiety by math anxiety



Note. High and low values are +1/-1 standard deviations around the mean.

GRE-Q performance was not predicted by drug condition (β = -0.514, p = 0.258), nor any trait anxiety measures (i.e., Beck Anxiety Inventory (β = 0.136, p = 0.374),

Cognitive Test Anxiety Scale (β = 0.078, p = 0.791), Abbreviated Math Anxiety Scale (β = -0.087, p = 0.777), and Anxiety Sensitivity (β = 0.258, p = 0.111)).

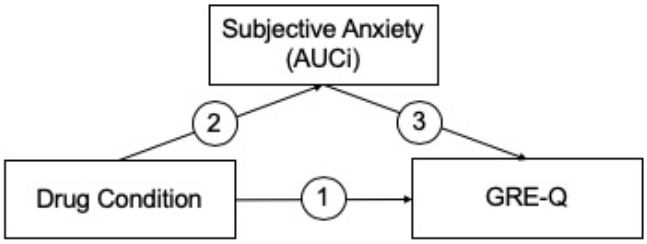
Condition Comparisons by Measurement Period

Subjective anxiety as measured by visual analogue scales did not differ across drug conditions during pre GRE-Q administration (t(140) = -0.165, p = 0.869, d = 0.028), post GRE-Q administration (t(142) = 1.298, p = 0.196, d = 0.216), or recovery (t(141) = 1.396, p = 0.165, d = 0.233).

Mediation of GRE-Q Performance

Mediation analysis revealed that changes in subjective anxiety across drug conditions did not impact GRE-Q performance (Figure 10). Specifically, a series of simple linear regressions showed that (1) drug condition did not significantly predict GRE-Q performance (β = -0.095, p = 0.258); (2) drug condition significantly predicted subjective anxiety (β = -0.197, p = 0.018); and (3) drug condition and subjective anxiety did not predict GRE-Q performance when both were entered into the regression model (p = 0.452).

Figure 10. Mediation model for GRE-Q performance



EXPLORATORY ANALYSES

Considering that GRE-Q performance was not significantly predicted by drug condition, nor any trait anxiety measures ($p > 0.05$), a multiple linear regression was computed to identify significant predictor(s). Results indicated that GRE-Q performance was significantly predicted by SAT-quantitative ($\beta = 0.811$, $p = 0.018$), and Wechsler Test of Adult Reading—a brief estimate of general intelligence ($\beta = 0.186$, $p = 0.040$), Table 4.

Table 4. Standardized Regression Coefficients Predicting GRE-Q Performance

	B	*SE*	*β*
(constant)	-1.865	3.257	–
Trait Anxiety (BAI)	.051	.041	.147
Anxiety Sensitivity (ASI-3)	-.064	.032	-.249
Math Anxiety (AMAS)	.009	.040	.023
Test Anxiety (CTAS)	-.033	.021	-.159
High School GPA	1.377	.794	.149
SAT Verbal	-.005	.003	-.501
SAT Quantitative	.008	.003	.811*
IQ (WTAR)	.029	.014	.186*
**p<.05, **p<0.01, ***p<0.001*			

Moreover, there was a trending, non-significant association between changes in acute self-reported anxiety (AUC_I) and trait anxious symptomatology (BAI) when controlling for other trait anxiety measures and previous achievement testing and scholastic performance ($p = .059$), Table 5.

Table 5. Standardized Regression Coefficients Predicting Subjective Anxiety

	B	*SE*	*β*
(constant)	73.402	82.909	–
Trait Anxiety (BAI)	1.981	1.038	.249~
Anxiety Sensitivity (ASI-3)	-.192	.808	-.032
Math Anxiety (AMAS)	-.711	1.015	-.077
Test Anxiety (CTAS)	.753	.526	.158
High School GPA	-20.258	20.214	-.095
SAT Verbal	.071	.083	.320
SAT Quantitative	-.084	.081	-.382
IQ (WTAR)	.300	.360	.082
*~p<.06, *p<.05, **p<0.01, ***p<0.001*			

Lastly, mediation analysis demonstrated that trait math anxiety did not explain differences in acute subjective anxiety across drug conditions. Specifically, a series of simple linear regressions showed that while (1) drug condition did significantly predict acute subjective anxiety (β = -0.197, p = 0.018); (2) drug condition did not predict trait math anxiety (β = -0.109, p = 0.201); and (3) drug condition and trait math anxiety did not predict acute subjective anxiety when both were entered into the regression model (p = 0.481).

DISCUSSION

The present study examined the effect of 14 mg intranasal testosterone administration on subjective anxiety and cognitive performance (using a quantitative section of the Graduate Record Examination, GRE-Q). Results revealed a statistically significant anxiolytic effect of exogenous testosterone relative to placebo as assessed by self-reported, repeatedly-measured, visual analogue scales. Moreover, this drug effect

was moderated by math anxiety (Abbreviated Math Anxiety Scale), such that exogenous testosterone had a statistically significant impact on subjective anxiety for participants with highest levels of math anxiety pre-drug administration. Drug effect on self-reported anxiety was not moderated by any other measures (i.e., trait anxiety, test anxiety, or anxiety sensitivity). No difference in GRE-Q performance was found between testosterone and placebo conditions. Instead, GRE-Q performance was more accurately predicted by correlates of general intelligence, including quantitative SAT performance, and Wechsler Test of Adult Reading (WTAR).

To date, this is the first study to demonstrate an anxiolytic effect of testosterone administration on self-reported measures in response to a stressor. In contrast, previous studies have only reported behavioral effects of exogenous testosterone. These include diminished preconscious selective attention to threatening stimuli (van Honk et al., 2005), reduced gaze avoidance in individuals with social anxiety disorder (Enter, Terburg, et al., 2016), reduced fear-potentiated startle response (Hermans et al., 2006), and increased behavioral approach (Enter et al., 2014; Enter, Spinhoven, et al., 2016). Considering that clinical assessment and diagnosis of anxiety is typically accomplished via a patient's subjective report of his or her anxiety, rather than laboratory-based behavioral assessments, the outcome of the present study provides a more clinically valid test of the putative effects of exogenous testosterone and has significant implications for intervention. For example, lower academic anxiety was associated with higher liking and valuing of school, and greater academic motivation (Gottfried, 1985), particularly among children of lower social economic status (Dunn, 1968). Given that lower anxiety is associated with more behavioral

approach (Enter et al., 2014; Enter, Spinhoven, et al., 2016), individuals who are otherwise paralyzed by academic anxiety may experience less avoidance in response to scholastic stressors, and have additional opportunities to demonstrate mastery.

Considering that the quantitative section of the Graduate Record Exam is composed exclusively of mathematical problem sets, it is not surprising that the drug effect on self-reported anxiety was significantly moderated by math anxiety (Abbreviated Math Anxiety Scale). Contrary to study hypothesis, the drug effect was not moderated by more generalized test anxiety (i.e., Cognitive Test Anxiety Scale). This may be due to the broader specificity of test anxiety as a construct, relative to math anxiety. Replication of the present study using the verbal section of the GRE would be helpful in distinguishing the predictive power of the various moderators.

Based on this set of findings, it appears that GRE-Q performance is not controlled by momentary changes or trait anxiety, but seems to be a reflection of *g* (a measure of general intelligence), similar to other scholastic admissions tests, such as the SAT. In fact, a correlation of .82 has been found between SAT scores and *g* scores computed from an IQ test battery (Frey & Detterman, 2004; Sackett, Borneman, & Connelly, 2008). Like other admission tests, the Graduate Record Exam was designed to assess different aspects of cognition, including verbal reasoning, quantitative reasoning, and critical thinking. Previous research suggests that other than significant alterations in brain health (e.g., atrophy or organic changes due to disease or toxin), no interventions have been shown to have long-term effects on measures associated with *g* (e.g., Heart Start Program, Neisser, 1986). In fact, Kamphaus (2005) suggest that *g* accounts for 40 to 50 percent of the

between-individual performance differences on a given cognitive test. In light of these considerations, it is not surprising that drug administration did not yield a significant effect on GRE-Q performance. For future extension of the present findings, it would be important to include tasks that are less correlated or informed by *g*. For example, working memory tasks like Digit Span or Letter-Number Sequencing from the Wechsler Adult Intelligence Scale battery. Previous research indicates that individuals are more likely to allocate working memory resources to threat-related information (e.g., possibility of failure in the context of test anxiety), and thus have trouble with both encoding and recall (Ashcraft & Kirk, 2001; Ashcraft & Krause, 2007; Ikeda, Iwanaga, & Seiwa, 1996; Shackman et al., 2006; Stout, Shackman, Pedersen, Miskovich, & Larson, 2017). It is hypothesized that individuals randomized to the placebo condition will have worse performance on working memory tasks than those randomized to the testosterone condition due to greater resources allocated to test anxiety.

Although this study utilized a female-only sample, it is worth noting possible effects of testosterone administration on subjective anxiety in males. Only a handful of studies have examined the effect of exogenous testosterone on self-reported mood outcomes in young, healthy, eugonadal men. Pope, Kouri, and Hudson (2000) found that 600 mg of weekly testosterone administration led to increased ratings of manic symptoms in men. The authors cautioned that this effect was not uniform across individuals. Specifically, whereas a few developed prominent effects, most participants showed little psychological change (Pope et al., 2000). Similarly, Bhasin et al. (2001) did not detect differences in mood in a sample of healthy young men randomized to various dosages of

testosterone administration (0, 25, 50, 125, 300, 600 mg) over the course of 20 weeks. Lastly, no differences in subjective anxiety were detected between male participants randomized to placebo and testosterone conditions in Study 2. Based on these findings, it is theorized that the effect of testosterone administration on self-reported anxiety in women will likely not extend to men.

In conclusion, the present study demonstrates an anxiolytic effect of testosterone on self-reported anxiety in a large female sample. Taken together, these findings contribute to a better understanding of potential mechanisms associated with the development and maintenance of anxiety symptoms, and may help inform more efficacious treatments.

Chapter 5: General Discussion

This dissertation examined the effects of a novel intranasal testosterone spray on physiological, psychological, and cognitive outcomes across three studies. Taken together, findings are consistent with greater literature on the anxiolytic effects of testosterone in humans and animals.

Study 1 provided the methodological foundation for Studies 2 and 3 by establishing the utility, effectiveness, and pharmacokinetic profile of a novel intranasal testosterone spray in healthy men and women. Results from 23 participants (35% female) indicated that total and free testosterone concentrations increased in a linear fashion at 15- and 30-minutes post testosterone administration, peaking at 30 minutes, and declining 60 minutes following administration. Study 2 leveraged the utility of this novel spray preparation, and investigated the effect of intranasal testosterone on stress-evoked (Trier Social Stress Test) subjective anxiety in a double-blind, placebo-controlled trial. Results from 104 participants (48.1% female) revealed a statistically significant drug by sex interaction, in which women—as expected—experienced significantly higher levels of subjective anxiety in the placebo condition compared to men; a sex difference that was eliminated in the drug condition. Further, women randomized to the testosterone condition experienced significantly lower levels of recovery (but not overall) anxiety from the Trier Social Stress Test relatively to women in the placebo condition. Study 3 extended the anxiolytic effects of testosterone in women from Study 2, and assessed the effect of intranasal testosterone on acute subjective anxiety *and* cognitive performance (quantitative GRE) in a larger,

female-only sample (N = 150). Results revealed a statistically significant anxiolytic effect of intranasal testosterone relative to placebo. Moreover, this drug effect was moderated by trait anxiety, such that exogenous testosterone exerted the greatest anxiolytic effects for participants with highest levels of pre-drug administration math anxiety. No difference in quantitative GRE performance was found between drug conditions. Instead, quantitative GRE performance was predicted by correlates of general intelligence, including quantitative SAT performance, and verbal intelligence.

While the anxiolytic effect of testosterone was limited to the recovery period in Study 2, it generalized across the study when the female sample size was increased by approximately 188% (from N of 52 to 150) in Study 3. It seems that one possible explanation for the lack of elicit self-reported effects of exogenous testosterone in previous studies (O'Connor, Archer, Hair, & Wu, 2002; Terburg, Aarts, & van Honk, 2012; Adriaan Tuiten et al., 2002; van Honk et al., 2005) may be due to limited power associated with small samples sizes. Moreover, the nature of the Trier Social Stress Test may have made detecting subjective differences difficult. The large floor effects across visual analogue scales might have obscured the effect in this small sample study. In fact, meta-analysis of recovery findings from Study 1 and overall findings from Study 2 demonstrated a significant mean effect between placebo and testosterone conditions (z = -1.632, p = .021).

POSSIBLE UNDERLYING NEUROBIOLOGICAL MECHANISMS

Human neuroimaging studies suggest that the anxiolytic effects of testosterone may be the result of its influence on connectivity between the prefrontal cortex and subcortical

regions (e.g., amygdala), particularly in the modulation of approach and avoidance behaviors (Volman et al., 2011). In a sample of age matched male participants, a negative feedback loop is observed between orbitofrontal cortex and amygdala in healthy controls, compared to a positive, excitatory connection in patients with social anxiety (Sladky et al., 2015).

Exogenous testosterone studies in healthy females show that testosterone administration rapidly reduces functional coupling of the amygdala with the orbitofrontal cortex, and enhanced amygdala coupling with the thalamus (van Wingen et al., 2010) Specifically, this decoupling decreases behavioral inhibition and emotional rumination by either inhibiting signals from the amygdala to the orbitofrontal cortex, or redirecting those signals away from the orbitofrontal cortex (van Wingen et al., 2011). It has been suggested that the mechanism underlying this effect is likely a function of one or more of testosterone's metabolites (van Wingen et al., 2010). For example, testosterone exerts a variety of downstream effects via its aromatization to estradiol. The enzyme aromatase—responsible for metabolizing testosterone into estradiol in the brain—is capable of rapid aromatization of testosterone due to its proximity to the presynaptic terminals, resulting in behavioral changes within minutes of the onset of fluctuations in circulating testosterone levels (Balthazart & Ball, 2006).

TRANSLATIONAL IMPLICATIONS

Current evidence suggest that a large subset of individuals diagnosed with an anxiety disorder do not recover following evidence based psychological or

pharmacological treatments (Blanco et al., 2003; Gould et al., 1995, 1997; Hofmann & Bögels, 2006), highlighting the need for new treatment strategies that enhance remission rates. A novel line of research has shown that treatment effects are augmented by pairing exposure therapy with a pharmacological agent (Hofmann et al., 2014; Otto et al., 2009, 2005; Singewald et al., 2015; Smits et al., 2010). Given the present findings in women, combined with previous research showing that exogenous testosterone promotes social approach behavior in individuals with social anxiety disorder (Enter, Spinhoven, et al., 2016), it would be interesting for future investigations to explore whether the addition of testosterone as a pharmacological enhancer may augment the efficacy of exposure therapy in anxiety disorders.

In particular, the addition of testosterone administration may reduce the amount of time it takes for individuals to tackle items of higher intensity on their fear hierarchies in exposure therapy. Early success in exposure therapy has been shown to increase confidence and motivation, and a foster greater sense of mastery (E. A. Hembree & Cahill, 2007). Over time, smaller doses of testosterone nasal spray may be used to achieve similar anxiolytic effects, until no exogenous testosterone is needed.

Unlike benzodiazepines or beta-blockers such as propranolol, both typically prescribed for the treatment of acute anxiety, testosterone does not exert depressant effects on the central nervous system, eliminating acute cognitive impairment, and potentially dangerous physiological consequences such as bradycardia or hypotension.

LIMITATIONS

As with any study, the present findings must be interpreted in light of study limitations. First, all three studies were conducted using convenience samples consisting of participants relatively similar in terms of age, body mass, and gonadal status. Replication of findings using heterogeneous samples is needed. Second, the intranasal administration of testosterone resulted in post-nasal drip, which prohibited salivary testosterone samples from being effectively assayed due to contamination, and therefore no salivary data is available. Third, menstrual cycle phase for female participants were collected via self-report. Future replications would benefit from more rigorous assessment of female participants' hormonal status.

FUTURE DIRECTIONS

The present research examined the effect of intranasal testosterone on physiological, psychological, and cognitive outcomes; it does not however, address *how* testosterone exerts its influence. Existing neuroimaging studies (e.g., van Wingen et al., 2010) point to possible causal mechanisms underlying its anxiolytic effects (e.g., Sladky et al., 2015; Volman et al., 2011). Future clinical neuroendocrinological research should incorporate methods from various fields to create a more complete picture of the mechanism and effect of intranasal testosterone in humans. For example, radioactive tracers used in positron emission tomography (PET) studies can help track the pathway of testosterone molecules inside the brain in real time.

Another avenue for future research is the search for moderators, including evaluation of family history and genotyping for specific genetic profiles associated with anxiety disorders. Moreover, replications of the present research with high-risk samples, such as in individuals with remitted anxiety disorders will help to generalize the current research findings to more ecologically valid populations.

Relatedly, it would be helpful to examine individual differences in enzyme metabolization in future replications of the present set of studies. For example, individual differences in the expression of aromatase, an enzyme catalyst involved the conversion of testosterone to estradiol (an estrogen) may explain variability in the efficacy of testosterone administration in women. It is hypothesized that women with higher levels of aromatase will experience greater anxiolytic effects of testosterone due to greater conversation to estradiol. Several existing studies have demonstrated both endogenous and exogenous aromatase in prolonging the effects testosterone in humans (Cherrier et al., 2005; Nathan et al., 2001).

Finally, both Studies 2 and 3 examined the effect of exogenous testosterone using a between-subjects design. Given that anxiety is future-oriented, it would be helpful to explore the effect of intranasal testosterone on performance and willingness to approach using a more conventional clinical trial extending over 6 or more weeks.

CONCLUSIONS

Collectively, these three studies aim to contribute to a broader understanding of the anxiolytic effects of testosterone, and with it, the potential for testosterone to act as a novel pharmaceutical in the treatment of anxiety.

Appendix

Figure 11. Total serum testosterone concentrations by sex (N = 20)

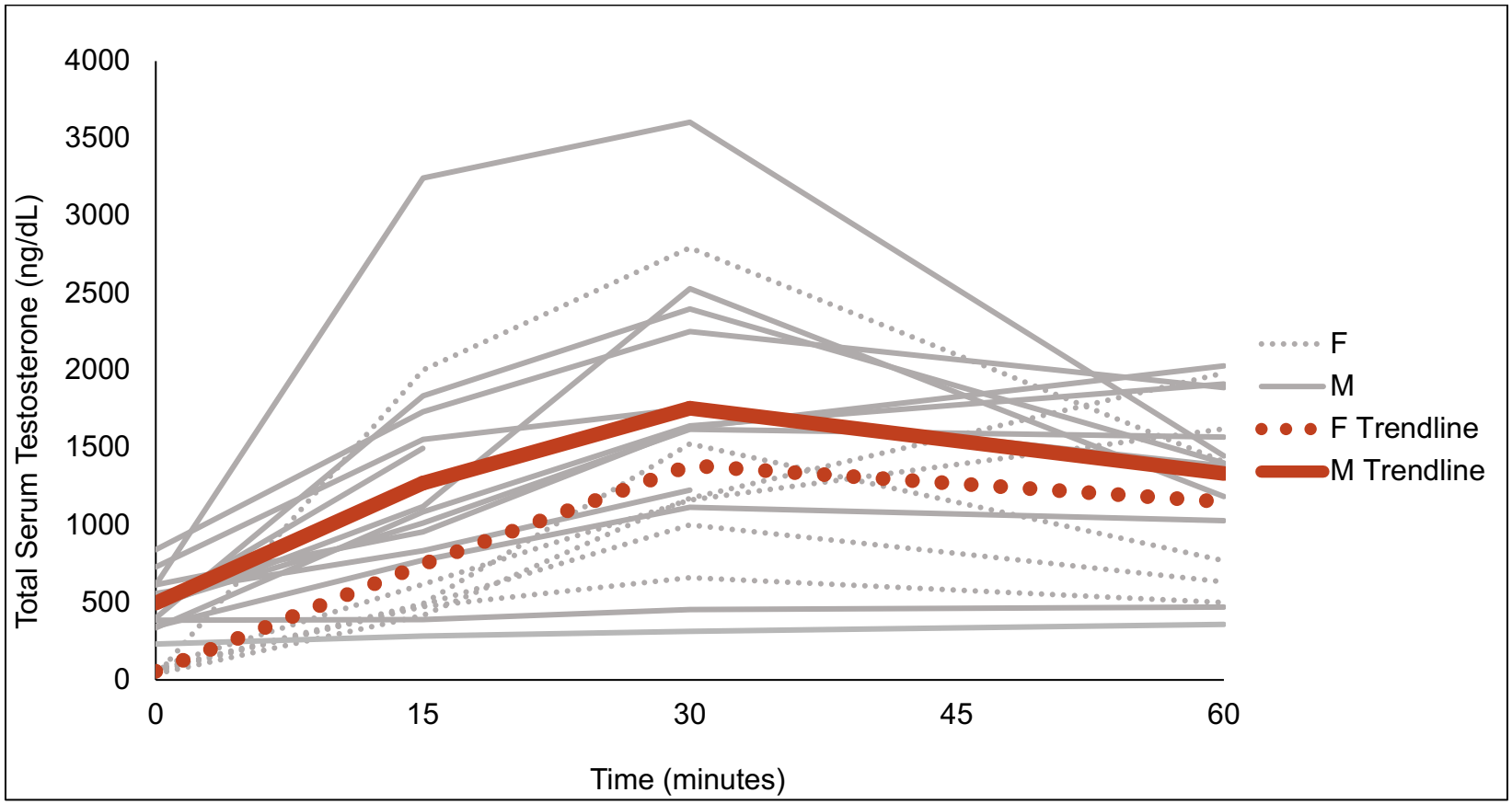


Figure 12. Free serum testosterone concentrations by sex (N = 20)

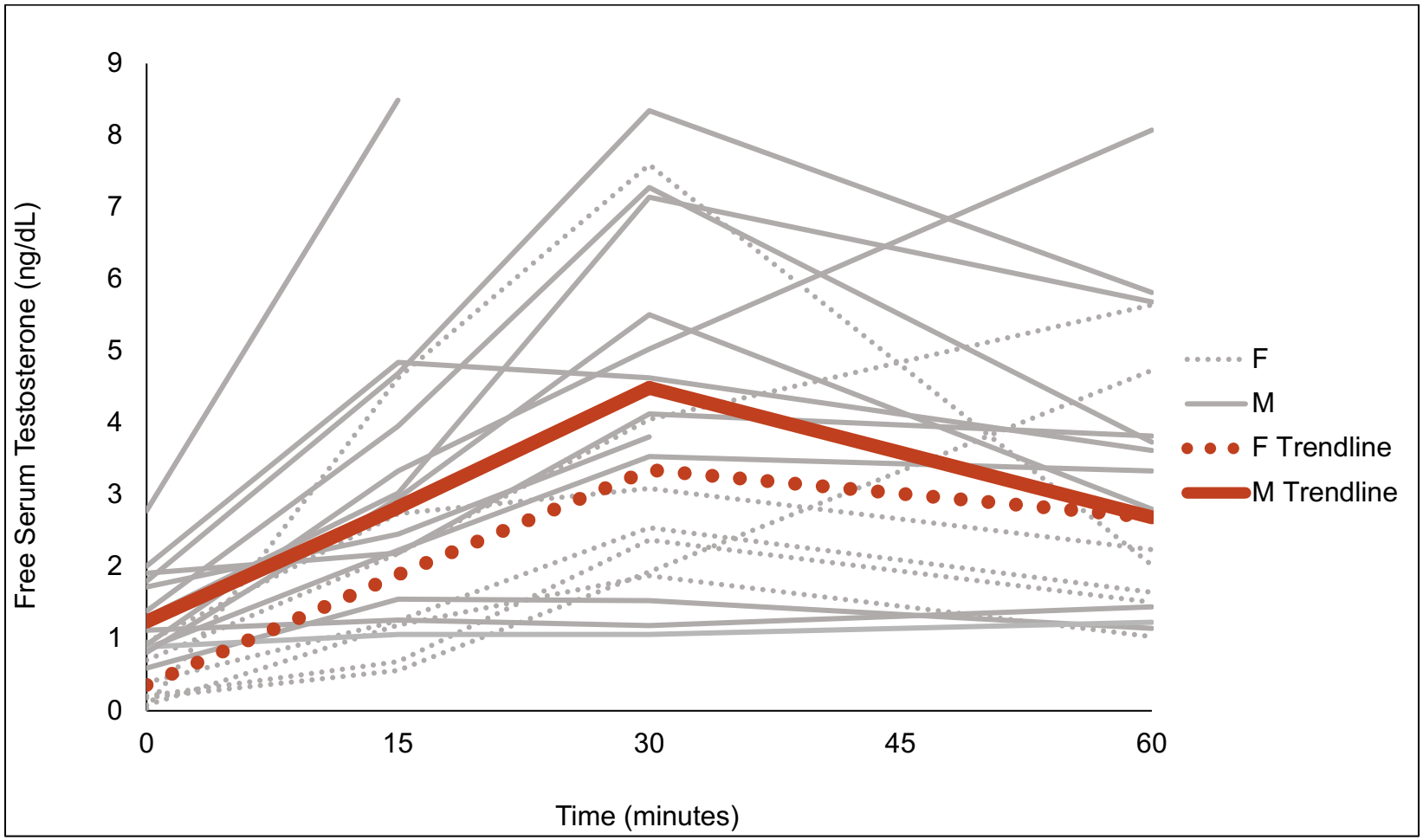


Figure 13. Subjective anxiety by sex, drug condition, and time (N = 104)

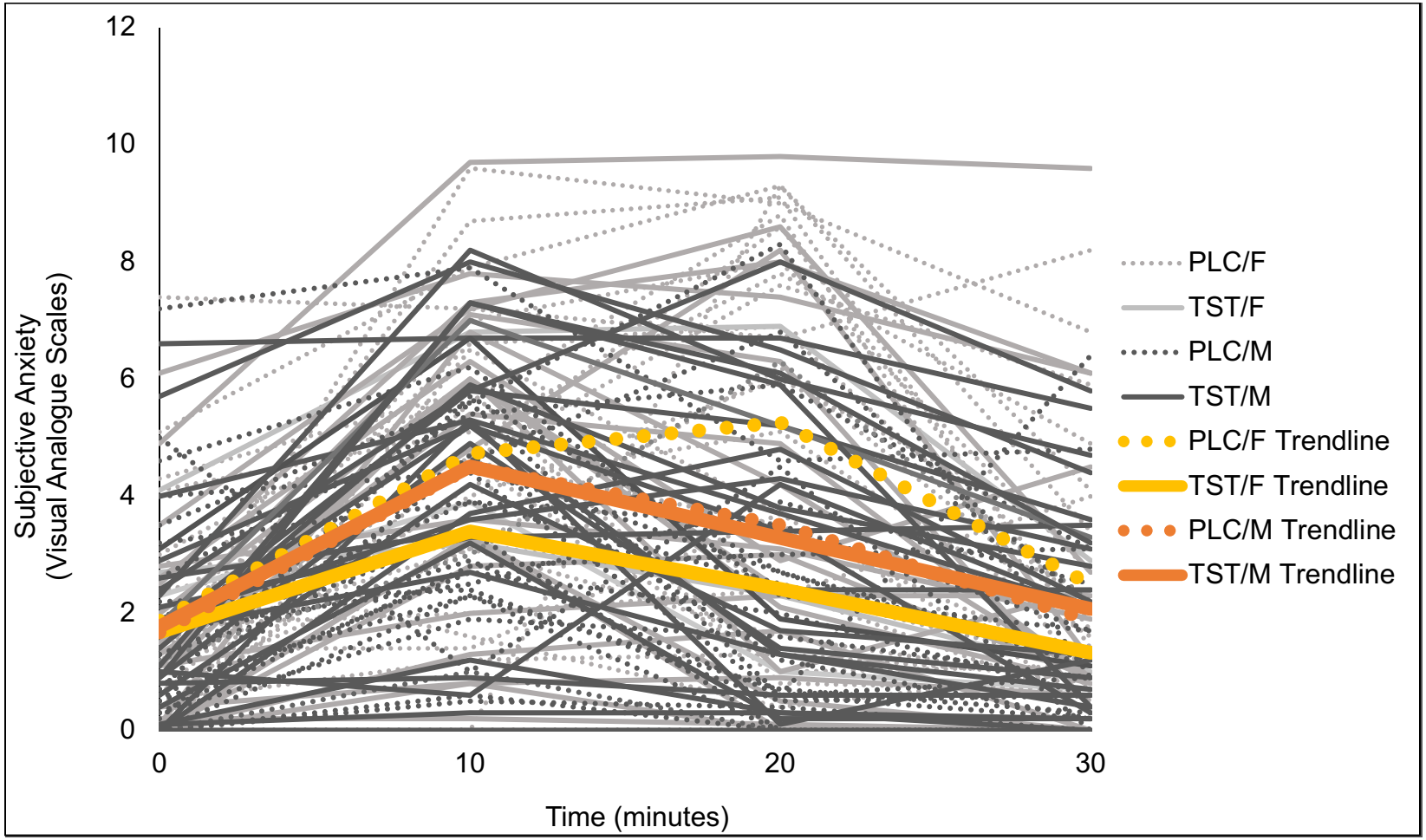
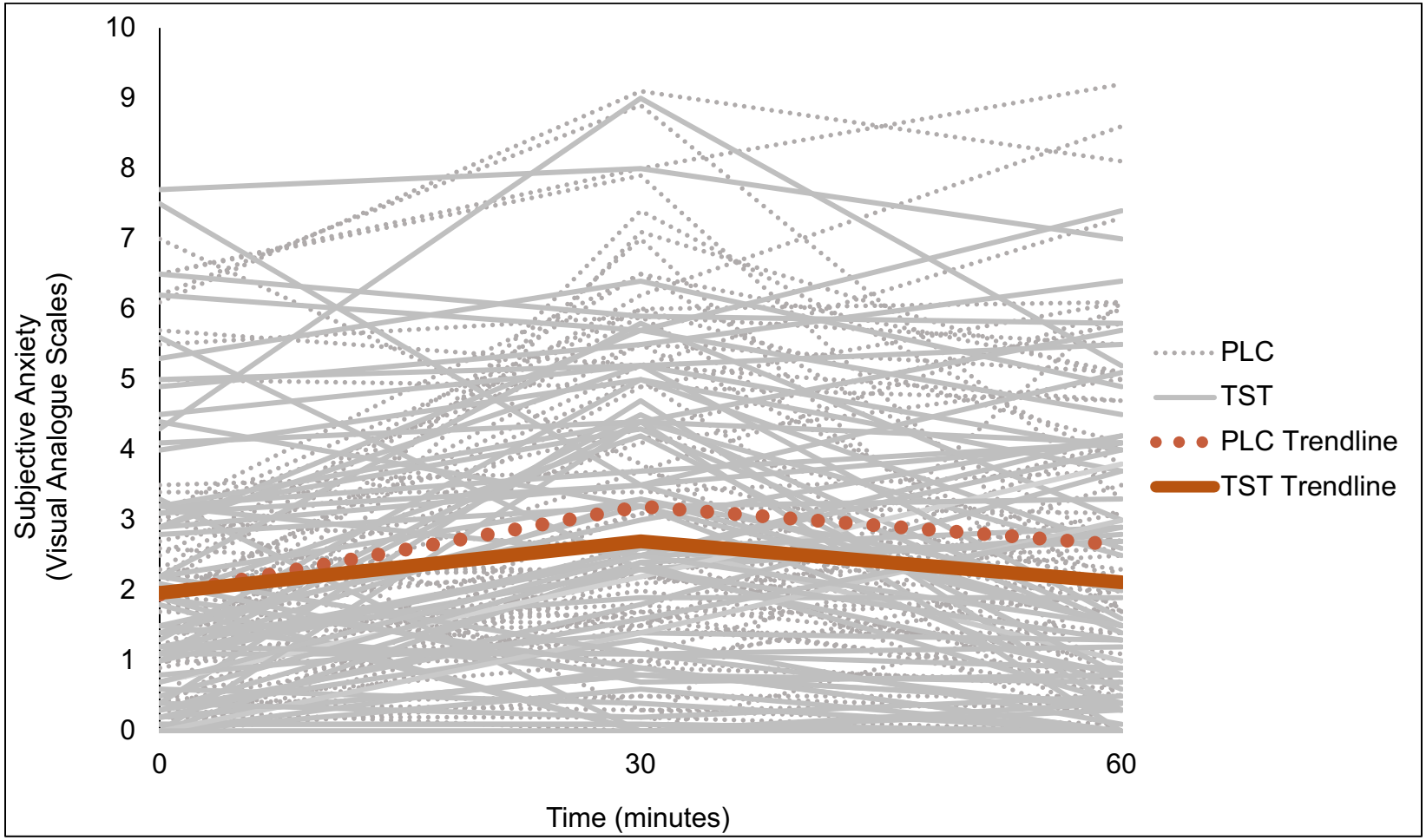


Figure 14. Subjective anxiety by drug condition across time (N = 150)



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