Testosterone responses to competitive interactions and facial displays of emotion: a social neuroendocrinology perspective

by

Samuele Zilioli

M.A. (Psychology), Università Cattolica del Sacro Cuore, 2009
B.A. (Psychology), Università Cattolica del Sacro Cuore, 2006

Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in the

Department of Psychology

Faculty of Arts and Social Sciences

SIMON FRASER UNIVERSITY

Summer 2014

Approval

Name: **Samuele Zilioli**

Degree: **Doctor of Philosophy (Psychology)**

Title of Thesis: ***Testosterone responses to competitive interactions and facial displays of emotion: a social neuroendocrinology perspective***

Examining Committee: **Chair:** Rachel Fouladi
Associate Professor

Dr. Neil V. Watson
Senior Supervisor
Professor

Dr. Ralph Mistlberger
Supervisor
Professor

Dr. Mario Liotti
Supervisor
Professor

Dr. George Alder
Supervisor
Senior Lecturer

Dr. Charles Crawford
Internal Examiner
Professor Emeritus
Department of Psychology

Robert A. Josephs
External Examiner
Professor, Department of Psychology
The University of Texas at Austin

Date Defended/Approved: July 8, 2014

Partial Copyright Licence

Ethics Statement

The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

a. human research ethics approval from the Simon Fraser University Office of Research Ethics,

or

b. advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University;

or has conducted the research

c. as a co-investigator, collaborator or research assistant in a research project approved in advance,

or

d. as a member of a course approved in advance for minimal risk human research, by the Office of Research Ethics.

A copy of the approval letter has been filed at the Theses Office of the University Library at the time of submission of this thesis or project.

The original application for approval and letter of approval are filed with the relevant offices. Inquiries may be directed to those authorities.

Simon Fraser University Library
Burnaby, British Columbia, Canada

update Spring 2010

Abstract

Testosterone levels are not static but fluctuate in response to environmental inputs, including social signals. Acute changes are particularly observed in response to evolutionarily salient social interactions such as antagonistic encounters and exposure to potential mates. The overarching goals of this dissertation are (1) to examine whether facial displays of emotions are effective social behavioral signals that modulate testosterone (Study 1); (2) to better understand the interplay between motivational, situational and physiological factors in shaping androgen release in competitive situations (Study 2 and Study 3); and, (3) to investigate the short-term and longer-term functional consequences of testosterone responses to competition (Study 3). In Study 1, I found that both men and women had an increase in testosterone when exposed to faces of the opposite sex, while only women had an additional increase in testosterone when presented with angry faces. In Studies 2 and 3, I found that testosterone responses to shifts in social status (win vs. loss) (1) were modulated by situational (i.e. familiarity of the task and number of competitions) as well as physiological (i.e. basal levels of cortisol) factors, and (2) had long-term – *but* not short-term- functional consequences on behaviors related to the competitive task. These results are discussed within a comparative perspective, drawing parallels with the Competition Effect, the Winner Effect and the Challenge Hypothesis observed in non-human animals. Possible evolutionary mechanisms underlying these phenomena are discussed as well.

Keywords: Testosterone; Social Neuroendocrinology; Challenge Hypothesis; Cortisol; Facial Expressions of Emotion, Winner Effect.

This thesis is dedicated to my parents.
For their unconditional love, support and encouragement.

Acknowledgements

I would like to thank everyone who made this work possible. Thanks to my supervisor Dr. Neil Watson for encouragement and financial support. I am grateful to him for his patient guidance, insightful comments and suggestions, and for being a stainless example of the passionate researcher and enthusiastic teacher I intend to be.

Thanks for the members of my committee, especially Dr. Mario Liotti, who has provided valuable support over the years of my PhD.

Thanks to all fantastic volunteers and colleagues in the Behavioural Endocrinology Lab, for their contributions to the collection and analysis of the data - without you, none of this research would have been possible.

Lastly, I would like to thank all my friends and family for everything else. A special thanks goes to my partner, Julie. Her continual support made this work possible.

Table of Contents

List of Tables

List of Figures

List of Acronyms

HPG	Hypothalamic-Pituitary-Gonadal
HPA	Hypothalamic-Pituitary-Adrenal
LH	Luteinizing Hormone
GnRH	Gonadotropin–releasing Hormone
SHBG	Sex Hormone Binding Globulin
GBRs	Y-aminobutyric acid $(GABA)_A$/benzodiazepine receptor complexes
BMS	Biosocial Model of Status
FDEs	Facial displays of emotion
BIS	Behavioral Inhibition System
BAS	Behavioral Activation System
FACT	Facial Affect Comparison Task
PANAS	Positive and Negative Affective Scale
SOS	Salimetrics Oral Swabs
ELISA	Enzyme-linked immunosorbent assay
CE	Competition Effect
MRT	Mental Rotation task

Chapter 1.

Introduction

1.1. Hormones and Behavior

Hormones contribute to phenotypic plasticity, the ability of an organism to change its phenotype in response to the environment, by transducing external and interoceptive stimuli into changes in cell functions. Thus, by conjoining environment and an organism's physiology, the endocrine system ultimately regulates behavior (Dufty, Clobert, & Moller, 2002). Although endocrine signaling pathways require an interconnected network of diverse components (enzymes, receptors, hormones and binding proteins), fluctuations in circulating hormone concentrations are clearly crucial in the expression of endocrine-mediated phenotypes (Rosvall et al., 2012). These endocrine activation profiles have been shaped by natural selection and are on display when the organism is coping with environmental challenges, ranging from temperature changes to social interactions. Endocrine profiles can be quantified, depending on the phenotype of interest, over short-term or long-term time scales.

1.1.1. Testosterone

To any scientists interested in behavior, testosterone is one of the most interesting hormones because of its pleiotropic antagonistic effects on various behavioral, physiological and morphological traits. Mainly regulated by the hypothalamic-pituitary-gonadal (HPG) axis, testosterone is a sex steroid from the androgens group with pronounced effects on skeletal muscles, body composition and sexual function (Mooradian, Morley, & Korenman, 1987) but also fundamentally implicated in the expression of social behaviors (Booth, Granger, Mazur, & Kivlighan, 2006). In men, testosterone secretion by testicular Leydig cells is stimulated by the pulsatile release of

the luteinizing hormone (LH) from the anterior pituitary gland, which in turn is regulated by the pulsatile secretion of gonadotropin–releasing hormone (GnRH) from the hypothalamus. This hormone cascade pathway is regulated by negative feedback (i.e. each hormone feeds back to inhibit the surge for more of the same hormone), which guarantees the maintenance of hormone levels within a particular appropriate physiological range. In women, testosterone is secreted by the ovarian stroma, which contains interstitial cells resembling those of the testis. In men and women, tissues in both the zona fasciculate and the zona reticularis of the adrenal cortex provide an additional source of testosterone, suggesting an involvement of the hypothalamic-pituitary-adrenal (HPA) axis in androgen production. Regardless its origin, once testosterone is released in the blood, it travels via the bloodstream to target tissues throughout the body. For tissues, the biologically available testosterone is the sum of the free testosterone and albumin-linked testosterone. The large proportion of testosterone bound to the carrier protein sex hormone binding globulin (SHBG) is biologically unavailable as it cannot enter the cells and interact with the androgen receptors. Salivary testosterone provides a measure of bioavailable testosterone that highly correlates with plasma bioavailable testosterone (Shirtcliff, Granger, & Likos, 2002).

Because testosterone has many sources, studies measuring it without concomitant measures of LH, and GnRH for that matter, do not allow definitive statements on the specific pathway of testosterone secretion. For example, studies on human competition report increases in salivary testosterone within fifteen minutes (Archer, 2006), and, although testosterone lags LH release by 10-20 minutes (Veldhuis et al., 1987; Coquelin & Desjardins, 1982) some authors have proposed that other faster pathways - independent of the HPG axis, such as sympathetic activation - might be responsible for this phenomenon (Chichinadze & Chichinadze, 2008). More studies are required to shed light on the pathways through which testosterone is secreted in response to social stimuli.

Physiological details of hormone action are beyond the scope of this dissertation; some discussion of mechanisms is needed. Given its lipophilic nature, testosterone passess through the extracellular membrane and inside the cell it directly or indirectly (through its metabolites) binds to specific receptor proteins (e.g., androgen receptor). Next, this steroid-receptor complex increases or decreases the synthesis of specific

proteins after it binds to DNA. In brief, testosterone regulates gene transcription and expression and therefore influence cells development and differentiation. These slow actions (> 30 minutes) are known as genomic mechanisms and can be distinguished from the non-genomic actions of androgens (Foradori, Weiser, & Handa, 2008), which are more rapid -acting within seconds to few minutes- and operate via membrane-bound receptors interactions. Testosterone is able to cross the blood-brain barrier and interact with various brain nuclei, such as the hypothalamus, the hippocampus, the amygdala and areas of the mesocorticolimbic pathway (Simerly, Swanson, Chang, & Muramatsu, 1990; Kritzer, 2004). Thus, it is likely that both genomic and non-genomic androgen effects influence behavioral phenotypes. A brain pathway were both these effect are evident is the mesocorticolimbic/reward pathway (Kritzer, 1997; Frye, 2007). Genomic actions induce genes transcription responsible for the production of a wide array of enzymatic, structural and receptor proteins. Superimposition on intracellular androgen receptors on the reward circuit is seen in both direct and indirect neural substrates that mediate the effects of positive incentives, suggesting genomic effect of testosterone. For example, androgen receptors are seen in the nucleus accumbens, central tegmental area and substantia nigra, and, in greater amount, in areas that supply afferent projections to midbrain dopamine nuclei, such as the bed nucleus of the stria terminalis, the lateral septal nucleus and the medial preoptic nucleus (Kritzer, 1997). Besides, elevated anabolic–androgenic steroids dosages upregulate androgen receptors in some parts of the reward circuitry, such as the ventral tegmental area (Frye, 2007).

Steroids exert their effects also through non-genomic ways. For example, they can regulate changes in membrane permeability, act on membrane receptors, indirectly activate intracellular steroid receptors and regulate γ-aminobutyric acid $(GABA)_A$/benzodiazepine receptor complexes (GBRs). In the case of testosterone, several studies have shown that its metabolites (e.g., 5α-reduced metabolites) may mediate its fast action on the reward circuit (i.e. euphorogenic effects) by acting via GBRs and dopaminergic neurons in the nucleus accumbens (Frye, 2007).

This would be of particular interest in the current dissertation, which, among others, investigates the topic of functional significance of fast testosterone fluctuations in response to evolutionary salient social contexts.

1.1.2. Human Social Neuroendocrinology

In the broader field of behavioral endocrinology, the study of the relationship between hormones and behavior, human social neuroendocrinology represents that branch concerned with behavioral systems and social contexts that modulate human neuroendocrine function and its link to behavior.

The fact that circulating androgen levels are highly heritable (~70%) (Hong et al., 2001) does not indicate that testosterone concentrations are static; on the contrary, they vary on long-term and short-term scales. For example, as in other species (Muroyama, Shimizu, & Sugiura, 2007), humans show seasonal variation in androgens, with peaks reached in Autumn (Van Anders, Hampson, & Watson, 2006; Stanton, Mullette-Gillman, & Huettel, 2011). In addition to seasonality, there is diurnal variation in testosterone secretion, with the highest levels around waking period followed by a constant decline throughout the day. At sleep time testosterone reaches its lowest concentration and starts rising again during the sleep phase until it reaches the highest point immediately before waking. Interestingly, it has been shown that variation between morning and afternoon testosterone taps into factors critical to engaging with a social system, such as attention to threatening stimuli (i.e. angry faces) (Wirth & Schultheiss, 2007).

Lastly, and of central importance for the present work, testosterone fluctuates in response to environmental/social contexts. In parallel with results in other species, testosterone concentrations rise in heterosexual men conversing with members of the opposite sex (Roney, Lukaszewski, & Simmons, 2007), women exposed to video-clips of attractive men (Lòpez, Hay, & Conklin, 2009), during sexual intercourse (Dabbs & Mohammed, 1992), interaction with an infant (van Anders, Tolman, & Volling, 2012), and in response to competition (Zilioli & Watson, 2012). These fast androgen fluctuations have been hypothesized to fine-tune ongoing and future behaviors (Mazur & Booth, 1998), similarly to what is observed in other species such as birds (McGlothlin, Jawor, & Ketterson, 2007) and rats (Gleason & Marler, 2010). However, only recently has the association between socially-induced testosterone fluctuations and behavior been explored (Mehta & Josephs, 2006; Carré, Putnam, & McCormick, 2009; Apicella, Dreber, & Mollerstrom, 2014). This body of research shows that hormones and behavior are

inextricably linked, with endocrine profiles influencing and being influenced by behavioral systems.

This dissertation presents experimental data that probe distinct aspects of this complex relationship between the social environment and the endocrine axes. Acute changes in testosterone are explored within two evolutionarily salient behavioral domains –intrasexual competition and exposure to potential mates- and novel hypotheses regarding their functional significance are tested. Across various species, including primates, intrasexual selection often takes the form of male-to-male competition (Andersson, 1994), so Study 2 (Chapter 3) and Study 3 (Chapter 4) investigate testosterone reflexes in response to competitive interactions among men. On the other hand, Study 1 (Chapter 2), which examines testosterone response to facial expressions of emotions, comprises both men and women. Lastly, in Study 3, testosterone reactivity is correlated with short-term and long-term cognitive abilities hypothesized to favor success in a competition. Because hormones affect behavior in a probabilistic and context-dependent fashion, understating how testosterone concentrations at rest and, particularly, acute increases (or decreases) ensued from social experiences modulate behaviors is of crucial importance for both basic and applied research. As to the former, isolating and integrating endocrine causal variables with observable behavior helps building more comprehensive models of everyday social phenomena. Further, because of the profound impact of hormonal fluctuations on mental and physical health, these models inform related fields, such as psychopathology and health psychology. On the applied side, this research contributes to edify pioneering endeavours, such as personalized medicine, in which medical decisions and practices are customized to the individual patient.

1.2. Testosterone and Evolution: Theoretical Frameworks

In evolutionary biology, life history theory is concerned with the trade-offs individuals face when allocating limited resources (energy, nutrient and time) toward fitness optimization (Kaplan & Gangestad, 2005). Once sexual maturity is reached the basic trade-off is that of mating effort (resources invested to attract/protect mates, increasing opportunities for reproduction) and parenting effort (resources invested in

raising already-conceived offspring). Because testosterone is closely linked to somatic growth and development (i.e. anabolic effects), sexual differentiation (i.e. androgenic effects), and reproduction, it is considered to be a crucial mediator of life-history trade-offs (Muehlenbein & Bribiescas, 2005). In the following section, I will argue in favor of this hypothesis by reporting studies mainly conducted in humans.

1.2.1. Testosterone and life history theory

The link between testosterone and mating effort is obvious; testosterone augments male reproductive success by modulating morphological, physiological and behavioral phenotypes. For example, testosterone controls development of physical characteristics such as muscle mass and strength (Bhasin et al., 1996), voice pitch (Puts, Apicella, & Cardenas, 2012), and body (Kasperk et al., 1997) and face features (Lefevre, Lewis, Perrett, & Penke, 2013) that can be attractive to females (Weatherhead & Robertson, 1979; Valentine, Li, Penke, & Perrett, 2014) and advantageous when confronting same sex rivals (Archer, 2006). Testosterone is also responsible for spermatogenesis, and promotes sexual motivation (Sherwin, Gelfand, & Brender, 1985) and courtship behavior (Slatcher, Mehta, & Josephs, 2011). Moreover, testosterone increases reproductive access to potential mates by intimidating, deterring or defeating same-sex rivals (Andersson, 1994). This can take the form of direct male-to-male confrontation (Archer, 2006) and aggression (Archer, 1991) or indirect competition whereby individuals focus more on resource production/accumulation functional to pursue social dominance (Mazur & Booth, 1998). In humans, direct evidence of the link between androgens and reproductive fitness are observed in studies on fatherhood showing that single men with high testosterone level are more likely to become fathers in the short term (Gettler, McDade, Feranil, & Kuzawa, 2011) and that testosterone concentration in a father positively predicts self-reported lifespan reproduction (Pollet, Cobey, & van der Meij, 2013).

Although there is marked interspecies variability (Hau, 2007), simultaneous adverse effects on certain aspects of the immune system (Muehlenbein & Bribiescas, 2005; Furman et al., 2014) and paternal care (Mascaro, Hackett, & Rilling, 2013) offset the increase in reproductive fitness associated with prolonged exposure to testosterone.

For example, in a recent study, Mascaro and colleagues found that fathers with high levels of testosterone had lower scores on a paternal investment scale and weaker neural reactivity in response to viewing pictures of their own child (Mascaro et al., 2013). Other studies showed that testosterone is inversely related to relationship quality and divorce rates for married couples (Booth & Dabbs, 1993) and predicts polygyny (Alvergne, Faurie, & Raymond, 2009) in polygynous societies. On the other hand, low testosterone levels are usually associated with greater paternal involvement in primates (Clark & Galef, 1999), including humans (Muller, Marlowe, Bugumba, & Ellison, 2009).

These results perfectly match studies on other species whereby testosterone administration suppresses parental behavior (Katharina Hirschenhauser & Oliveira, 2006) as well as evidence found in non-human animals (Wingfield, Hegner, Dufty, & Ball, 1990) and humans (Gray, Kahlenberg, Barrett, Lipson, & Ellison, 2002; Gettler et al., 2011) showing that transition to fatherhood (and motherhood; Kuzawa, Gettler, Huang, & McDade, 2010) is associated with a significant drop in circulating androgens.

An additional cost associated with high testosterone levels is immunosuppression (Muehlenbein & Bribiescas, 2005). The immunocompetence handicap hypothesis suggests that a conspicuous investment in secondary sexual characteristics increases a male's immediate reproductive success at the expense of survival (Folstad & Karter, 1992). Although the effects of testosterone on the immune system depend on the type of function considered, vary considerably between species, and are often indirect (for example, glucocorticoids-mediated; Evans, Goldsmith, & Norris, 2000; Rantala et al., 2012), this hypothesis has been confirmed not only in birds and reptiles, but also mammals (Roberts, Buchanan, & Evans, 2004), including humans (Alvergne et al., 2009). Further, complementary evidence supports the idea that infection down-regulates the HPG axis (Simmons & Roney, 2009).

In summary, testosterone regulates adaptive phenotypic plasticity by promoting reproductive success at the expense of survival. These pleiotropic antagonistic effects regulating energy-allocation processes make it a fundamental proximate mechanism of vertebrates' life histories.

1.2.2. Basal Testosterone and Testosterone Reactivity

Most of the studies reported so far in favor of this hypothesis (i.e. testosterone as proximate mechanism of life history trade-offs in humans) have looked at testosterone concentrations at rest. However, basal androgen level is only one of the many components of an endocrine signaling network, which comprises also enzymes, other hormones, receptors, hormone binding proteins and transporters. Further, an important distinction can be made between basal testosterone levels and testosterone reactivity/responsiveness (McGlothlin et al., 2007; Nyby, 2008; Gleason, Fuxjager, Oyegbile, & Marler, 2009; McGlothlin et al., 2010).

Across taxa, rapid alterations of testosterone are observed in response to multiple social contexts. The highly conserved nature of testosterone fluctuations following competitive challenges raises the question of its functional/ultimate significance (Nyby, 2008). In other words, what functions does this phylogenetically ancient physiological mechanism serve? Rats and birds are two species where testosterone reflexes (discussed in section 1.3) and their function (discussed in section 1.4) have been most studied.

In rats, testosterone reactivity has been studied either by looking at socially induced fluctuations in endogenous testosterone (Marler, Oyegbile, Plavicki, & Trainor, 2005; Oyegbile & Marler, 2005) or by experimentally mimicking these physiological responses through testosterone injections (Trainor, Bird, & Marler, 2004). Although the latter approach allows researchers to look at the mechanisms of testosterone actions and overcome the limitations of the correlational methodology of the former approach, it also wipes out phenotypic variation (i.e. individual differences) in testosterone responsiveness. Recent work with birds has tried to combine these two approaches by experimentally examining individual differences in testosterone reactivity (McGlothlin et al., 2007; McGlothlin et al., 2008).

Avian studies have shown that testosterone is a crucial physiological mechanism regulating the relative amount of energy invested into either parental or mating effort (Ketterson & Nolan, 1992). However, the majority of the studies have focused their attention on baseline testosterone (Alatalo, Hoglund, Lundberg, Rintamaki, & Silverin,

1996) or experimentally manipulated testosterone (Ketterson, Nolan, Wolf, & Ziegenfus, 1992) without addressing questions about individual natural variation in testosterone. New research by Ketterson and colleagues (McGlothlin et al., 2007; McGlothlin et al., 2008; McGlothlin et al., 2010) – followed by matching work in rats (Gleason & Marler, 2010)- has explored individual variation in testosterone levels by measuring testosterone before and after injections of gonadotropin-releasing hormone (i.e. GnRH challenge). Because the short-term testosterone increases following a GnRH challenge resemble those produced naturally in response to social stimuli, this method allows the investigation of individual variation in the HPG axis responsivity (McGlothlin et al., 2007). Interestingly, testosterone responses correlated positively with aggressive behavior and negatively with parental behavior (McGlothlin et al., 2007). More recently, it was also found that birds that experienced a medium to high GnRH-induced testosterone increase displayed optimal patterns of both survival and reproduction (McGlothlin et al., 2010). Notably, this pattern of findings shows that selection acts not only on baseline and seasonal androgen levels but also on short-term testosterone natural variation. In humans, it is therefore important not only to look at the relationship between individual differences in baseline testosterone and behavior, but also at individual differences in testosterone reactivity and their association with behavior.

In the next section, I will describe two evolutionary relevant behavioral systems where testosterone responses are observed, namely competitive interaction and exposure to potential mates. Adopting a comparative approach, representative studies in both humans and other species will be reported.

1.3. Testosterone release in evolutionary relevant social contexts

1.3.1. Testosterone, competition and the Challenge Hypothesis

In a wide range of vertebrates, including humans, intraspecific competition, especially in the form of male-to-male antagonistic encounter, is an ecologically relevant context that modulates androgen release (Wingfield et al., 1990; Hirschenhauser & Oliveira, 2006). Acute spikes in testosterone are observed as early as 10 minutes after

the onset of a social conflict (Wingfield & Wada, 1989). Testosterone release following intrasexual competition is a consistent response within and across species that has been maintained by evolution despite speciation. Initially applied to avian species, the Challenge Hypothesis postulates that male breeding season baseline testosterone would rise to a maximum physiological level in situations of intense mating effort, such as in the presence of other aggressive male conspecifics (Wingfield et al., 1990). With appropriate modifications (for example, many species are continuous breeders), and not without exceptions (Thompson & Moore, 1992), the main idea behind the Challenge Hypothesis (i.e. that social challenge feeds back onto androgen levels) has been extended to a variety of invertebrates (Scott, 2006) and vertebrates (for a review, see Hirschenhauser & Oliveira, 2006), including fishes (Hirschenhauser, Taborsky, Oliveira, Canario, & Oliveira, 2004), amphibians (Houck & Woodley, 1995), reptiles (Greenberg & Crews, 1990) and mammals (Oyegbile & Marler, 2005), embracing non-human (Girard-Buttoz, Heistermann, Krummel, & Engelhardt, 2009) and human primates (Archer, 2006). However, among species variability exists in terms of situational factors able to moderate this phenomenon. In this regard, the most powerful moderator of the challenge effect seems to be the outcome of a social conflict. In some species (mostly birds), regardless the outcome of the confrontation, the presence of a hostile opponent is enough to provoke a rise in testosterone (Wingfield et al., 1990). In many species, however, testosterone fluctuates in concert with changes in social status, such that winning competitions leads to an increase in circulating testosterone and/or losing leads to a net decrease in testosterone (Lloyd, 1971; Bernstein, Rose, & Gordon, 1974; Dixson, 1980; Oliveira, Silva, & Canàrio, 2009). In striking harmony with this idea, tied fights are not accompanied by any significant change in testosterone (Oliveira, Carneiro, & Canàrio, 2005). This phenomenon, known as the “Competition Effect” or the “Winner-Loser Effect”, is one of the main predictions of the biosocial model of status (BMS) (Mazur & Booth, 1998), which posits a dynamic, bidirectional relationship between human testosterone and status. According to the model, testosterone encourages status-seeking behaviors and changes in status alter testosterone concentrations. Thus, in competitive interactions it is predicted that winners will experience a rise in testosterone relative to losers and that these testosterone changes will in turn guide individuals towards or away from future attempts at gaining status (Mazur & Booth, 1998). Most human studies testing this hypothesis have examined sports competitions -

such as soccer (Oliveira, Gouveia, & Oliveira, 2009), tennis (Mazur & Lamb, 1980), and volleyball (Edwards & Kurlander, 2010). Consistent with the predictions of the BMS, many of these studies show a competition effect (or winner-loser effect).

Such effects are seen in competitors not only following a contest, but also when reviewing previous contests on video; in one example, hockey team members showed an increase in salivary testosterone after viewing a previous game that they had won (Carré & Putnam, 2010). Impressively, even purely vicarious competition effects have been observed, in the testosterone responses of sports fans witnessing wins or losses of their favorite teams. For example, in a study of soccer fans watching a World Cup match, fans that rooted for the winning team showed an increase in testosterone after the match relative to fans who rooted for the losing team (Bernhardt, Dabbs, Fielden, & Lutter, 1998). Similarly, on the night of the 2008 US presidential election, people who supported the losing candidate (McCain) dropped in testosterone relative to people who supported the winning candidate (Obama) (Stanton, Beehner, Saini, Kuhn, & LaBar, 2009). These results, which can somehow be connected to the "audience effect" (Gyger, Karakashian, Dufty, & Marler, 1988), perfectly match findings in other species such as cichlid fish (*Oreochromis mossambicus*) whereby male spectators of an aggressive interaction experienced a rise in androgens compared to control bystanders not exposed to the fight (Oliveira, Lopes, Carneiro, & Canario, 2001).

Interestingly, the competition effect can be further modulated by additional contextual factors, such as the location of the dispute (the so called "home advantage", Neave & Wolfson, 2003), with victories in a familiar environment -i.e. home cage for rats; (Fuxjager, Mast, Becker, & Marler, 2009) and home sport venue in humans (Carré, 2009)- being associated with greater testosterone responses compared to victories "away from home".

The interplay between situational variables regulating challenge-induced testosterone pulses reaches its maximum complexity when considering multiple experiences of winning (or losing). Seminal work from Marler and colleagues investigated this phenomenon proposing it as the physiological substrate of the winner effect -the increased probability of winning an aggressive encounter following previous victories (discussed below) (Oyegbile & Marler, 2005; Fuxjager & Marler, 2010). For

example, Oyegile and Marler (2005) showed that endogenous testosterone in California mice (*Peromyscus californicus*) experiencing multiple wins was higher compared to conspecific that experienced fewer or no social victories. Similarly, Huhman et al. (1991) found that reiterated experiences of social defeat in male hamsters led to a suppression of plasma testosterone when compared to male hamsters that were not engaged in any conflict. Surprisingly, experiments on androgen responses to multiple competitive interactions are absent in humans. Research is needed to establish how repeated wins or losses influence HPG reactivity. Study 3 of this dissertation examine this phenomenon by measuring salivary testosterone in pairs of male participants engaging, on two consecutive days, in head-to-head competitions.

A second order of intervening variables refers to what I call motivational factors. With this broad term I denote both intraspecies and interspecies differences that stems from ontogeny, phylogeny, ecological conditions and their interaction. For example, a species' structure in relation to sexual behavior (i.e. mating system) is determined by both phylogenetic and ecological factors (Emlen & Oring, 1977) and strongly modulates the testosterone response to antagonistic encounters (Wingfield et al., 1990; Fuxjager & Marler, 2010). Accordingly, in its original formulation, the Challenge Hypothesis made different predictions regarding testosterone secretion for monogamous and polygamous species (Wingfield et al., 1990). In polygamous species, generally characterized by low levels of paternal care, androgens are likely to be high throughout the breeding season as males frequently court females and compete with other males; whereas, in socially monogamous male birds, which provide more paternal care, testosterone oscillations are expected only during territorial intrusions. In fact, because of the trade-off between mating and paternal efforts, which appears to be mediated by testosterone concentrations, androgens cannot be kept at the maximum physiological level in males with high level of parental investment (Wingfield et al., 1990).

Motivational factors also encompass sociobiological and, biopsychological individual differences, such as coping style (Koolhaas, de Boer, Buwalda, & van Reenen, 2007), social status (Gould & Ziegler, 2007) or life-history strategies (Schwabl, 1996; Del Giudice, Ellis, & Shirtcliff, 2011). For example, Gould and Ziegler (2007) found that among Ring-tailed lemurs (*Lemur catta*), a species of primates with extreme reproductive seasonality, mating season testosterone was higher in high-ranking males

compared to the low-ranking males. In humans, various motivational variables in the androgenic activation to competition have been proposed, such as personality traits (Schultheiss, Campbell, & McClelland, 1999), mood (McCaul, Gladue, & Joppa, 1992) and physiological state (Mehta & Josephs, 2010).

Although there is relatively little evidence that mood directly mediates testosterone changes after winning or losing (McCaul et al., 1992), aspects of personality and personal differences in causal attribution appear to play a role. Schultheiss et al. (1999) and Schultheiss and Rohde (2002) suggest that the intensity of the individual's intrinsic drive to enhance their own status relative to others, termed ''implicit power motivation'', may be a crucial moderator of the effect of competitive outcome on hormonal responses. Studying teams of basketball players, Gonzalez-Bono et al. (1999, 2000) found that positive changes in testosterone (post-competition testosterone minus pre-competition testosterone) were more pronounced in those winners that attributed success in the contest to their own involvement and skills. This finding has clear implications for possible differences in the endocrine impact of individual competitions — where the attribution of the outcome to one's own abilities is unambiguous — versus team contests, where the perception of one's own contribution to the outcome is diluted by the performance of the other team members.

A distinct but related issue is personal involvement; evidence indicates that a subject's evaluation that a competition is important for status or social ranking may lead to higher personal involvement, leading to greater activation of the HPG axis (Salvador, 2005). Indeed, excepting some studies of competition in very strenuous sports, in which physical exertion may have confounded the neuroendocrine competition effect (Edwards et al., 2006), competitive situations in which subjects' personal investment is believed to be greatest appear to be most effective in eliciting a competition effect on circulating testosterone (Mazur et al., 1992; Bernhardt et al., 1998). The joint feature of these naturalistic studies was to engage men in common meaningful competitive situations close to their everyday life experiences. This aspect may be missing in some laboratory studies of the competition effect, where less engaging contests have been used (see, for example, Mazur et al., 1997; Mehta and Josephs, 2006; van Anders and Watson, 2007). It is possible that lack of familiarity, perceived unimportance for status, and/or decreased

involvement in some of these tasks, has resulted in inconsistent evidence of a competition effect (Archer, 2006).

An additional moderator of the competition effect is physiology, namely the impact of other systems such as the autonomic nervous system or other endocrine axes, on testosterone secretion. For example, sustained physical activity can buffer testosterone secretion likely in relation to the activity of other endocrine axis, such the HPA axis (Maestu, Jurimae, & Jurimae, 2005). Notably, the same buffering effect of the HPA axis on the HPG axis has been recorded in non-physical competition (Mehta & Josephs, 2010). In their seminal work Mehta and Josephs found that among losers of a laboratory competition, a combination of basal cortisol and testosterone predicted changes in testosterone after the contest. High pre-competition testosterone was associated with a decrease in testosterone only in those losers whose pre-competition cortisol concentrations were also high. This pattern was not found in subjects with low basal concentrations of cortisol.

Using a novel experimental paradigm, Study 1 of this dissertation investigates whether angry facial display of emotions, evolutionary ancient social cues of potential status-challengers and/or physical aggressors (i.e. angry males) (Sell, Tooby, & Cosmides, 2009), would impact testosterone secretion in ways similar to what is observed during competitive interaction. On the other hand, directly using a head to head competition, Study 2 of this dissertation investigates the possible interactions between the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) stress axis in predicting transient changes in testosterone after social victory or defeat, using a familiar competitive task.

1.3.2. Testosterone release in response to potential mating opportunities

An encounter with a potential mate is another evolutionary salient behavioral context where rapid testosterone alterations are observed. Luteinizing hormone (LH) increases in response to female stimuli (Arthur Coquelin & Bronson, 1979), including the mere sight of a female conspecific (Katongole, Naftolin, & Short, 1971) and exposure to female urinary pheromones (Clancy, Singer, Macrides, Bronson, & Agosta, 1988;

Richardson et al., 2004). Further, in males of a variety of mammalian taxa -- including sheep (Borg, Esbenshade, Johnson, Lunstra, & Ford, 1992), rats (Bonilla-Jaime, Vazquez-Palacios, Arteaga-Silva, & Retana-Marquez, 2006; Macrides, Bartke, & Dalterio, 1975) and monkeys (Ziegler, Schultz-Darken, Scott, Snowdon, & Ferris, 2005; Cerda-Molina et al., 2006) -- exposure to female conspecifics results in elevated testosterone. Two reproductive situations seem to be particularly responsible for these fluctuations: (1) the initial exposure to a female –even in the absence of female physical stimulation (Amstislavskaya & Popova, 2004)- and (2) the time following ejaculation if the interaction led to mating (Coquelin & Desjardins, 1982; Nyby, 2008). Interestingly, although being sexually experienced leads to a more robust androgenic response, it is not a requirement for these responses to take place (Clancy et al., 1988). However, as in the case of testosterone fluctuations in response to male-to-male antagonistic encounters, different motivational factors, such as mating system and mating status (Ziegler et al., 2005), novelty of the female stimulus (Coquelin & Bronson, 1980) or social status (Surbeck, Deschner, Schubert, Weltring, & Hohmann, 2012), modulate this hormonal reflex. Another similarity with competitive interactions is the timeline of this physiological response: Testosterone levels rise within 10 min of encountering a female, peak within half hour, and return to basal levels within an hour (Coquelin & Desjardins, 1982).

Exposure to mate relevant stimuli leads to similar endocrine responses in humans too (Roney, Mahler, & Maestripieri, 2003; Roney et al., 2007; Lòpez et al., 2009). For example, in their pioneering work, Roney and colleagues (2007) asked male undergraduates to provide salivary samples both before and after a brief social interaction with a female confederate. Compared to men interacting with another man or sitting alone, men interacting with a female confederate showed a significantly greater increase in testosterone. Similar results were obtained by Lòpez and collaborators (2009), who demonstrated that exposure to a video montage showing courtship interaction between a highly socially and parentally attractive man and a young woman caused testosterone accumulation in a female sample. In accordance with the hypothesis that cues of potential mating opportunities induce increases in testosterone, Miller and collaborators (2012) found that the ratio of opposite-sex (i.e. potential mates) to same-sex individuals during a physical competition was associated with increases in

salivary testosterone in both men and women. Lastly, at least in men, this phenomenon seems to be strongly moderated by motivational and physiological factors (Roney et al., 2003; van der Meij, Buunk, van de Sande, & Salvador, 2008; Roney, Simmons, & Lukaszewski, 2010). For example, sexual experience (Roney et al., 2003; but see, van der Meij et al., 2008) and dominant-aggressive personality (van der Meij et al., 2008) have both been found to modulate testosterone responses to potential mates, such that increases in testosterone after interaction with a woman were more pronounced in aggressive-dominant men (van der Meij et al., 2008) and sexually experienced men (Roney et al., 2003). Likewise, men with low cortisol concentration and a more responsive androgen-receptor genotype (as inferred from the number of CAG codon repeats in the androgen receptor gene) have been found to have larger testosterone accumulations to potential mates (Roney et al., 2010).

Studies on the link between testosterone reactivity and sexual activity have yielded mixed results. For example, it is not clear whether non-physical stimulation affects testosterone reactivity analogously in men and women. Watching erotic movies causes men's testosterone (Pirke, Kockott, & Dittmar, 1974; Hellhammer, Hubert, & Schurmeyer, 1985; Rowland et al., 1987; Carani et al., 1990; Stoleru, Ennaji, Cournot, & Spira, 1993; Redoute et al., 2000) and LH (LaFerla, Anderson, & Schalch, 1978; Rowland et al., 1987; Carani et al., 1990; Stoleru et al., 1993) to increase; however, simply engaging in sexual thoughts does not seem to have an effect on testosterone secretion (Goldey & van Anders, 2012). In women, on the other hand, the opposite seems to be true, with a boost in testosterone occurring when imagining a positive sexual encounter with an attractive men (Goldey & van Anders, 2011), but not when watching an erotic movie (Van Anders, Brotto, Farrell, & Yule, 2009) - at least when not accompanied by masturbation to orgasm (Exton et al., 1999). Notably, these findings mirror sex differences in neural activations to erotic stimuli; three studies reported greater male neural reactivity in response to visual presentation of erotic stimuli (Hamann, Herman, Nolan, & Wallen, 2004) (Sabatinelli, Flaisch, Bradley, Fitzsimmons, & Lang, 2004; Gizewski et al., 2009), but no sex differences were observed in response to seductive voices of the opposite sex (Ethofer et al., 2007). Lastly, whether olfactory stimuli influence testosterone secretion in men is still a matter of debate (Miller & Maner, 2010; Roney & Simmons, 2012; Cerda-Molina, Hernandez-Lopez, Claudio, Chavira-

Ramirez, & Mondragon-Ceballos, 2013). This pattern of results suggests that, similarly to neural responses, male androgenic reactivity to erotic stimuli may be modality specific.

Early negative findings on the association between penis-vagina intercourse and testosterone increase might be explained by the low sample sizes employed (Fox, Ismail, Love, Kirkham, & Loraine, 1972; Stearns, Winter, & Faiman, 1973; Lee et al., 1974); in fact, most more recent studies have found that partnered sexual activity leads to both transient (in both men and women; Dabbs & Mohammed; van Anders, Hamilton, Schmidt, & Watson, 2007) and prolonged testosterone accumulation (in men only; Kraemer et al., 1976; Hirschenhauser, Frigerio, Grammer, & Magnusson, 2002). Lastly, the effect of masturbation on androgen secretion remains controversial (Purvis, Landgren, Cekan, & Diczfalusy, 1976; Kruger et al., 1998; Exton et al., 1999).

Study 1 of this dissertation investigates testosterone reactivity to those facial emotional expressions that signal potential mating opportunities, such as happy expressions in women (Tracy & Beall, 2011; Penton-Voak & Chang, 2008).

1.4. Functional significance of testosterone release in evolutionary relevant social contexts

Socially induced acute changes in testosterone represent a phylogenetic conserved phenomenon, which suggest sadaptive functions (Nyby, 2008). Focusing specifically on challenge-induced testosterone release, in this section, I will first describe the main hypotheses that have been proposed about the functional significance of testosterone reflexes by comparing non-human animals, especially rats and birds, with human research. Then, I will provide a rationale behind the specific hypotheses on the functional significance of androgen release tested in Study 3 of this dissertation.

1.4.1. Hypotheses on adaptive function for testosterone release in response to competitive interactions

The Challenge Hypothesis posits that contest-related testosterone releases mediate trade-offs between parental and mating effort (Wingfield et al., 1990). In avian

species, although not without exceptions, both experimental and correlation empirical evidence support this hypothesis. For example, various studies using experimental elevation of testosterone via subcutaneous implants have shown increase in mating behavior at the expense of parental behavior (Silverin, 1980; Ketterson et al., 1992; Van Roo, 2004; but see, Van Duyse, Pinxten, & Eens, 2002). Likewise, a positive association between mating success and circulating levels of testosterone has been observed (Alatalo et al., 1996; Mills, Grapputo, Koskela, & Mappes, 2007; but see, Brown, Brown, Raouf, Smith, & Wingfield, 2005). Further, recent studies demonstrated that individual variation in the responsiveness of the HPG axis -i.e. variation in the capacity of the HPG axis to generate acute increase in testosterone in response to a GnRH challenge (McGlothlin et al., 2007)- positively predicts aggression during a simulated territorial intrusion and correlates negatively with nestling feeding (McGlothlin et al., 2007). The magnitude of GnRH-induced testosterone increase is also associated with amount of tail white, a secondary sexual characteristic used by males in courtship and territorial defense (McGlothlin et al., 2008). In sum, confirming the Challenge Hypothesis (Wingfield et al., 1990), males with higher testosterone reactivity are less invested in parental effort and more highly invested in mating-acquisition behavior such as courtship and aggression (but see, McGlothlin et al., 2010).

In rats, three hypotheses have been proposed for the functional significance of contest-related androgen elevations (Gleason et al., 2009). First, testosterone could enhance an individual's ability to win future encounters by increasing aggression (Trainor et al., 2004; Oyegbile & Marler, 2005). Second, competition-related testosterone surges could reinforce and/or stimulate learning processes associated with the contest, such as the preference for the competition location (Martinez, Guillen-Salazar, Salvador, & Simon, 1995) or the behavioral strategies that led to a victory (Marler et al., 2005). Third, testosterone might affect behaviors, other than aggression, that are associated with winning, such as persistence in search behavior (Andrew & Rogers, 1972; Wingfield, 1994).

In California mice, consecutive wins on consecutive days result in an increase in testosterone on the final testing day and a higher probability to win the final aggressive encounter (Oyegbile & Marler, 2005). This phenomenon is known as the Winner Effect (or the Winner-Challenge Effect) and it is mediated by androgens (Fuxjager, Oyegbile, &

Marler, 2011; Oliveira et al., 2009). Because in rodents the outcome of a fight depends on the fast display of various attacks (e.g., initiating biting or chasing) (Eisenberg, 1962) the winner effect generally has been framed in terms of social aggression. However, post-encounter androgens offer a biological substrate for a variety of behaviors that are not necessarily confined to aggression (Wingfield, 1994; Martinez et al., 1995; Fuxjager et al., 2010).

In humans, changes in testosterone after winning a competition predict immediate aggressive behaviors (Carré, Campbell, Lozoya, Goetz, & Welker, 2013). Despite the broad appeal of the aggression-enhancing hypotheses, there are two important limitations in this area of research that have not been adequately addressed in humans. First, none of the alternative hypotheses reported above have been tested. For example, testosterone is implicated in cognition (O'Connor, Archer, Hair, & Wu, 2001; Ackermann et al., 2012) and it could be expected that testosterone surges following a challenge might modulate winning by acting on learning of contest-related information (Marler et al., 2005). A second limitation concerns the fact that all studies so far have looked at changes in behaviors occurring right after the competitive interaction, overlooking long-term behavioral outputs that might be correlated with testosterone changes on previous days (Oyegbile & Marler, 2005; Wright, Edwards, Fleming, & Dolan, 2012).

As to the former, Study 3 focuses on cognitive abilities ancestrally relevant in male-to-male combat (i.e. intrasexual selection) and mate choice (i.e. intersexual selection), namely spatial abilities. At least three hypotheses link sexual selection to spatial abilities: the “male range” hypothesis (Gaulin & FitzGerald, 1986; Jacobs, Gaulin, Sherry, & Hoffman, 1990), the “male warfare” hypothesis (Alexander & Culligan, 1979; McDonald, Navarrete, & Van Vugt, 2012) and the “female choice” hypothesis (Hawkes, 1991). In many species, including humans, males outperform females on spatial tasks (Gaulin & FitzGerald, 1986; Kimura, 2000). According to the “male range” hypothesis this difference emerges when one sex has a larger range than the other, a pattern that results from the mating system. For example, in polygamous species, but not monogamous species, males can improve reproductive success by expanding their ranges and remembering the locations of multiple females (Jacobs et al., 1990). It derives that male competition select for enhanced spatial ability, with testosterone

playing a crucial role in explaining between sexes (a possibly within sex) variation (Gouchie & Kimura, 1991; Janowsky, 2006). The “male warfare” hypothesis suggests that the function of range size expansion is to cause conflicts with other males (i.e. outgroup) in order to reduce competition for resources and capturing females (Alexander & Culligan, 1979; McDonald et al., 2012). Lastly, the “female choice” hypothesis posits that women would use hunting success, which strongly depend on spatial abilities, as an important mate choice criterion (Hawkes, 1991). In brief, compelling evidence suggests that sexual selection acted as a selective pressure on spatial cognition. In Study 3, I investigate testosterone response to antagonistic encounter as one of the underlying physiological mechanisms that might modulate this behavior.

In terms of long-term effects, Study 3 focuses on how testosterone influences learning of contest-related information. Recent findings in rodents showed that androgen deprivation, induced through gonadectomy, led to impaired memory performance in hippocampus-dependent tasks, and that this impairment could be prevented by testosterone replacement (Edinger & Frye, 2007). Similarly in humans, endogenous and exogenous testosterone seems to improve learning, markedly on a long-term timescale (Wright et al., 2012). In a two-day experiment, wherein subjects performed the same tasks on each day, Wright and colleagues found that testosterone administration induced learning between sessions (i.e. subjects who received testosterone performed better on the second day compared to subjects who received placebo), suggesting that testosterone is able to influence “off-line” consolidation processes (Robertson, Pascual-Leone, & Miall, 2004). The possibility that testosterone reactivity to antagonistic encounters modulates learning has not yet been investigated. In Study 3 I test whether testosterone reactivity on the first day would be associated with any improvement on the competitive task performed on the second day.

1.5. Review of the dissertation experiments

In summary, testosterone levels are not static but fluctuate in response to environmental inputs, including social signals. Acute spikes are particularly observed in response to evolutionary salient social interactions such as antagonistic encounters and exposure to potential mates. The first goal of this dissertation is to further examine this

phenomenon using facial display of emotions as potential eliciting stimuli, serving as proxies for social interactions (e.g., van Honk et al., 2000). From an evolutionary point of view, facial displays of emotions represent potent, species-specific social signals that help individuals coordinate their responses, so as to improve their inclusive fitness and shape group hierarchies. For example, happy faces are indicative of affiliative intentions from conspecifics (Knutson, 1996). On the other hand, angry faces are thought to convey threat and signal imminent dominance challenges (Dimberg and Öhman, 1996). Further, previous studies indicate that there are sexually dimorphic aspects in the evaluation of emotional expressions. For example, Becker et al. (2007) found that evaluations of anger, happiness and sex in facial displays of emotions were not mutually independent. Because connections between feminine facial features and happy displays and masculine features and angry displays were found, it was concluded that people make automatic associations between gender and specific emotions. Penton-Voak and Chang found that smiling (the key behavioral component of happy expression) increased attractiveness judgments in female targets but not males (Penton-Voak & Chang, 2008). Conversely, angry male faces are associated with high degrees of threat (van Honk et al., 2000).

This body of research hint that facial emotions might affect testosterone fluctuations in similar ways to what observed in response to antagonist encounters and interactions with potential mates. Notably, these effects might vary as a function of the sex of the stimulus face. This possibility has not been tested yet. Using a simple paradigm, Study 1 examines the effects of emotional content and sex of facial stimuli in modulating endogenous testosterone fluctuations, as well as sex differences in the endocrine responses to faces.

Socially induced changes in testosterone are not identical among individual but rather moderated by motivational (e.g., mating system and personality), situational (e.g., win vs. lose and location of the dispute) and physiological (e.g., current pathogenic stress) factors. The second goal of this dissertation is to further investigate this phenomenon by focusing on physiological factors and in particular on the buffering effects of the HPA axis, responsible for glucocorticoids secretion, on the HPG axis. In other words, Study 2 tests the hypothesis that the androgenic response observed within the context of male-to-male competition is moderated by cortisol, the primary stress

hormone of the body, fundamentally implicated in the regulation of cardiovascular, metabolic, homeostatic, and immune system functions (Miller, Chen, & Zhou, 2007). The integration between these two endocrine axes is well documented (for reviews, see (Johnson, Kamilaris, Chrousos, & Gold, 1992; Viau, 2002), and, considering the opposing effects of androgens and glucocorticoids (Crawford, Liu, Kean, Bleasel, & Handelsman, 2003), it seems that crosstalk between the HPA and HPG systems may take the form of reciprocal inhibition (Viau & Meaney, 1996; Tilbrook, Turner, & Clarke, 2000; Ciechanowska, Lapot, Mateusiak, & Przekop, 2010). Consistent with this view, we hypothesize that individuals with high basal levels of cortisol would experience less of an increase in testosterone when winning a competition compared to those individual with initial low levels of cortisol.

Because reflexive testosterone release in mating and competitive situations has been recorded in a wide variety of taxa, it is expected that it would play some functional role in regulating behavior. Maintaining constantly high levels of testosterone can be costly and maladaptive (Wingfield, Lynn, & Soma, 2001), therefore fast rise in androgens might have been selected to modulate ongoing and/or future behaviors implicated in survival and reproduction (Nyby, 2008). Recent human studies provide compelling support for the idea that testosterone increments after a contest predict short-term aggressive behavior (Carré et al., 2009; Carré et al., 2013). However, it remains unclear whether other behavioral domains, such as cognition, are influenced by these acute increases. Further, long-term effects of testosterone reactivity have not been investigated yet. To fill this gap, Study 3 examines whether testosterone reactivity to repeated head-to-head competitive interactions correlates with short-term and long-term cognitive abilities hypothesized to favor success in a competition.

Chapter 2.

STUDY 1: Testosterone Reactivity to Facial Display of Emotions in Men and Women

Note: This section is based on the following article, with permission: Zilioli, S., Caldbick, E. & Watson, N.V., (2014). Testosterone Reactivity to Facial Display of Emotions in Men and Women. *Hormones and Behavior* 65, 461-468.

2.1. Introduction to Study 1

In social mammals, most social behaviors can be viewed as belonging to two broad categories that control social organization: dominance behaviors, which often involve conflict between individuals, and affiliative behaviors, which bring individuals together in a prosocial manner (Wilson, 1975). The steroid hormone testosterone plays an important role in regulating both types of behaviors, through its modulatory actions on both cortical and subcortical brain mechanisms (for examples, see Stanton, Wirth, Waugh, & Schultheiss, 2009; Mehta & Beer, 2010).

One way in which the relationship between hormones and social behaviors has been investigated in humans is through presentation of facial stimuli, such as angry and happy faces, serving as proxies for social interactions (e.g., van Honk et al., 2000). Facial displays of emotion (FDEs) are perceived as being closely tied to the emotional experiences of the displaying individual, and thus are decoded as a paralinguistic communication channel reflecting the individual's emotional state (Ekman & Friesen, 1971). Cross-cultural similarities have been reported in the recognition and production of facial expressions in both adults and children, generally supporting theories about their universality (Izard, 1994). Hence, from an evolutionary point of view, FDEs represent potent, species-specific social signals that help individuals coordinate their responses, so as to improve their inclusive fitness and shape group hierarchies. For example, happy

faces are indicative of affiliative intentions from conspecifics (Knutson, 1996). On the other hand, angry faces are thought to convey threat and signal imminent dominance challenges (Dimberg & Öhman, 1996). Approach or avoidance behaviors in response to these ritualized displays seem to depend on individual and contextual differences in motivational stance. For instance, the threat conveyed by an angry face may be perceived as more intimidating by a submissive person, who in response may avert his or her gaze away from the potential competitor. In dominant individuals the same FDE might be perceived as a provocation or dominance challenge, giving rise to a face-to-face competition for status. In this context, testosterone seems to help regulate the processing of FDEs, by affecting these motivational dimensions (van Honk et al., 2000).

In contrast to the work exploring the impact of hormonal status on processing of affective facial displays, less attention has been paid to the reverse relationship: the impact of processing social affective cues on hormonal responses, and the functional significance of such responses (van Anders & Watson, 2006b). In other words, how does the perception of FDEs affect testosterone levels? To our knowledge only one previous study, more than a decade ago, has indirectly addressed this issue (van Honk et al., 2000). In a between subject design, van Honk and colleagues compared endocrine responses of young men to two different versions of an emotional Stroop task, used to assess selective attention to male angry faces. The researchers found that the individual stance towards angry faces (vigilance vs. avoidance) was associated with testosterone reactivity in the subliminal presentation (i.e. backward-masked); specifically, participants engaging in vigilance behavior showed an increase in testosterone when subliminally exposed to angry faces. Supraliminal, consciously-perceived angry stimuli lacked an equivalent effect on testosterone. Clearly, more systematic data on endocrine reactivity to FDEs is needed, and in both sexes.

2.1.1. Sex differences in Emotion Processing

A number of striking sex differences in electrophysiological responses (Mazurski, Bond, Siddle, & Lovibond, 1996; Bradley, Codispoti, Sabatinelli, & Lang, 2001) and fMRI activation (e.g., Killgore & Yurgelun-Todd, 2001; Fine, Semrud-Clikeman, & Zhu, 2009; Whittle, Yücel, Yap, & Allen, 2011) to emotional stimuli have emerged. In general,

women exhibit stronger overall activation in response to negative cues -- that is, unpleasant, traumatic and *some* threatening stimuli (e.g. fearful faces) -- whereas men tend to show stronger activation in response to positive affective stimuli and different threatening stimuli (e.g. cues of dominance). These sex differences appear to be especially evident with regard to emotional reactivity (the subject's threshold, extent and intensity of affective arousal) (Williams et al., 2005; Wrase et al., 2003) and emotion regulation (the subject's effort to manage, inhibit and enhance emotions) (Mak, Hu, Zhang, Xiao, & Lee, 2009). For example, when fMRI and skin conductance were recorded during processing of fearful faces, men showed an attenuation of activation in brain regions associated with emotional processing (i.e. amygdala) and in the sympathetic nervous system, from early to late phases of the experiment. In contrast, women generally showed increased amygdalar activity, persisting for the entire course of the experiment, possibly indicative of a higher resistance to extinction of emotional arousal (Williams et al., 2005).

Increased female (vs. male) activation in subcortical (i.e. amygdala) and prefrontal (i.e. orbitofrontal cortex) regions has been also observed in response to static angry faces (McClure et al., 2004). This result, however, was restricted to a small sample of healthy adults and was not extended to adolescents tested in the same study (McClure et al., 2004). Recently, a larger study using brief video clips of neutral faces evolving into angry expressions seemed to counter this conclusion by showing that amygdalar responses to angry faces were more accentuated in male than female adolescents (Schneider et al., 2011). Although it is plausible that these discrepancies derive from methodological differences, they may simply reflect a developmental switch (adolescent vs. adults) in men's sensitivity to cues of dominance, such as angry faces of other males. More research is needed to answer this question. The hypothesis that men are generally more sensitive to status-threatening stimuli is supported by other studies, wherein greater neural (Schienle, Schafer, Stark, Walter, & Vaitl, 2005) and psychophysiological (Mazurski et al., 1996) activation was recorded in males exposed to pictures of attacks by humans or non-human animals (Schienle et al., 2005), or specifically angry faces of other males (but not females) (Mazurski et al., 1996).

There is better agreement among studies investigating responses to positive emotional stimuli. For example, Wrase and colleagues (2003) found that depictions of

positive affect caused a stronger amygdalar response in men than women. Similarly, Killgore et al. (2001) reported a sex difference in lateralized amygdalar activation during viewing of happy facial expressions, with men showing relatively greater right amygdala activity compared to women. Pro-sexual imagery generates complementary results, such as enhanced amygdala and hypothalamus activations in men viewing heterosexual sexual activity (Hamann et al., 2004), and greater sympathetic arousal (i.e. skin conductance) in men compared to women when viewing erotic pictures (Bradley, Codispoti, Sabatinelli, et al., 2001). Whether such effects extend to the endocrine system, with FDEs bringing about complementary modulations of circulating hormones, has not been empirically explored.

Given the importance of testosterone for sexually-selected traits, and its important role in regulating social emotional behavior (van Anders & Watson, 2006b), the current study was designed to explore possible sex differences in testosterone responses to same-sex and opposite-sex FDEs signaling either threat (i.e. angry faces) or affiliation (i.e. happy faces). The Challenge Hypothesis (Archer, 2006; Wingfield et al., 1990) -- which is mainly concerned with males, but might extend to females in less sexually-dimorphic species (Ketterson, Nolan, & Sandell, 2005) -- builds on the observation that testosterone secretion prepares the body to face imminent adaptive challenges relating to dominance. For example, testosterone is implicated in defense of resources (e.g. food, territory, offspring, status) that determine mate value and reproductive success. Accumulation of testosterone is thus observed both in response to dominance challenges such as intra-sexual competition (see for example, Bateup, Booth, Shirtcliff, & Granger, 2002; Zilioli & Watson), or conspecific signs of threat (van Honk et al., 2000), as well as in situations involving exposure to sexual stimuli, such as interactions with potential mates (Lòpez et al., 2009; Roney et al., 2007).

Taken together, the extant data and theoretical frameworks provide for certain sex-specific hypotheses regarding testosterone reactivity to orthogonal FDEs. Specifically, for men we expect that happy faces of women would induce a rise in testosterone compared to happy male faces or neutral male faces. This would be in line with both the Challenge Hypothesis and the fact that males show a greater emotional activation in response to positive stimuli. A similar activation could be also observed in the case of men watching faces of potential status-challengers and/or physical

aggressors (i.e. angry males) (Sell et al., 2009). However, the null finding reported by the only previous experiment on steroid reactivity in response to FDEs (van Honk et al., 2000), wherein men that consciously and unconsciously perceived angry faces did not show increased secretion of testosterone, argues against this hypothesis.

In women, we expect that angry male faces, as evolutionarily salient signals of potential physical aggression (McDonald et al., 2012), and angry female faces, as potential status-challengers, might be associated with endocrine activity when compared to happy female faces or neutral female faces. As an alternative hypothesis women increase in testosterone might be restricted to faces of potential status challengers (i.e. angry females). An increase in testosterone concentration in response to angry males -- potentially associated disposition toward anger/aggression --might in fact be maladaptive, given sex differences in body size and physical strength.

Lastly, contrary to expectations with men, women are predicted to not experience a significant increase in testosterone when exposed to happy faces of the opposite sex. Indirect evidence suggests that women's testosterone responses to potential mates might be more selective than in men (van der Meij et al., 2008; Lòpez et al., 2009). This would be in keeping with the conclusion reached earlier of a blunted response to positive/arousing emotional cues in women.

In summary, because angry faces might signal imminent challenges, it is possible that an increase in testosterone would be observed in both sexes when exposed to threatening stimuli (i.e. angry male faces for men and angry female and male faces for women). Further, given preliminary clues in the literature that relate to sex differences in steroidal and neural reactivity to positive emotional expressions of the opposite sex, it is plausible that only men would be affected by positive affective cues of females (i.e. smiling faces). In order to test these possibilities, we evaluated testosterone reactivity in response to photographs of emotional faces in a large sample of young people.

2.2. Methods

2.2.1. Participants

Two hundred undergraduate participants (92 men, M = 20.04 years, SD = 2.7 years; and, 108 women, M = 19.87 years, SD = 2.22 years) were recruited from the Department of Psychology undergraduate participant pool at Simon Fraser University, and received course credit for participation. Screening at the beginning of the testing session disqualified 2 participants due to consumption of food immediately prior (1 female and 1 male). Three participants (one male) were excluded due to current use of medications. Furthermore, because hormonal contraceptives blunt hormone responses to emotionally-relevant stimuli (Lòpez et al., 2009), data from twenty-one women reporting current use of hormonal contraceptives were discarded. Lastly, we excluded participants who reported a same-sex sexual orientation (four men and two women) (Roney et al., 2007) due to the nature of the experimental facial stimuli. The final sample was thus reduced to 168 subjects (86 males). Fifty-five percent of the participants identified themselves as Asian, 34% as White/Caucasian, 11% as other. All procedures were reviewed and approved by the Simon Fraser University Research Ethics Board. Participants provided written informed consent prior to participation, and were advised that they could withdraw at any time.

2.2.2. Procedure

On arrival, participants received study information and provided informed consent. This initial phase, lasting approximately 10 minutes, was followed by collection of a baseline saliva sample (T1). All samples were collected between 13:30– 19:00 h to control for diurnal rhythms in testosterone secretion (Dabbs, 1990). Five minutes after the collection of the first saliva sample, participants, who were tested individually in separate rooms, were instructed to fill out the BIS/BAS questionnaire, which measures various aspects of an individual's motivational systems with respect to appetitive behavior and avoidance of undesirable situations (Carver & White, 1994). The behavioral inhibition system (BIS), which is related to sensitivity to punishment and avoidance motivation), and the behavioral activation system (BAS), which is related to sensitivity to reward and approach motivation, were measured as potential modulators of

the neuroendocrine response under investigation as hinted by previous reports (e.g., (Vermeersch, T'Sjoen, Kaufman, & Vincke, 2009). Once completed, instructions were given for the subsequent task, the Facial Affect Comparison Task (FACT) (see below for more details), which lasted for 15 minutes. Following the FACT participants completed the Positive and Negative Affective Scale (PANAS, Watson, Clark, & Tellegen, 1988) while providing an alternate saliva sample for use in a different study (Zilioli and Watson, 2014). Through twenty mood descriptors, the PANAS assesses the individual's current emotional state on two general dimensions: positive affect and negative affect. Lastly, participants completed a non competitive cognitive task for a different study and viewed a neutral video (a documentary about Ireland, serving as a filler task) and at exactly 25 minutes after the completion of the FACT, participants provided a saliva sample (T2) and completed demographic measures (e.g. relationship status, medications, menstrual cycle information, height, weight, age, education, sexual orientation and ethnicity). The timing of the T2 testosterone sample (25 minutes post-FACT) was based on recent work in our lab in which significant differences in testosterone response were found 30 minutes after the experimental manipulation (Zilioli & Watson, 2014).

2.2.3. Facial Affect Comparison Task (FACT)

Stimuli consisted of photographs (12.5 cm x 17 cm) of 70 Caucasian individuals (35 males) displaying 3 facial expressions (neutral, anger and happiness; see Figure 2.1 for examples), taken from a standardized set (Lundqvist, Flykt, & Öhman, 1998). We did not collect new data on the level of attractiveness of the faces in our FACT task, because they are drawn from an existing database of facial stimuli (Lundqvist et al., 1998), previously rated for attractiveness by a large sample of male and female undergraduates (Oosterhof and Todorov, 2008).

Figure 2.1. Sample stimuli taken from the Karolinska dataset.

Participants were randomly assigned to one of the following experimental conditions, identifying the type of stimuli to which they were exposed: angry female faces, happy female faces, angry male faces, happy male faces, or neutral faces (male faces for men, female faces for women). The FACT consisted of a 15-minute long forced-choice task, in which two faces were randomly selected from the same list (e.g., angry female faces) and the pair was presented together side by side. Because faces were chosen at random, it is possible that participants rated the same pair more than once. In all conditions, participants were instructed to choose which of the two faces presented was “more emotionally intense” (simply “more intense” in the neutral conditions). Each trial had a maximum duration of 5 seconds, after which the computer would display the next pair of faces regardless of whether participants had been responded to the trial. In any case, the rating itself was unimportant; the task was designed simply to keep participants engaged in processing facial displays of the same emotion.

2.2.4. Saliva samples and hormone assays

Saliva samples were collected using Salimetrics oral swabs (SOS; Salimetrics LLC, State College PA) placed under the tongue, according to vendor usage instructions for testosterone determinations (this location is not recommended for some analytes, such as α-amylase and SIgA, that show differential glandular secretion rates). According to the vendor, the SOS device consists of "an inert food-grade polymer" individually validated for use in specific assays that include salivary testosterone determinations in both men and women. Unlike cotton swabs, SOS devices show high volume recovery and measurement accuracy properties that compare well with passive drool techniques, according to the vendor.

Samples were chilled immediately following collection, and then frozen within 1 h and held at -20 °C until assay. Samples were assayed in our laboratory using competitive enzyme immunoassays for testosterone (Salimetrics LLC, State College, PA). The average intra-assay coefficient of variation for these assays was 5.56% and the inter-assay coefficients for high and low controls were respectively 4.3% and 8.2%. Subjects for whom the coefficient of variation exceeded 15% between duplicates, indicating unreliable assay results between duplicates, were excluded from analyses (three women, two T1 and one T2). Additionally, one saliva sample was lost for one man. Therefore, testosterone data were available for 85 men and 79 women.

2.2.5. Data Analysis

A factorial ANCOVA with Sex (male vs. female participants), FACT sex (same sex vs. opposite sex) and FACT expression (angry, happy, or neutral) was run on positive affect, negative affect and testosterone reactivity, which was measured as percentage change from T1 to T2 (for a similar procedure, see Jimenez, Aguilar, & Alvero-Cruz, 2012). However, interpretation of these results is ambiguous in the absence of an opposite – sex neutral condition; for this reason, additional models were run where neutral condition data were excluded. Moreover, in order to assess whether testosterone actually increased in response to the FACT we ran additional mixed ANOVAs/ANCOVAs with TIME (T1 and T2), FACT sex (same sex vs. opposite sex) and FACT expression (angry, happy). These analyses were run on men and women

separately. Assumptions underlying covariate analysis (covariate and treatment effect independence and homogeneity of regression slopes) were checked. The threshold for statistical significance in all analyses was set at a *p* value of .05 (two-tailed, in the case of t-tests). Cohen's d and partial eta squared were used as the effect size estimates.

2.3. Results

2.3.1. Preliminary analyses

Previous research has shown that relationship status is linked with basal testosterone in both sexes (van Anders & Goldey, 2010). In our sample, single men showed higher concentrations (n= 48, M = 125.19 pg/mL, SD = 43.44 pg/mL) than men in a relationship/dating (n=37, M = 117.3 pg/mL, SD = 38.41 pg/mL), but this difference was not significant [*t* (83) =.873, *ns*]. Single women, however, showed significantly higher concentrations (n= 50, M = 67.97 pg/mL, SD = 19.02 pg/mL) than women in a relationship/dating (n=29, M = 55.54 pg/mL, SD = 20.64 pg/mL) [*t* (77) =2.713, *p* <.01, d = 0.62]. Relationship status was thus introduced as a covariate in analyses involving women.

The time of the day of sample collection did not correlate with basal testosterone in men [*r* = -.168, *p* =.123], but was significantly correlated in women [*r* = -.275, *p* =.014]; thus, it also was introduced as a covariate in the analyses involving women.

About 15% of the female sample failed to provide sufficiently precise information about their previous menses onset or the average duration of their menstrual cycle. Nevertheless, following Liening et al.'s method (Liening, Stanton, Saini, & Schultheiss, 2010) we tested whether testosterone varied as a function of the days since the prior menses onset[1]. A multiple linear regression was run with the following predictors: time of

[1] For three women, the calculated days since the prior menses onset were greater than 35 days. Because of this information and its mismatch with the average menstrual cycle length reported, it is likely that these women had an anovulatory menstrual cycle and therefore were excluded from the menstrual cycle analyses. Their inclusion, however, did not change the pattern of results.

the day the saliva was collected, relationship status, days since the prior menses onset and days since the prior menses onset squared. As expected, collection time and relationship status were significant predictors (β = -.275, t =-2.477, $p < .05$; β = -.319, t =-2.907, $p < .01$). However, confirming Liening et al.'s results (2010) neither days since the prior menses onset (β = -.409, t = -.975, *ns*) nor days since the prior menses onset squared (β = .710, t =1.698, *ns*) were. For this reason, menstrual cycle information (i.e. days since the prior menses) was not included as a covariate.

Furthermore, in women, because regularity of menstrual cycle onset has been shown to be linked to testosterone secretion (van Anders & Watson, 2006a), we collected information on this variable. As in van Anders and Watson (2006), women self-reported the regularity of their menstrual cycles with respect to actual versus predicted date of onset (e.g. perfectly regular, varies by 1-2 days, varies by 3-4 days, varies by 5-6 days, varies by 7 days or more, completely unpredictable). Basal testosterone seemed not to vary as a function of cycle regularity [F (5,73) = .68, *ns*]; therefore, regularity of menstrual cycle onset was not included as a covariate in the analyses.

2.3.2. Sex differences in basal testosterone and mood

Male basal testosterone concentrations were typical for this population (M = 121.76 pg/mL, SD = 41.28 pg/mL). Although there is disagreement about typical absolute values for salivary testosterone in women (see for example, Goldey & van Anders, 2011), our results (M = 63.41 pg/mL, SD = 20.41 pg/mL) are comparable to recent studies with similar samples and ELISA determinations (59.4 pg/mL, in Caruso et al., 2012; 58.7 pg/mL, in Lòpez et al., 2009; ~ 60 pg/ml, in Jimenez et al., 2012). As expected, testosterone was significantly different between sexes [t (124.72) = 11.596, $p < .001$, d = 1.79]. One-way ANOVAs revealed that at baseline testosterone was independent from the assigned experimental condition in both women and men [F (4,74) =1.01, *ns*; F (4,80) =.67, *ns*].

The factorial ANOVA for positive affect revealed a significant main effect of Sex [F (1,154) = 5.12, $p < .05$, $\eta_p^2 = .03$], indicating that men's self-reported mood was more positive (M = 2.76, SD = .75) than women's (2.51, SD = .77) when collapsing across all FACT conditions. This effect, however, disappeared when we excluded the neutral

conditions [*F* (1,135) = 2.65, *ns*]. The factorial ANOVA for negative affect revealed a significant FACT sex by FACT expression interaction [*F* (1,154) = 9.87, $p < .01$, $\eta_p^2 = .06$], indicating that more negative mood was reported after viewing angry faces compared to happy or neutral faces of the same sex [*F* (2,88) = 4.01, $p < .05$, $\eta_p^2 = .08$]. No differences in negative affect emerged after viewing faces of the opposite sex [*F* (1,66) = 2.68, *ns*]. The same pattern of results emerged when we excluded the neutral conditions.

2.3.3. Testosterone Response to FDEs

The Sex (male vs. female participants) X FACT sex (same sex vs. opposite sex) X FACT expression (angry, happy, or neutral) factorial ANCOVA with relationship status and collection time as covariates revealed a main effect of FACT sex [*F* (1,152) = 11.46, $p = .001$, $\eta_p^2 = .07$], indicating that in those men and women exposed to faces of the opposite-sex testosterone percentage increased compared to the testosterone percentage of those men and women exposed to faces of the same sex. When the opposite – sex neutral conditions were excluded, the omnibus 2 (Sex) by 2 (FACT sex) by 2 (FACT expression) ANCOVA, with relationship status and collection time as covariates, confirmed the main effect of FACT sex [*F* (1,133) = 10.64, $p = .001$, $\eta_p^2 = .07$]. Additional analyses keeping men and women separated were run.

In men, a 2 (FACT sex) X 2 ANOVA (FACT expression) revealed the expected main effect of FACT sex [*F* (1,70) = 7.81, $p < .01$, $\eta_p^2 = .10$], but no main effect of FACT expression [*F* (1,70) = .63, *ns*]. Further, a significant t-test indicated that men exposed to female faces (M = 16.61, SD = 21.9) had a higher testosterone increase compared to those men in the neutral condition (M = 4.81, SD = 11.3) [*t* (33.667) =2.37, $p < .05$, d = 0.68] (Figure 2.2).

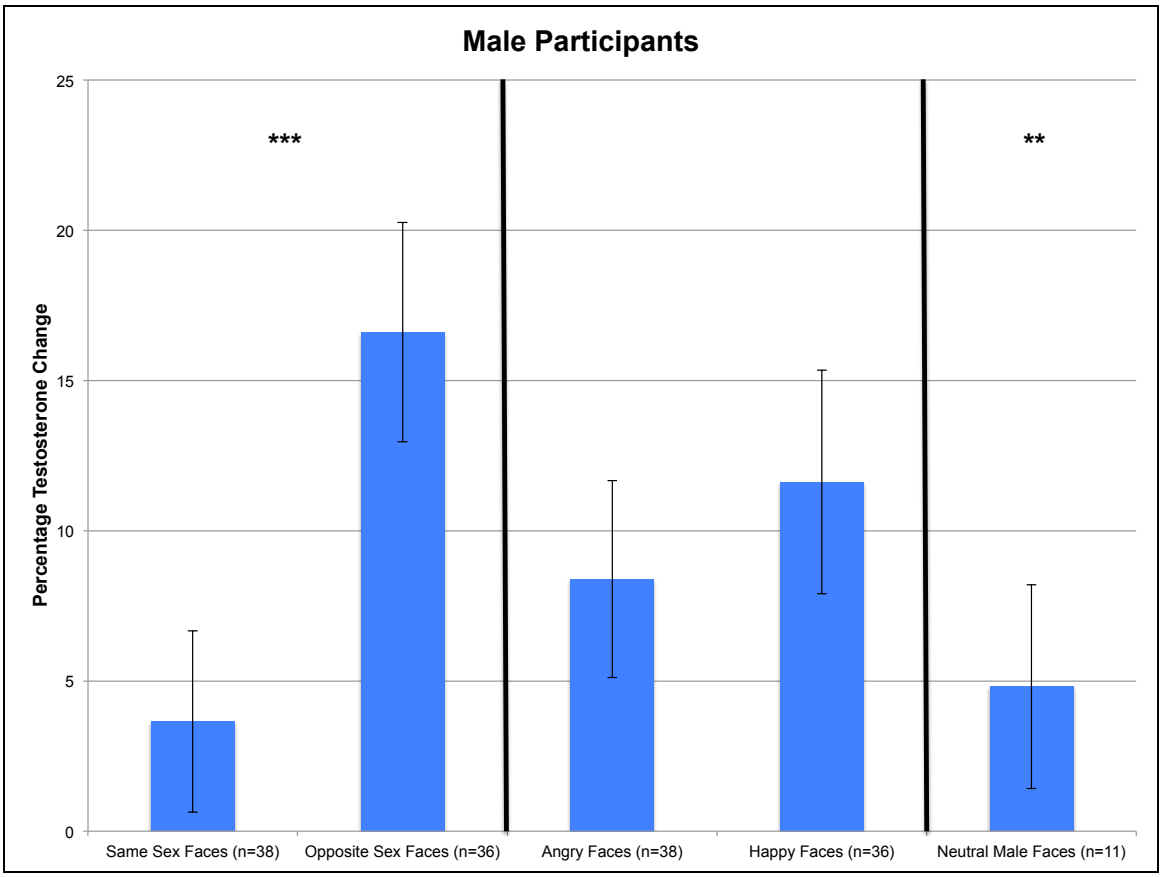

Figure 2.2. Means (SEM) for male testosterone percentage change as a function of FACT sex (same sex vs. opposite sex) and FACT expression (happy, angry and neutral). '**' above the Neutral Male Faces group indicates a significant difference in testosterone response between the Neutral Male Faces group and the Opposite Sex Faces group. The total number of participants per condition is reported in brackets. '**' indicates a significant difference within a factor at $p < .05$. '***' indicates a significant difference within a factor at $p < .01$.

A 2 (TIME) X 2 (FACT sex) X 2 (FACT expression) ANOVA revealed a TIME by FACT sex interaction [F (1,70) = 9.23, $p < .01$, $\eta_p^2 = .12$] indicating that men responded with an increase in testosterone when exposed to female faces regardless of their emotional expression. A repeated measure ANOVA with TIME as factor showed no significant difference between pre- and post-testosterone in those men in the neutral condition [F (1,10) = 1.27, *ns*] (Figure 2.3).

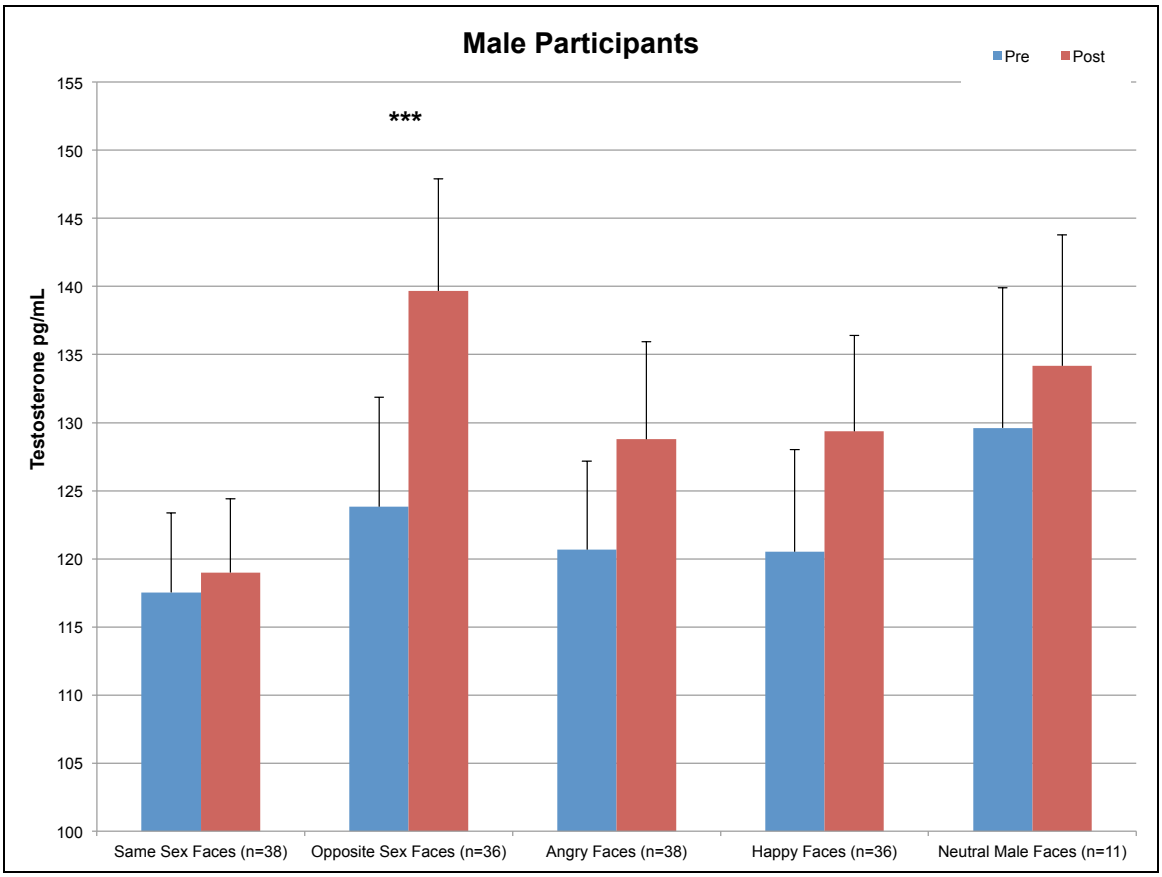

Figure 2.3. Mean (SEM) salivary concentrations of testosterone for our male sample pre- and post-FACT condition presentation. The total number of participants per condition is reported in brackets. '***' indicates a significant change pre- and post-FACT at $p < .01$.

In women, a 2 X 2 ANCOVA, with relationship status and collection time as covariates revealed significant main effects for both FACT sex [F (1,63) = 4.4, $p < .05$, $\eta_p^2 = .07$] and FACT expression [F (1,63) = 4.37, $p < .05$, $\eta_p^2 = .07$]. This pattern of results indicates that testosterone percentage change was higher in those women who were exposed to faces of the opposite sex compared to the testosterone percentage change of those women exposed to faces of the same sex. It also indicates that testosterone percentage change was higher in those women who were exposed to angry faces compared to happy faces regardless of the sex of the stimuli. A one-way ANCOVA, with relationship status and collection time as covariates, revealed that testosterone percentage change in women exposed to angry faces was not different than testosterone percentage change of women in the neutral condition [F (1,39) = 2.64, p =

.112, η_p^2= .06]; however, when comparing the testosterone response in women exposed to male faces and the testosterone response in those women exposed to neutral female faces a trend towards significance was found [*F* (1,40) = 3.69, *p* = .062, η_p^2= .09] (Figure 2.4).

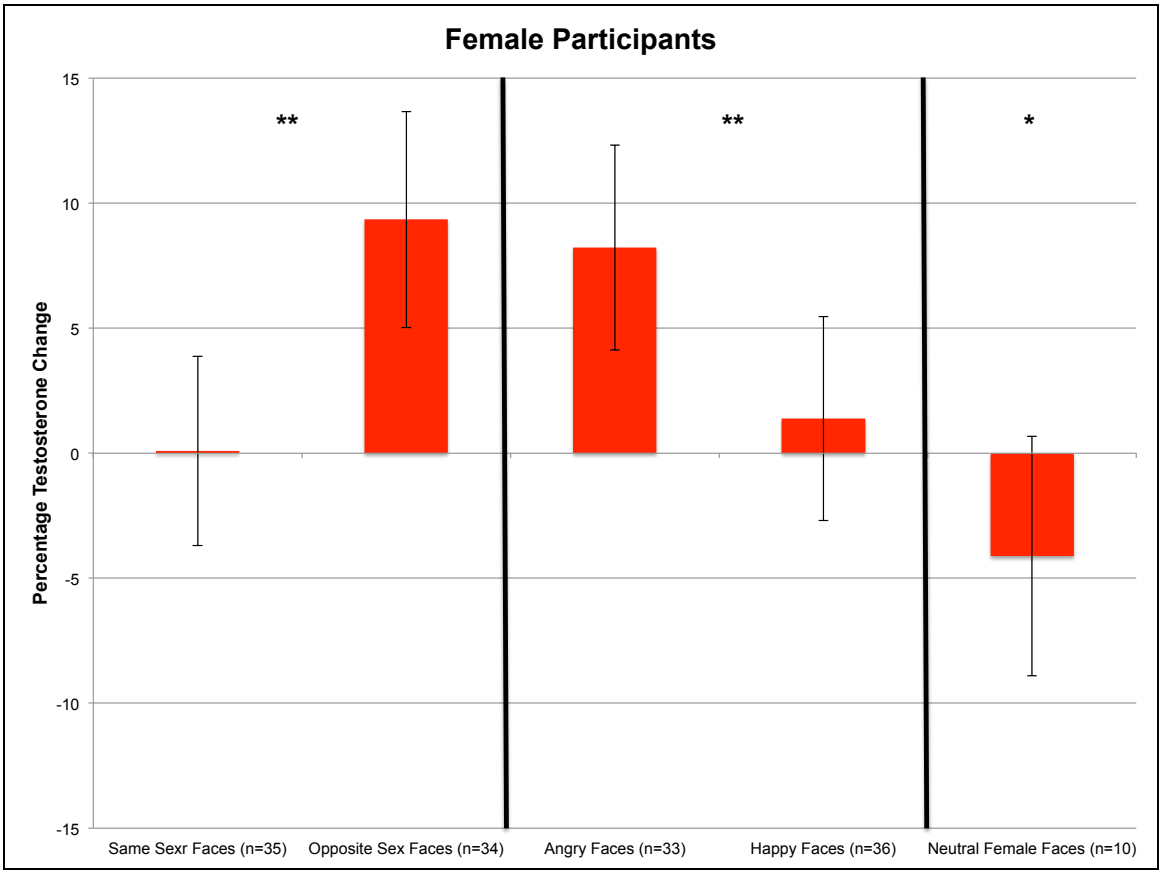

Figure 2.4. Means (SEM) for female testosterone percentage change as a function of FACT sex (same sex vs. opposite sex) and FACT expression (happy vs. angry). '*' above the Neutral Female Faces group indicates a trend towards significance (*p* = .062) when comparing the testosterone response in the Neutral Female Faces group with the testosterone response in the Opposite Sex Faces group. The total number of participants per condition is reported in brackets. '**' indicates a significant difference within a factor at *p* < .05.

A 2 (TIME) X 2 (FACT sex) X 2 (FACT expression) ANCOVA with relationship status and collection time as covariates revealed a TIME x FACT sex [*F* (1,63) = 6.19, *p* < .05, η_p^2= .09] as well as a trend towards significance for the TIME x FACT expression interaction [*F* (1,63) = 3.75, *p* = .057, η_p^2= .06]. These findings indicate that women not only had a higher testosterone response to faces of the opposite sex, but they also

experienced an increase in testosterone when observing angry faces compared to happy faces regardless of the sex of the stimuli. Lastly, a repeated measures ANCOVA with TIME as factor, and relationship status and collection time as covariates showed no significant difference between pre- and post-testosterone in those women in the neutral condition [F (1,7) = 2.25, ns] (Figure 2.5).

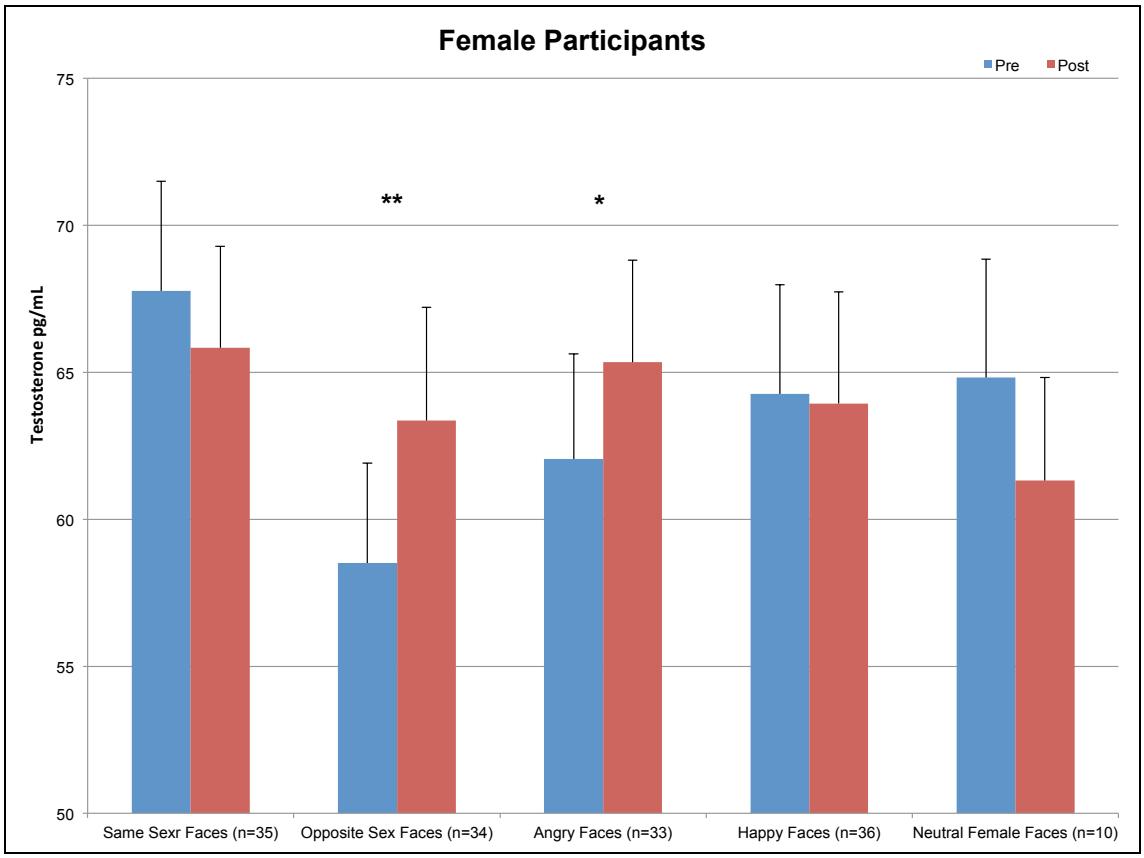

Figure 2.5. Mean (SEM) salivary concentrations of testosterone for our female sample pre- and post-FACT condition presentation. The total number of participants per condition is reported in brackets. '**' indicates a significant change pre- and post-FACT at $p < .05$. '*' indicate a trend towards significance at $p = .057$.

Finally, bivariate correlations, controlling for sex and relationship status, were run on the entire sample to test whether the BIS/BAS (approach versus avoidance) were associated with testosterone percentage reactivity to faces of the opposite sex. None of the partial correlation coefficients reached significance [r, all $p > .09$] even with the most liberal alpha value (.05, not corrected for multiple comparison). In women, the same bivariate correlations were run between BIS/BAS scales and negative affect and

testosterone reactivity to angry faces. Again, none of the partial correlation coefficients reached significance [*r*, all *p* >.1]. Although negative mood was higher in women viewing angry faces of other women and in men viewing angry faces of other men, no association was found between negative mood and testosterone reactivity in these experimental conditions (*r* = -.235, *p* =.38; *r* = -.131, *p* =.62).

2.4. Discussion

Although previous reports have examined the effect of circulating testosterone on motivational tendencies (i.e. approach and avoidance) towards various FDEs, this is the first study to report on the reciprocal phenomenon: the impact of these fundamental social signals on testosterone secretion, comparing men and women. In a between-subject design, participants were asked to rate emotional intensity of orthogonal facial displays (i.e. happy or angry faces) of people of either the same sex or the opposite sex. Our results clearly show that, regardless of the emotional content expressed, extended and uninterrupted exposure to faces of the opposite sex compared to exposure to faces of the same sex was accompanied by an increase in salivary testosterone in both men and women. Moreover, women experienced an additional specific neuroendocrine response, with an increase in testosterone occurring only when viewing faces of angry individuals compared to happy individuals, regardless of the sex. These effects seemed to be independent of individual differences in basic motivational systems, as measured through the BIS/BAS scales, as well as mood.

Our initial hypothesis that greater hypothalamic-pituitary-gonadal (HPG) axis activation would be evident in men exposed to smiling women was confirmed, but in a modified form. A fundamental prediction of the Challenge Hypothesis is that testosterone will rise in situations implicated in mating effort (Archer, 2006; Wingfield et al., 1990). Empirical evidence seems to suggest that happy faces of females are relevant mating signals for men. For one thing, happy faces convey interpersonal intent of social affiliation (Knutson, 1996), and seem to be more efficiently detected than other FDEs (i.e. angry faces) (Becker, Anderson, Mortensen, Neufeld, & Neel, 2011). More specifically, men judge such emotional expressions in females as the most sexually attractive ones (Tracy & Beall, 2011). Consequently, the testosterone increase exhibited

by men exposed to happy female faces might be comparable to the rapid steroidal responses observed in more direct mating effort situations, such as brief social interactions with young women (Roney et al., 2007).

The finding that men experience an increase in testosterone when viewing happy female FDEs was complemented by a similar testosterone activation in men attending to faces of angry females, suggesting that in fact men show a more generalized hormonal response to faces of women, regardless of the specific emotion displayed. It is possible that in our task, extended exposure to the same facial expressions (i.e. a series of angry female faces) might have caused a shift in attention from the emotion being expressed to the sex and attractiveness of the faces being judged. Thus, angry female faces, which probably convey much less direct threat to men than the faces of angry males (Goos & Silverman, 2002; Becker et al., 2007; Sell et al., 2009), might have been perceived as potential mates similarly to the case of happy female faces. In both instances, being primed with the presence of new indirect mating opportunities could have been sufficient to induce reactive changes in testosterone that are thought to facilitate context-adaptive behaviors such as status competition (Ronay & von Hippel, 2010) and courtship behavior (van der Meij, Almela, Buunk, Fawcett, & Salvador, 2012).

The data collected from women paralleled the phenomena observed in men: testosterone accumulation accompanied exposure to faces of the opposite sex, regardless of the emotional content. The mechanisms behind this response may be identical to the ones in place for men, with rapid steroidal secretion reflecting an anticipatory response to direct (i.e. happy faces) and indirect (i.e. angry faces) mating signals. In this regard, our initial hypothesis that women would show a more selective androgenic response to potential mates might need to be reconsidered. Lòpez and collaborators (2009) found that exposure to a video montage showing courtship interaction between a highly socially and parentally attractive man and a young woman caused testosterone accumulation in a female sample. In our experiment, faces of smiling men might be subtle signals of these qualities.

Women also experienced a significant increase in testosterone when observing angry faces of other women and men. In contrast to men, previous experimental evidence suggests that women are physiologically responsive to angry faces of both

sexes (Mazurski et al., 1996). Whether this testosterone accumulation is a result of activation of the HPG axis or hypothalamic-pituitary-adrenal (HPA) axis, or both (Burger, 2002), it can be framed within the Challenge Hypothesis. In this interpretation, the testosterone response is a physiological reaction to imminent potential challenges, perhaps relating to territory defense and/or offspring protection (Bos, Hermans, Montoya, Ramsey, & van Honk, 2010). For instance, Bos and colleagues found that testosterone administration augmented neural activation in women exposed to baby cries, implying an involvement of this steroid in protective preparatory responses (Bos et al., 2010). This speculation is congruent with other studies involving exogenous testosterone (Hermans, Ramsey, & van Honk, 2008; van Honk et al., 2001), wherein women receiving a single dose of sublingual testosterone showed either heart beat acceleration (van Honk et al., 2001) or activation of brain areas involved in reactive aggression in response to angry faces only (Hermans et al., 2008). Likewise, the present results can be interpreted in terms of proneness to fight, likely through a testosterone-induced reduction of fear (Wirth & Schultheiss, 2007; Stanton et al., 2009).

Lastly, testosterone accumulation was not observed in men viewing angry male faces. This is in keeping with the sole previous experiment on testosterone reactivity in response to FDEs (van Honk et al., 2000), wherein men that consciously perceived angry faces did not show any hormonal change. The authors proposed that cortical processing -- likely at long exposure times -- might have caused inhibition of more automatic initial responses to threatening facial displays. A similar process may have been at work in our experiment, where participants were passively exposed to the same facial expression for 15 min. Future studies might investigate whether a different methodology (i.e. subconscious presentation of FDEs) and/or an earlier collection of saliva samples would yield positive findings in this regard. It is uncertain why women still experienced a testosterone increase in the same condition. It is plausible that this sexually dimorphic endocrine response to threating stimuli might be linked to sex difference in emotional regulation. For example, it has been suggested that during regulation of negative emotion, women tend to recruit more prefrontal brain areas associated with affective processing compared to men, who show a stronger activation of prefrontal areas mainly associated with cognitive processing (Mak et al., 2009). Moreover, women display more prolonged neural activation in response to negative

stimuli (Williams et al., 2005). These distinct processing mechanisms might account for the sexual dimorphic endocrine reactivity observed in response to angry male face as presented in our experiment.

This initial study of testosterone and FDEs was subject to several limiting factors that could be addressed in future work. For example, it would be useful to contrast static (still) versus dynamic (video) emotional stimuli to corroborate our results using the rich alternate social stimulus of a live facial display. Future studies would also benefit from broader control of potential mediating variables (e.g. attractiveness of the stimulus faces, which might have contributed to our results given the self-paced nature of the task) and moderating variables (e.g. phase of the menstrual cycle, or use of contraceptives), and investigation of other emotional signals associated with threat (e.g. fear and disgust).

An additional limitation of the current report was the presence for each sex of only one emotionally neutral group. Inclusion of neutral faces of both sexes would allow conclusive direct tests of a possible main effect of FACT sex in men and women as well as helping disambiguate our findings with respect to challenge, threat, and mating signals. In probing these additional neutral conditions, future studies would require a larger number of participants per condition.

In the present report, each condition included around twenty people: while this resembles sample sizes in previous studies on hormone responses to emotionally-relevant stimuli (Lòpez et al., 2009; Goldey & van Anders, 2011), given the variability in the size of the testosterone responses observed, it might have not provided sufficient power to detect more subtle effects (i.e. interaction effects). Methodologically, the standardized facial stimuli that we employed lack the ethnic diversity seen in our sample, so although angry and happy faces universally signal threat and affiliation independently of ethnicity, evaluation of race-associated effects of stimuli might be beneficial in future work.

Lastly, the *absolute* values for free testosterone that we obtained for women seem quite high; while this is of limited concern for the *relative* proportional measures reported here, we note that it remains to be determined if the combination of Salimetrics

ELISA kits and SOS swabs produce results that are elevated relative to other techniques, and whether it affects sexes comparably.

In summary, we found that men and women show elevated salivary testosterone following extended exposure to faces of the opposite sex, regardless of the apparent emotional content of the faces. Furthermore, women experienced an additional androgenic response to angry expressions compared to happy expressions. Taken together, these findings add emotional facial stimuli to the collection of social signals that modulate endocrine status.

Chapter 3.

STUDY 2: The hidden dimensions of the competition effect: Basal cortisol and basal testosterone jointly predict changes in salivary testosterone after social victory in men

Note: This section is based on the following article, with permission: Zilioli, S. & Watson, N.V., (2012). The hidden dimensions of the competition effect: Basal cortisol and basal testosterone jointly predict changes in salivary testosterone after social victory in men. *Psychoneuroendocrinology* 37, 1855-1865.

3.1. Introduction to Study 2

Competition encompasses the large suite of behaviors employed by motivated individuals who are engaged in inter-individual conflict aimed at attaining the same goal or reward. In many animal species, certain forms of competition are sexually-selected traits; in particular, inter-male competition is often crucial, allowing an individual to obtain or maintain high positions in social hierarchies, and thus gain greater access to resources and mates, and acknowledged dominance over conspecifics with lower status (Altmann, Sapolsky, & Licht, 1995; Barinaga, 1996; Blanchard et al., 1995; Ellis, 1995). Although the precise form of social hierarchies varies between mammalian taxa, reaching high complexity in the multidimensional ranking systems of some species (such as humans), hierarchies structured around unequal distribution of specific resources – mates, territory, food, etc. – are the most common (Chase, Tovey, Spangler-Martin, & Manfredonia, 2002; Magee & Galinsky, 2008).

These dominance struggles appear to affect hormone concentrations in many mammals. In species ranging from mice to nonhuman primates and humans, testosterone (T) has been observed to fluctuate in concert with changes in social status,

such that winning competitions leads to an increase in circulating testosterone (or reduced decrease relative to others), and/or losing leads to a net decrease in T (Lloyd, 1971; Bernstein et al., 1974; Dixson, 1980; Lloyd, 1971). This "Competition Effect" – hypothesized to be part of a broader biosocial system for establishing dominance status (Mazur & Booth, 1998) – has been demonstrated in a number of previous studies, using both sport and laboratory contests (for reviews, see van Anders & Watson, 2006b; Archer, 2006; Salvador & Costa, 2009). However, a number of other studies have reported null results in similar competition tasks (see, for example, Gonzalez-Bono, Salvador, Ricarte, Serrano, & Arnedo, 2000; Salvador, Simon, Suay, & Llorens, 1987; Suay et al., 1999; Gonzalez-Bono et al., 2000); in fact, T has even been reported to *decrease* in winners in some cases (Filaire, Maso, Sagnol, Ferrand, & Lac, 2001). It is unclear why the results have been equivocal. Furthermore, some studies find that changes in post-competition T (relative to pre-competition baseline) are equivalent regardless of the competition outcome (van der Meij, Buunk, Almela, & Salvador, 2010), a pattern that is more consistent with the logically related but conceptually distinct Challenge Hypothesis (Wingfield et al., 1990; Archer, 2006; van Anders & Watson, 2006b; Wingfield et al., 1990), and possibly more prevalent among female competitors (Hamilton, van Anders, Cox, & Watson, 2009).

Two explanations have been proposed to account for the inconsistent results in the literature: (1) the idea that individual psychological variables, such as mood, appraisal and personality, might intervene as moderators or mediators of the effects of the competition outcome (for reviews, see Archer, 2006; Stanton & Schultheiss, 2009; Archer, 2006; Salvador & Costa, 2009), and; (2) the idea that responsivity of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates T secretion, may be modulated by interactions with other neuroendocrine systems -- especially the hypothalamic-pituitary-adrenal (HPA) axis (Mehta & Josephs, 2010) -- that are themselves sensitive to environmental variables.

3.1.1. Psychological mediators in the psychoneuroendocrinology of competition

Several psychological processes have been proposed as mediators or moderators of differential neuroendocrine activation in competition (Archer, 2006).

Although there is relatively little evidence that mood directly mediates T changes after winning or losing (McCaul et al., 1992), aspects of personality and personal differences in causal attribution appear to play a role. Schultheiss and collaborators (1999; 2002) suggest that the intensity of the individual's intrinsic drive to enhance their own status relative to others, termed "implicit power motivation", may be a crucial moderator of the effect of competitive outcome on hormonal responses. Studying teams of basketball players, Gonzalez-Bono and colleagues (Gonzalez-Bono et al., 2000; Gonzalez-Bono, Salvador, Serrano, & Ricarte, 1999; Gonzalez-Bono et al., 2000) found that positive changes in T (post-competition T minus pre-competition T) were more pronounced in those winners that attributed success in the contest to their own involvement and skills. This finding has clear implications for possible differences in the endocrine impact of individual competitions – where the attribution of the outcome to one's own abilities is unambiguous – versus team contests, where the perception of one's own contribution to the outcome is diluted by the performance of the other team members.

A distinct but related issue is personal involvement; evidence indicates that a subject's evaluation that a competition is important for status or social ranking may lead to higher personal involvement, leading to greater activation of the HPG axis (Salvador, 2005). Indeed, excepting some studies of competition in very strenuous sports, in which physical exertion may have confounded the neuroendocrine competition effect (Edwards, Wetzel, & Wyner, 2006), competitive situations in which subjects' personal investment is believed to be greatest appear to be most effective in eliciting a competition effect on circulating T (Mazur, Booth, & Dabbs, 1992; Bernhardt et al., 1998; Mazur et al., 1992). The joint feature of these naturalistic studies was to engage men in common meaningful competitive situations close to their everyday life experiences. This aspect may be missing in some laboratory studies of the competition effect, where less engaging contests have been used (see, for example, Mazur, Susman, & Edelbrock, 1997; Mehta & Josephs, 2006; van Anders & Watson, 2007). It is possible that lack of familiarity, perceived unimportance for status, and/or decreased involvement in some of these tasks, has resulted in inconsistent evidence of a competition effect. This possibility has not been investigated further.

3.1.2. Dual-hormone hypothesis

In its usual form, the competition effect hypothesis focuses exclusively on T reactivity in dominance manipulations. Recently, a modulating effect of HPA axis activity on HPG axis responses to competition has been proposed to account for conflicting reports (Dabbs et al., 1991; Mehta & Josephs, 2010; Popma et al., 2007; Terburg, Morgan, & van Honk, 2009). For example, in a clinical sample of 103 adolescents, Popma and collaborators (2007) found that overt aggression was positively correlated with T only in those subjects with a low cortisol baseline. The same effect was not found for subjects with high concentrations of cortisol at baseline. Likewise, Mehta and Josephs (Mehta & Josephs, 2010) observed that cortisol moderated the association between T and assessed dominance in participants assigned to a leadership position in a role playing task, with an attenuation of the relationship between dominance and T observed in high-cortisol individuals. Moreover, among losers of a competition, a combination of basal cortisol and T predicted both willingness to compete again (behavioral response), and changes in T before and after the contest (hormonal response). High pre-competition T was associated with a decrease in T only in those losers whose pre-competition cortisol concentrations were also high. This pattern was not found in subjects with low basal concentrations of cortisol.

3.1.3. Current study

The current study was designed to evaluate the dual-hormone hypothesis of the competition effect in the context of a commonplace competition: videogaming. Recreational videogaming is a highly involving activity that lacks the confounding effects of physical exertion, and we sought to heighten ecological validity and personal involvement by employing a variant of a familiar and challenging commercial videogame, Tetris, in an apparent head-to-head competition for monetary reward (for a detailed description of the game and its history, see Fahey, 2003). While a few previous studies have examined endocrine responses in computer-based competitions, those studies have tended to employ unfamiliar and sometimes artificial tasks that may be less personally involving than a commercial videogame (examples include reaction time tasks (Gladue, Boechler, & McCaul, 1989; McCaul et al., 1992); vocabulary tasks, (Schultheiss et al., 1999; Mehta & Josephs, 2006; Schultheiss et al., 1999; van Anders &

Watson, 2007), and a computer based intelligence task (van der Meij et al., 2010). The few studies that have probed endocrine responses to "real" commercial videogames have produced inconclusive results: in one case, a null result was attributed to the use of an uninvolving tennis-like game (Mazur et al., 1997), while in another study a very involving commercial game was employed (Unreal Tournament, published by GT Interactive), but the study's focus was on group phenomena in teams of players, not individual performance or the competition effect *per se* (Oxford, Ponzi, & Geary, 2010). As we discussed earlier, team competition studies are fundamentally different, in that personal attribution of results is less clear (i.e., a loss or a win might be perceived by the subject to be mostly due to the performance of other team members). Furthermore, teams were not randomly assigned to win or loss conditions in the Oxford, et al. (2010) study, so it is impossible to know to what extent hormone concentrations, game skills, and competition outcome were confounded together.

Here, we hypothesize that: (1) that in an ecologically valid and engaging videogame competition, pre- and post-test salivary immunoassay would reveal that baseline cortisol has a moderating effect over net increases of T in winners (Burnstein, Maiorino, Dai, & Cameron, 1995; Tilbrook et al., 2000; Viau, 2002), with high basal cortisol associated with a blunted competition effect and no rise in T after a victory, and; (2) that baseline cortisol would have a lower impact in losers experiencing the net decrease in testosterone that is predicted under the biosocial model (Mazur & Booth, 1998). This is premised on the assumption that the induced net decline in testosterone in losers would mask or otherwise negate the restriction attributable to baseline cortisol (Elefther & Church, 1967) . An extension of the dual hormone hypothesis is that (3), the strength of a competition effect on post-competition T may be best predicted by joint status of T and cortisol at pre-competition baseline, with high-T and low cortisol winners likely to show the largest competition effect (Terburg et al., 2009; Mehta & Josephs, 2010; Terburg et al., 2009). This would be complementary to the previously-reported maximal joint effect of high basal testosterone and high basal cortisol on net post-loss testosterone decrements in losers (Mehta and Josephs, 2010). We also examined related aspects of psychological mediation of endocrine responses to competition, such as the expected negative relationship between perceived control and cortisol (Dickerson

& Kemeny, 2004) and the supposed positive association between changes in T and self-assurance (Burgoon, Johnson, & Koch, 1998).

3.2. Methods

3.2.1. Participants

Seventy-six male undergraduates (mean age=19.9 years, SD=2.27) served as participants, in exchange for course credit in an introductory psychology class. Six participants were excluded due to recent use of medications with endocrine activity, leaving a total of 70 participants. All procedures were subject to review and prior approval by the Simon Fraser University Research Ethics Board.

3.2.2. Competition Task

The commercial version of Tetris involves a speeded puzzle in which complex shapes, dropping down the screen, must be rotated and fitted together into rows. As the game unfolds, the rate at which blocks drop increases, resulting in a high concentrations of engagement by the player. For our purposes, a modified version of Tetris was professionally developed (Advanced Technology Solutions, Milan), in which pairs of participants believed they were competing against one another via two linked computers. The game retained the high concentrations of graphical and auditory detail found in the commercial version. The most important modification was the addition of a script module that allowed us to manipulate the outcome of the task such that winner and loser conditions were randomly assigned rather than determined by skill, unbeknownst to the participants. Further intensifying the competition, the participants were told, just before the beginning of the contest, that the winner would receive a $10 cash prize. While the Tetris game itself was not actually linked between the computers, the experiment was individually scripted so as to synchronize the progress and outcomes of the competitors. An additional modification was that if the screen filled with blocks the game did not terminate (as in the commercial version) but rather the screen would shift the blocks down allowing the player to continue competing for the predetermined amount of time. Throughout the competition, both participants were able to assess their performance in

relation to their opponent's score via scripted messages that contained different sorts of feedback. At completion of the competition, the message "you win!" on a colourful background was displayed on the winner's screen, while the loser's screen displayed "you lose!" on a drab background.

3.2.3. Procedure

Pre-competition phase. Upon arriving, each pair of competitors was greeted by a male experimenter, and each participant was directed to one of two small rooms, where they completed an informed-consent form and a simple questionnaire sampling bio-demographic information (e.g., height, weight, sexual orientation, educational level and ethnic background) as well as other control variables (i.e. sleep habits). In addition, an *ad hoc* self report measure of experience playing videogames was formulated based on a previous study (Terlecki & Newcombe, 2005). Subjects also immediately provided a baseline saliva sample (time zero sample). During this period participants were given instructions for the competition task and were informed about the cash prize. Five min after the collection of the time zero saliva samples, participants were instructed to begin the game on the experimenter's mark, after which doors to the two participants' rooms were shut for the duration of the competition task.

Competition phase. After competing for exactly 15 min (by which time the game's scripted outcomes had just been reached), the participants' rooms were opened and the experimenter called for the winning participant to step out of his room and claim the competition prize (walking past the room of the "loser" to do so). The winning participant was audibly congratulated by the experimenter and then returned to his room.

Post-competition phase. Following the competition participants completed the mood, attribution and demographic measures, described below, and viewed a neutral video (a documentary about Ireland, serving as a filler task) (Riadfahmy, Read, Walker, & Griffiths, 1982; Riadfahmy, Read, Walker, Walker, & Griffiths, 1987; Oliver C Schultheiss et al., 2005). At exactly 30 minutes after the completion of the Tetris competition, participants provided a second saliva sample (T1) and were given a printed debriefing form to read. All testing occurred between 1400h and 1900h to control for diurnal hormone fluctuations (Campbell, Walker, Riadfahmy, Wilson, & Griffiths, 1982;

Bremner, Vitiello, & Prinz, 1983; Campbell et al., 1982; Dabbs, 1990; Horrocks et al., 1990).

3.2.4. Paper-and-pencil questionnaires

Mood. Immediately following the competition task, subjects completed the PANAS-X (Watson & Clark, 1994). This instrument includes the two higher order scales found in the older PANAS (Watson et al., 1988), along with more selective affective subgroups: basic negative emotion scales (Fear, Sadness, Guilt, Hostility), basic positive emotion scales (Joviality, Self-Assurance, Attentiveness) and other affective states (Shyness, Fatigue, Serenity, Surprise).

Attribution survey. In order to examine participants' attributions for the competition outcome, we created an *ad hoc* survey using 5-point Likert-type questions assessing the role of personal ability and luck, as well as open questions (e.g., *"Why do you think you have lost?"*) (Gonzalez-Bono et al., 1999; Gonzalez-Bono et al., 2000; Gonzalez-Bono et al., 1999). The attribution survey was also designed to: (1) check for suspicions about the rigged nature of the contest; (2) provide general feedback from participants about the competition and the experiment up to that point; and, (3) explore whether the experimental manipulation had an impact on other psychological processes, such as confidence and perceived control over the competition outcome (e.g.,*"How much control did you have over whether you won or lost"*) (McCaul et al., 1992).

3.2.5. Saliva samples and hormone assays

Participants were instructed to abstain from eating, drinking, smoking, or brushing their teeth for one hour before testing. Saliva samples were collected using oral swabs (Salimetrics LLC, State College, PA) placed under the tongue. Samples were chilled immediately following collection, and then frozen within one hour and held at -20°C until assay. Samples were assayed in duplicate using competitive enzyme immunoassays for testosterone and cortisol (Salimetrics LLC, State College, PA). The average intra-assay coefficient of variation was 3.9 % for T and 4.01% for cortisol, and inter-assay coefficients averaged across high and low controls were 11.43% for T and 5.7% for cortisol.

3.2.6. Statistical analyses

Possible differences between winners and losers on socio-demographic variables and hormonal concentrations before the competition were assessed using independent t-tests. Pearson product-moment correlations were used to assess associations between continuous variables. The competition effect was tested via repeated-measures ANOVA. *Post hoc* comparisons between pre-task (i.e. baseline) and post-task T concentrations were conducted separately for winners and losers using paired sample t-test. Keeping these two groups separate, linear multiple regression analyses (see Results section for details) were carried out to test the dual hormone hypothesis. And lastly, simple-slope analyses were used for *post hoc* evaluation of dual-hormone effects (Aiken & West, 1991; Cohen, Cohen, West, & Aiken, 2003). All tests are two-tailed ($\alpha = .05$) and were carried out using PASW Statistics 17.0.3.

3.3. Results

3.3.1. Hormone measures

Ten participants indicated suspicion about the competition manipulation, and therefore were removed from the analysis, leaving a sample of 60 participants (30 winners). Consistent with previous reports, distributions for baseline cortisol (C0) and post-test cortisol (C1) were positively skewed; consequently, we used a log transformation to normalize these variables (Mehta & Josephs, 2006; Wirth, Welsh, & Schultheiss, 2006). Two subjects differed by more than three standard deviations from the normalized mean, and thus met criterion for outliers (Kirk, 1995); cortisol values for these individuals were excluded. Baseline (T0) and post-competition T (T1) concentrations were normally distributed. One subject showed T concentrations more than three standard deviations over the mean, and his T measure was excluded. Cortisol unstandardized residuals, obtained when post-competition log-transformed cortisol (C1) was regressed on collection time (covariate) and baseline log-transformed cortisol (C0), were used as a measure of cortisol change, as described in Wirth (2006). This allowed us to partial out the variance associated with time of day, which was negatively correlated with baseline log-transformed cortisol ($r = -0.339$, $p < 0.01$) (Cronbach, 1970).

The same procedure was used to calculate change in T scores; however, time of day was not included in the regression since it did not correlate with baseline T (r = -0.228, p > 0.08). Descriptive statistics for baseline and post-competition cortisol (raw scores) and T concentrations are presented in Table 3.1.

Table 3.1. Descriptive statistics for raw hormone measure

	Winners and Losers (*n* T = 61; *n* C = 60)		**Winners (*n* T = 32 ; *n* C = 32)**		**Losers (*n* T = 29 ; *n* C = 28)**	
	M (SEM)	SD	M (SEM)	SD	M (SEM)	SD
Pre-competition testosterone (pg/mL)	111.6 (5.8)	45.4	110.3 (8.2)	46.3	113 (8.4)	45.2
Post-competition testosterone (pg/mL)	107.1 (5.1)	39.6	111.2 (7)	39.4	102.5 (7.4)	40
Changes in testosterone (pg/mL)[a]	-4.5 (2.4)	18.4	.9 (3.3)	18.4	-10.5 (3.1)	16.8
Pre-competition cortisol (μg/dL)[b]	.18 (.03)	.21	.15 (.02)	.11	.2 (.05)	.28
Post-competition cortisol (μg/dL)[c]	.13 (.01)	.1	.12 (.01)	.07	.13 (.02)	.13
Changes in cortisol (μg/dL)[d]	-.05 (-.02)	.13	-.03 (.02)	.08	-.08 (.03)	.17

[a] Post-competition testosterone minus baseline testosterone.
[b] Means, standard deviation and standard error of the mean (SEM) were calculated from the untransformed baseline cortisol distribution.
[c] Means, standard deviation and standard error of the mean (SEM) were calculated from the untransformed post-competition cortisol distribution.
[d] Means, standard deviation and standard error of the mean (SEM) were calculated from the untransformed change in cortisol distribution (post-competition cortisol minus baseline cortisol).

3.3.2. Preliminary analyses

The randomly assigned "winners" and "losers" did not differ on any bio-demographic variables [t-tests, mean p = 0.59]. They also did not differ with regard to past involvement with videogaming, physique (BMI), or preceding night's sleep. Independent-groups *t*-tests further confirmed that at baseline, winners and losers did not differ in their salivary concentrations of T [t (57) = 0.029, p > 0.98] or cortisol [t (56) = -0.540, p > 0.59]. Baseline hormone concentrations did not correlate with age, height, weight, BMI, educational level, sleeping and wake times, or any measure of involvement

with videogames [*r*, all p ≥ 0.09]. However, consistent with previous reports (Popma et al., 2007; Mehta, Jones, & Josephs, 2008) log-transformed baseline cortisol positively correlated with baseline T [*r* = 0.449, p < 0.001] (Table 3.2).

Table 3.2. Correlations among hormone measures *p< 0.05, **<0.001

	I	II	III
I. Pre-competition testosterone	-		
II. Pre-competition log-transformed cortisol	.449**	-	
III. Post-competition testosterone	.919**	.245	-
IV. Post-competition log-transformed cortisol	.479**	.824**	.331*

Mood. Statistical analyses confirmed that our manipulation was effective in provoking large differences between winners and losers in both negative affect [t (58) = -3.218, p < 0.01) and positive affect (t (58) = -3.623, p < 0.01]. In keeping with these findings, winners scored higher on all three basic positive emotion scales [attentiveness, t (58) = 2.542, p < 0.05; joviality, t (58) = 5.917, p < 0.001; and self-assurance, t (58) = 2.402, p < 0.05] whereas, losers reported higher scores on three of the four basic negative emotion scales [hostility, t (58) = -3.205, p < 0.01; sadness, t (58) = -3.969, p < 0.001; and guilt, t (58) = -5.012, p < 0.001]. Of the other affective states measured by the PANAS-X only surprise showed a significant difference, with winners scoring higher [t (58) = 2.616, p < 0.05].

Attribution survey. There was an inverted but complementary pattern between winners and losers in explaining their outcomes, with the winners crediting their own ability as the main cause of success [t (57) = 5.958, p < 0.001; one subject failed to answer this question] and the losers identifying luck as the main reason for their defeat [t (58) = 1.975, p = 0.053]. No effect was observed for the two remaining self-reported psychological processes (i.e. confidence and perceived control over the competition outcome).

3.3.3. Competition Effect.

The effect of the competition manipulation on the hormone profile of the participants was assessed via repeated-measure ANOVA, with outcome (victory or defeat) as a between-subjects factor and pre- and post-test testosterone (T0 and T1) as

a within subjects factor. In accordance with the predictions of the competition effect, there was a significant interaction between pre and post measures and outcome on T [F (1,57) = 4.794, p < 0.05; see Figure 3.1]. *Post hoc*, paired-samples t-tests revealed that post-test T was significantly lower than baseline T in losers [t (28) = 3.371, p < 0.01], whereas in winners there were no significant differences between pre- and post-task T [t (29) = .172, p > 0.86]. Repeating this analysis with pre- and post-test cortisol (C0 and C1) revealed a significant main effect of time, with a general decrease in cortisol between pre- and post-test [F (1,56) =22.518, p < .001] but no interaction between competition outcome and time was found [F (1,56) =1.801, p > 0.18], indicating that while cortisol declined from baseline over the course of the test session, it did so equivalently in winners and losers.

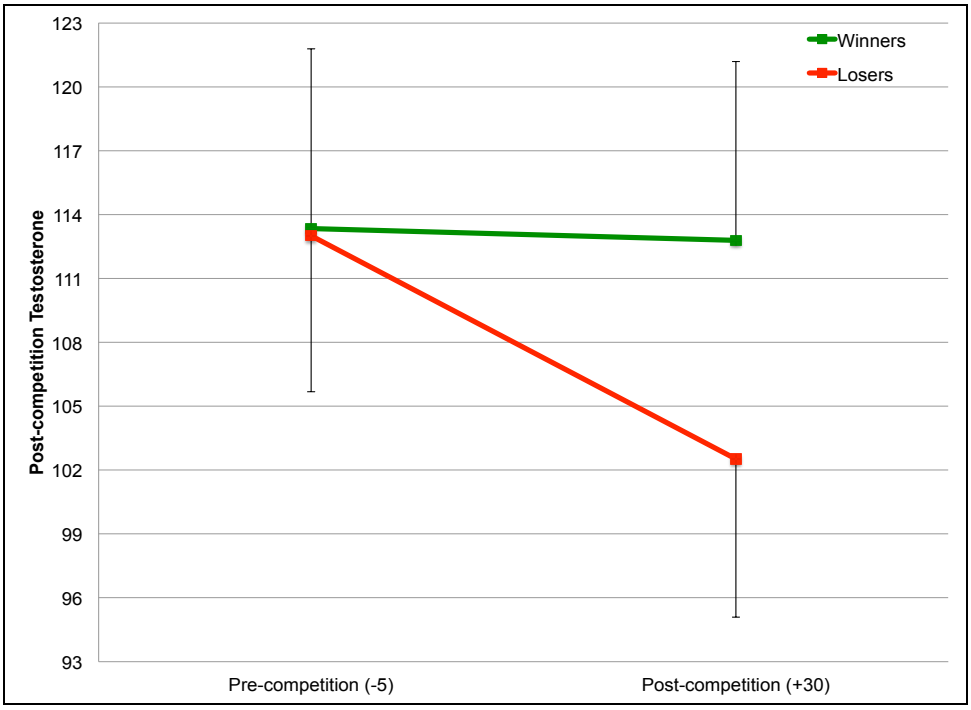

Figure 3.1. Mean testosterone (SEM) for winners (n=30) and losers (n=28) at baseline, post-competition, and the change from baseline to post-competition.

3.3.4. Dual-hormone hypothesis

The dual-hormone hypothesis posits that basal cortisol and basal T will have a conjunct effect on T fluctuations following a contest. Evidence supporting this hypothesis

has been reported in losers of a competition (Mehta and Josephs, 2010), and this mechanism may hold in winners as well.

So, in order to investigate the role of basal hormone status on testosterone changes following competition, separate analyses were run for winners and losers, using a multiple linear regression procedure as described in previous studies (e.g. Mehta et al., 2008; Mehta & Josephs, 2010; van der Meij et al., 2010). Accordingly, T1 was regressed on T0, which was entered as covariate in the first Model of the multiple regression. In Model 2, basal cortisol was entered as a predictor. The overall linear regression model was significant for both winners [R2 = .896, adjusted R2 = .888, F (2,27) = 116.240, p < .001] and losers [R2 = .850, adjusted R2 = .838, F (2,24) = 68.177, p < .001 (Table 3.3). Adding cortisol into the model significantly increased the amount of variance explained in winners at T1 (Δ F (1,27) = 11.608, p < 0.01, Δ R2 = .045) but not in losers (Δ F (1,24) = 3.644, p > 0.06, Δ R2 = .23) (Table 3.4). Time of saliva sampling was not included as a covariate because it correlated neither with basal cortisol nor T when winners and losers were considered separately.

Table 3.3. Regression Models examining the linear relationship between post-competition testosterone and baseline testosterone (T0), log-transformed baseline cortisol (LogC0), and their interaction in winners.

	Predictor (standardized beta coefficients and associated p-values)			
Model	T0 (pg/mL)	LogC0	T0 X LogC0	Regression Statistics
Model 1	1.058 (.000)	-.251 (.002)		F (2,27) = 116.240, p < .001
Model 2	1.138 (.000)	-.274 (.001)	-.140 (.049)	F (3,26) = 88.308, p < .001

Table 3.4. Regression Models examining the linear relationship between post-competition testosterone and baseline testosterone (T0), log-transformed baseline cortisol (LogC0), and their interaction in losers.

	Predictor (standardized beta coefficients and corresponding p values)			
Model	T0 (pg/mL)	LogC0	T0 X LogC0	Regression Statistics
Model 2	.969 (.000)	-.162 (.068)		F (2,24) = 68.177, p < .001
Model 3	1.018 (.000)	-.270 (.022)	-.153 (.148)	F (3,23) = 48.541, p < .001

Our second hypothesis predicted an interaction between baseline T and baseline cortisol, which we tested using the same multiple regression procedure, with the addition of the interaction term as Model 3. Once again the overall linear regression model was significant for both winners [R^2 = .911, adjusted R^2 = .900, F (3,26) = 88.308, p <.001] and losers [R^2 = .864, adjusted R^2 = .846, F(3,23) = 48.541, p <.001], with a significant effect of the interaction term only in winners (Table 3.3 and Table 3.4). Specifically, adding the interaction between baseline cortisol and baseline testosterone in winners increased the amount of variance explained in T1, Δ F (1,26) = 4.272 p < 0.05, Δ R^2 = .015; this did not occur in losers, Δ F (1,23) = 2.238, p > 0.14, Δ R^2 = .013. To interpret the significant interaction, we first conducted a simple slope analysis for basal T 1 SD below the mean and 1 SD above the mean (Aiken & West, 1991; Cohen et al., 2003). Subsequently, we graphed the interaction by plotting the changes in T scores 1 SD above and 1 SD below the means for basal T and basal cortisol (Figure 3.2). Changes in T scores were the unstandardized residuals of a regression analysis with basal T as the predictor and post-competition T as the dependent variable in winners. For baseline T 1 SD below the mean, the slope did not significantly differ from zero [b = -.097, t (26) = -.947, p > 0.35]. In contrast, a significant effect was found for baseline T 1 SD above the mean [b = -.453, t (26) = -3.775, p < 0.01], reflecting a significant negative association between basal cortisol and T changes at high concentrations of basal T. Taken together, these data indicate that for individuals with higher pre-competition T, – but not for low-T individuals – pre-competition cortisol predicted changes in T after victory. Specifically, in the high baseline T group, low pre-competition cortisol was associated with a larger increase in T following a victory.

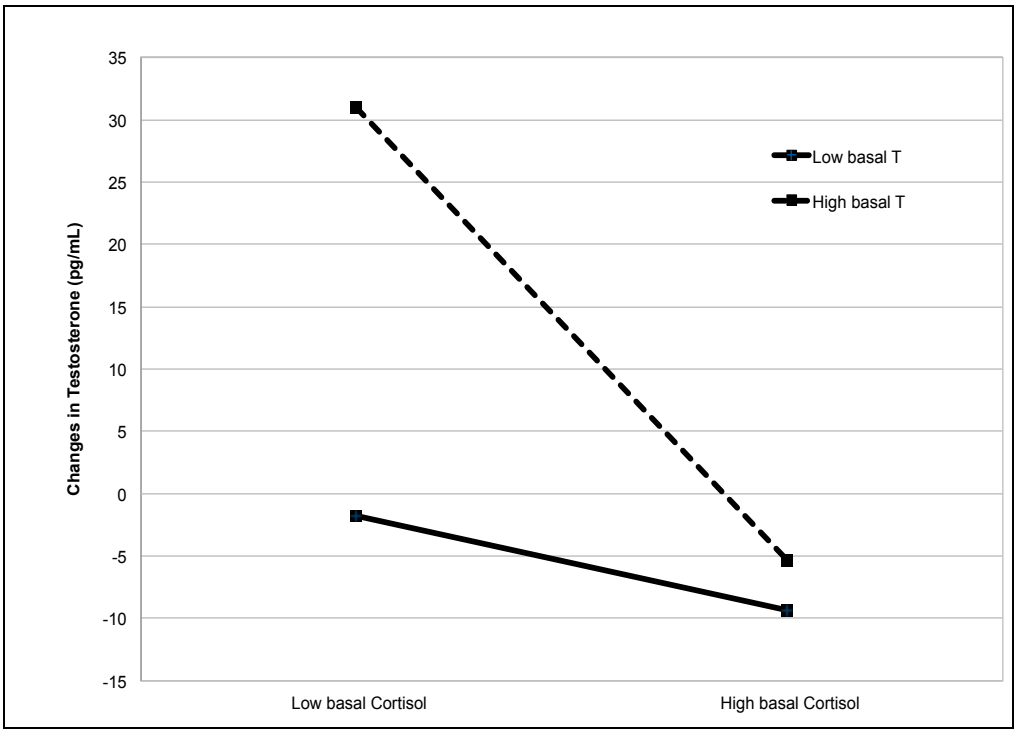

Figure 3.2. Changes in testosterone following victory, as a function of basal testosterone and cortisol levels (unstandardized residuals of a regression analysis, with basal T as the predictor and post-competition T as the dependent variable). Low = 1 standard deviation below mean; high = 1 standard deviation above mean. When pre-competition T was high, pre-competition cortisol was related to changes in T after victory, with greater T increase in those participants with low pre-competition cortisol.

At this point we would like to report how in subsequent analyses we tested for the specificity of the dual-hormone hypothesis to winners by including a three-way interaction between T, cortisol and the experimental condition (i.e. victory or defeat). This higher-order interaction did not reach significance [t (49) = -.326, *ns*], possibly due to restricted statistical power at this level of complexity.

At last, several interesting relationships between steroid hormone fluctuations and the self-reported psychological variables were observed. In particular, we found a negative correlation between self-reported perceived control over the outcome of the competition and change in cortisol [r = -0.306, p < 0.05]. This suggests that lower feelings of control are associated with an increase in cortisol over the test session, but not with pre-task (C0) "trait" concentrations of cortisol [r = 0.182, p > 0.17]. Changes in T did not correlate either with confidence as measured in the attribution survey [r = -0.153,

$p > 0.24$] or with self-assurance [$r = 0.027$, $p > 0.83$], but they correlated inversely with negative affect as measured by the PANAS-X [$r = -0.275$, $p < 0.05$]. When we computed a partial correlation coefficient between negative affect and T changes controlling for competition outcome, the relationship between mood (i.e. negative affect) and T changes was no longer significant ($r = -0.174$, $p > 0.18$), suggesting that the relationship between mood and T is outcome-specific.

3.4. Discussion

As in numerous prior reports (see, for example, Mazur et al., 1992; Stanton et al., 2009) we found that the simple form of the competition effect centered on diminished T: as a group, losing competitors experienced a decrease in T compared to winners, whose T concentrations remained constant (Figure 3.1). The success of our manipulation can be attributed to the familiarity and engaging nature of the task. We argue that along with the discrepancy of accessible resources -in our experiment only winners received a monetary reward-, a familiar contest is an additional condition for the manifestation of the competition effect. In other words, competitive interactions close to people's experience would be perceived as more important for status or social ranking, therefore more likely to induce differential HPG activations.

The apparent stability of winners' post-competition T concentrations can be interpreted in various ways. One possible interpretation is that a rise in T was hidden by the diurnal decline (Stanton et al., 2009). In other words, a competition-related burst in T and the circadian T cycle may have cancelled each other out. However, as described earlier we attempted to control for circadian variability in T by collecting data only in the afternoon, and indeed we found no significant correlation between time of the day and T assays in our data. Alternatively, an interaction between the HPG axis, which is the primary source of dynamic changes in T, and the HPA axis, whose main end product is cortisol, could also produce the observed pattern of results. The integration between the two axes is well documented (for reviews, see Johnson et al., 1992; Viau, 2002), and, considering the opposing effects of androgens and glucocorticoids (Mayer & Rosen, 1977; Chen, Wang, Yu, Liu, & Pearce, 1997; Crawford et al., 2003; Mayer & Rosen, 1977), it seems that crosstalk between the HPA and HPG systems may take the form of

reciprocal inhibition (Hayashi & Moberg, 1987; Burnstein et al., 1995; Viau & Meaney, 1996; Chen et al., 1997; Tilbrook et al., 2000; Ciechanowska et al., 2010). Consistent with this view we found that high baseline cortisol was associated with little or no increase in T, and low concentrations of basal cortisol were paired with larger increases in T. In other words, our dominance scenario induced positive T changes to a greater extent in winners with low cortisol. To our knowledge this is the first empirical evidence of an exclusive inhibitory effect of cortisol on T secretion in winners of a competition. This finding is not only in line with the predictions of the biosocial theory of status (Mazur & Booth, 1998), but it also helps shed light on the recurrent inconsistencies found in the literature on how opposite competition outcomes modulate sex steroids. Accordingly, the lack of an increase in T after winning non-physical contests in past research (Mazur et al., 1997; Mehta & Josephs, 2006, 2010; van Anders & Watson, 2007) may reflect the confounding influence of basal glucocorticoid status.

A buffering effect of cortisol on T secretion was not found in losers. Two explanations are possible. First, cortisol's inhibitory actions were more manifest in winners because of the larger pulses of luteinizing hormone (LH) and consequently T they experienced. In losers, the buffering effect of the stress axis against the reproductive axis might have been less evident because of the smaller pulses of LH (Elefther & Church, 1968; Bronson, Stetson, & Stiff, 1973; Elefther & Church, 1967, 1968). In essence, the lower LH production following social defeat made it already sufficiently low such that any inhibitory effect of cortisol would have been undetectable, as LH secretion had reached its floor. Alternatively, it might be that a similar but more subtle effect of baseline cortisol on T in losers would emerge only with a larger sample size. It remains that, according to the competition effect, losers should experience a decrease in T.

Our results indicate that T changes in winners depend on initial (i.e. basal) cortisol concentrations, but what about the initial T concentrations? An interesting finding of the present study was the unexpectedly complex nature of HPG - HPA interaction in regulating dynamic T changes after social victory. We found that when baseline T was low, initial pre-competition concentrations of cortisol were not related to changes in T from baseline to post-competition. However, additional variability was observed in individuals with high initial concentrations of T (Figure 3.2). Winners with high baseline

activity in both axes (i.e. high-T and also high cortisol) showed little or no change in T from baseline to post-competition. But when winners with high baseline T coupled with *low* baseline cortisol were analyzed separately, we found a large increase in T from pre-competition to post-competition. This finding suggests that individual responsiveness to a positive change in social status varies across a continuum that derives from a complex interaction of the two main steroid axes. Recent human studies seem to go in the same direction, showing how this interaction explains both behavioral outcomes, such as different types of aggression (Dabbs, Jurkovic, & Frady, 1991; Geniole, Carré, & McCormick, 2011; Popma et al., 2007; Geniole et al., 2011) and dominance (Mehta & Josephs, 2010), as well as physiological responses (Huovinen et al., 2009; Mehta & Josephs, 2010). For example, Mehta and Josephs (2010), looking at changes in T as a potential mediator of the relationship between baseline hormonal concentrations and dominance, found evidence of significant variation between people with high basal T concentrations compared to people with low T concentrations. A novel aspect of our study, in the context of this small literature, is that while most of the previous reports (Dabbs et al., 1991; Popma et al., 2007) investigated the behavioral implications (i.e. aggression) of a high-T/low cortisol profile in subjects subjugated within a status hierarchy (in Dabbs et al.'s study, subjects were young adult prisoners; and, in Popma et al.'s study participants were male adolescents referred to a diversion program for delinquents) our study showed that potentially similar mechanisms might take place also in subjects that achieve a socially dominant position. However, we failed to replicate the results of Metha and Josephs (2010), who found that a low cortisol/high-T profile predicted higher changes in T after *losing* (but not winning) a competition. This discrepancy might arise from aspects contingent to the experiments (for example, Mehta and Josephs's experimental manipulation was not successful in producing a rise in T among winners and their losers group showed a less restricted T response compared to ours), suggesting that rather than mutually exclusive these two results might be complementary.

Although precise understanding of the mechanisms underpinning the neuroendocrinology of dominance and status-seeking behaviors must await further research, we can make some initial observations about this system. The stability of T (Liening et al., 2010) and the positive correlation between its circulating concentrations

and status-seeking behaviors (for reviews, see Mazur & Booth, 1998; Archer, 2006; Mazur & Booth, 1998) has led some researchers to view T as an endocrine marker of individual differences in dominance (Josephs, Sellers, Newman, & Mehta, 2006; Sellers, Mehl, & Josephs, 2007). This theoretical framework might explain the lack of variability that we observed in those individuals with low baseline T – in an ancestral setting, individuals with low baseline T might have been males with relatively low dominance status who were less motivated to seek or respond to changes in status until a future time when they have more likelihood of success. It has been proposed that these individuals would feel uncomfortable when placed in high status positions or otherwise challenged (Josephs et al., 2006); this source of stress might also account for the lack of a T response after a social victory in these individuals. Given this reduced sensitivity to a competitive scenario, it would follow that pre-test cortisol, a major mediator of the organism's responses to threats to homeostasis, would have little or no effect on such individuals. In other words, T responses to status-relevant competition outcomes of people lacking in motivation towards dominant behaviors would not depend on concurrent cortisol state. On the other hand, cortisol state had a significant impact on high-T individuals, suggesting that the buffering effect of the stress axis on the reproductive axis applies only to this subsample. What this further implies is that even though high-T individuals are supposed to be more sensitive to positive status changes, their actual response critically depends on concurrent cortisol state, with low-cortisol individuals (possibly deriving from increased exposure to stress), experiencing T changes to a significantly greater extent. From an evolutionary perspective we can think of this interaction as an advantage for acquiring valued resources and preserving stable social groups. Acquisition and maintenance of high hierarchy position – necessary to facilitate social organization (Foss, 1998) – requires not only new dominance fights, whose likelihood may be governed by basal T (Mazur & Booth, 1998), but also the ability to sustain appropriate aggressive responses during and after competitive encounters, which has been proposed as the main function of the transient increase in T (Wingfield, Ball, Dufty, Hegner, & Ramenofsky, 1987). High-cortisol individuals, assumed to be undergoing stressful events, would be temporarily inhibited with regard to such dominance challenges (even if they had high baseline T). Overall, our results would be consistent with the operation of an adaptation that provokes increased competitiveness – via post-win T accumulation – mostly in those individuals that are both on a winning

streak (reflected in high baseline T) and free from stressors (such as illness, injury or social stress, reflected in circulating cortisol concentrations).

In summary, we found a significant interaction between the HPA and HPG axes status in modulating the competition effect in winners – randomly-determined videogame losers showed significantly decreased post-competition concentrations of T, compared to winners, especially those with a combination of higher baseline T and lower baseline cortisol. So our pattern of results suggests that the competition effect may be jointly determined by key characteristics of both the competitors and the competition. Specifically, the emergence of a competition effect may rely on a combination of (1) an ecologically-valid competition task that is believable and engaging (and thus amenable to subjects' attributions of locus of control), and (2) the baseline status of both the HPA and HPG axes of the participants, acting jointly.

Chapter 4.

STUDY 3: Testosterone across successive competitions: evidence for a 'winner effect' in humans

Note: This section is based on the following article, with permission: Zilioli, S. & Watson, N.V., (in press). Testosterone across successive competitions: evidence for a 'winner effect' in humans. *Psychoneuroendocrinology.*

4.1. Introduction

Hormones contribute to phenotypic plasticity, the ability of an organism to harmonize physiological and behavioral processes with environmental events. By conjoining environment and organism, the endocrine system thus ultimately regulates behavior through direct and indirect routes (Dufty et al., 2002). Although endocrine signaling pathways require an interconnected network of diverse components (enzymes, receptors, hormones binding proteins), fluctuations in circulating hormone concentrations are clearly crucial in the expression of endocrine-mediated phenotypes (Rosvall et al., 2012). These endocrine activation profiles are on display when the organism is coping with environmental challenges, ranging from temperature changes to social interactions, and can be quantified, depending on the phenotype of interest, over short-term or long-term time scales.

Mainly regulated by the hypothalamic-pituitary-gonadal (HPG) axis, testosterone is a sex steroid with pronounced effects on skeletal muscles, body composition (e.g. bone density) and sexual function (Mooradian et al., 1987) but also fundamentally implicated in the control of social behaviors more generally (Booth et al., 2006). In a wide range of vertebrates, including humans, intraspecific competition is an ecologically relevant context that modulates androgen release (Hirschenhauser & Oliveira, 2006;

Wingfield et al., 1990). In particular, male-to-male antagonistic encounters potently induce changes in androgenic tone, with acute spikes in testosterone observed as early as 10 minutes after the onset of a social conflict (Wingfield & Wada, 1989). Different interconnected facets of this phenomenon are under investigation. On the one hand, researchers are interested in unraveling those situational and motivational factors that affect the direction and/or strength of this neuroendocrine activation. On the other hand, the functional significance of this testosterone reflex has not been established: what behavioral changes do the observed endocrine responses propel? The presented study was designed to test novel hypotheses within this framework.

In many species testosterone fluctuates in concert with changes in social status, such that winning competitions leads to an increase in circulating testosterone and/or losing leads to a net decrease in testosterone (Lloyd, 1971; Bernstein et al., 1974; Dixson, 1980; Oliveira et al., 2009). In striking harmony with this idea, fights that result in a draw are not accompanied by significant changes in circulating testosterone concentration (Oliveira et al., 2005). We refer to this phenomenon as the "Competition Effect" (CE) (Samuele Zilioli & Watson, 2012). Interestingly, the CE can be extended to vicarious experience of victory (the so called "audience effect"; Bernhardt et al., 1998; Oliveira et al., 2001) and be further modulated by additional contextual factors, such as the location of the dispute (known as the "home advantage" (Fuxjager et al., 2009; Neave & Wolfson, 2003). The interplay between situational variables regulating challenge-induced testosterone pulses reaches its maximum complexity when considering multiple experiences of winning (or losing). Seminal work from Marler and colleagues investigated this phenomenon proposing it as the physiological substrate of the Winner Effect – defined as the increased probability of winning an aggressive encounter following previous victories (Fuxjager & Marler, 2010; Oyegbile & Marler, 2005). For example, Oyegbile and Marler (2005) showed that California mice (*Peromyscus californicus*) that experienced a repeated series of social victories had higher endogenous testosterone than conspecifics that experienced fewer or no social victories.

In humans, surprisingly, although some studies on athletes have looked at androgen responses to multiple competitive interactions (Crewther et al., 2013), no studies have investigated this phenomenon experimentally within a controlled laboratory

setting. Research is needed to establish how consecutive wins, losses, or a combination of both influence HPG reactivity. We examined this phenomenon in the current experiment by measuring salivary testosterone in pairs of male participants engaging, on two consecutive days, in head-to-head competitions on a previously validated laboratory task (Samuele Zilioli & Watson, 2012). Furthermore, although testosterone fluctuation following competitive challenges appears to be a well-conserved phenomenon across vertebrate taxa (at least in males), it is not clear what its subsequent function might be. In this regard, three hypotheses have been proposed (Gleason et al., 2009). First, testosterone could enhance an individual's ability to win future encounters by increasing aggression (Trainor et al., 2004; Oyegbile & Marler, 2005). Second, competition-related testosterone surges could reinforce and/or stimulate learning processes associated with the contest (Gleason et al., 2009). Third, testosterone might affect behaviors, other than aggression, that are associated with winning, such as persistence in search behavior (Andrew & Rogers, 1972; Wingfield, 1994). These hypotheses are not necessarily mutually exclusive.

In humans, a small but growing body of research suggests that contest-related testosterone reflexes map onto aggressive behavior (Carré et al., 2009; Carré et al., 2013; Carré, Iselin, Welker, Hariri, & Dodge, 2014), competitive motivation (Mehta & Josephs, 2006) (Carré & McCormick, 2008), courtship behavior (Leander van der Meij et al., 2012) and learning (Schultheiss & Rohde, 2002; Oliver C Schultheiss et al., 2005). For example, in a recent report, Carré and colleagues found that changes in testosterone after a competition, regardless of the outcome, predicted immediate reactive aggression (Carré et al., 2013). Thus, although available evidence supports the aggression-enhancing and competitive-enhancing hypotheses, more research is needed to shed light on the alternative possibilities identified above. For example, testosterone is implicated in cognition (Ackermann et al., 2012; O'Connor et al., 2001) and it could be expected that testosterone surges following a challenge might modulate winning by acting on learning of contest-related information (Marler et al., 2005). Pioneering work from Schultheiss and colleagues (2002; 2005) seemed to point in this direction by demonstrating that testosterone reactivity to a competitive interaction correlated with performance on an implicit learning task. Notably, these studies have restricted their analyses to changes in behaviors occurring immediately after the competitive interaction,

overlooking longer-term behavioral outputs that might be correlated with testosterone changes on previous days (Oyegbile & Marler, 2005; Wright et al., 2012).

In the current experiment the functional effects of testosterone reactivity were examined over both short-term and long-term timescales. Thus, participants were asked to complete a spatial ability task directly after competition (a short-term effect) on Day 1. It is well established that testosterone influences spatial cognition (O'Connor et al., 2001) and spatial cognition is an ancestrally relevant ability in male-to-male combat (Sherry & Hampson, 1997; Watson, 2001).

Longer-term effects were examined by evaluating influences of testosterone fluctuation on learning of contest-related information. Specifically, we tested whether testosterone reactivity on the first day would be associated with any improvement on the competitive task by measuring the difference between the score obtained on the second day and the score obtained on the first day.

4.2. Methods

4.2.1. Participants

Eighty-eight male undergraduates (mean age=20.38 years, SD=2.03) served as participants, following elimination of three subjects that failed to provide saliva samples and one subject that was aware of the hypothesis under study. Participants received course credit in exchange for their participation. Two participants reporting oral infections and bleeding gums, and two participants reporting use of medications were excluded, leaving a total of eighty-four individuals. All procedures were subject to review and prior approval by the Simon Fraser University Research Ethics Board.

4.2.2. Procedure

The study was conducted across two consecutive days. To reduce diurnal variability in testosterone, all testing occurred between 1300h and 1900h (Campbell et al., 1982). On the first day, upon arriving, each pair of competitors was greeted by a

male experimenter and asked to read and complete an informed-consent form. An additional description of the study was provided verbally, and, to further intensify the competition, participants were told that the winner would receive a $10 cash prize. Each participant, who was directed to one of two small rooms, was also reminded to come back on the next day, but it was not revealed that a second competition would be taking place. Before collecting the first saliva sample participants completed the BIS/BAS questionnaire (Carver & White, 1994), answered a few questions about the upcoming competition and completed a self-report measure of experience playing videogames (Terlecki & Newcombe, 2005). This initial phase, lasting approximately 10 minutes, mainly served as a buffer by providing time for any possible initial apprehension about the test session to dissipate. A baseline saliva sample was taken (T1), and five min after collection participants were instructed to begin the game on the experimenter's mark, after which doors to the two participants' rooms were shut for the duration of the competition task. This consisted of a custom-programmed head-to-head version of the well-known commercial videogame, Tetris, in which the outcome could be rigged to suit experimental needs (for a description of the task, see Zilioli & Watson, 2012). Although the software registered the actual score that each participant obtained, on the first day the outcome of each competition was rigged such that the "winner" and "loser" were randomly determined for the competing pair. Due to software malfunction scores for two participants were not saved. After competing for exactly 15 min, the participants' rooms were opened and the experimenter called for the "winning" participant to step out of his room and claim the competition prize (walking past the room of the "loser" to do so). The winning participant was audibly congratulated by the experimenter and then returned to his room. Following the competition participants completed the PANAS-X, (Watson & Clark, 1994), which assesses the individual's current emotional state on general negative and positive affect, basic negative emotions (e.g., hostility), basic positive emotions (e.g., joviality), and other affective states (e.g., fatigue). PANAS-X scores from two participants were lost due to computer malfunction. An attribution questionnaire designed to check for suspicions about the rigged nature of the contest and to receive general feedback from participants was also administered. Next, subjects completed a cognitive task, the Mental Rotation Test (MRT; see below for details). At exactly 20 minutes after the completion of the Tetris competition, participants provided a second saliva sample (T2). Collection took five minutes, after which, one participant at random

was dismissed while the other was dismissed few minutes later. This was done to avoid any social interactions between participants following the experiment that might influence their attitudes towards the experiment on the following day. Before being dismissed, participants were asked to come back to the lab at the same time the following day, not to drink alcohol and have a regular sleep.

A similar procedure was followed on the second day, with a few important modifications. First, upon arriving, subjects received a mood evaluation using the PANAS-X. Next, each participant provided a saliva sample (T3) and they were advised that a new round of competition would be conducted. The same task as in Day 1 was used, but this time the outcome of the contest was not randomly assigned, but rather determined by skill. Two steps allowed participants to unambiguously confirm their winner or loser status: (1) towards the end of the game a scripted message reminded each participant to check their score, and (2) at the conclusion of the competition the experimenter announced both names followed by their scores (rather than simply announcing a winner). After the completion of the Tetris competition, the winner was awarded $10 and the attribution questionnaire was administered. The fourth saliva sample (T4) was collected at exactly 20 minutes after the end of the contest. Participants provided biometric and demographic information and were given a printed debriefing form to read and sign. The study was conducted from May to November.

4.2.3. Mental Rotation Task

All participants completed a computerized version of the Mental Rotation Test (MRT, Peters et al., 1995). This test consisted of 24 items divided into two sets. For each question a computer image of a 3-dimensional target figure was presented, along with four comparison stimuli (two correct alternatives and two distractors). The task for participants was to hold the target item in their mind and imagine rotating it in one or more axes, in order to identify which of the comparison items could be the same object in a different spatial orientation. Participants were given 2 min to complete each 12-item set. One point was assigned for each correct response. MRT scores from seven participants were lost due to computer malfunction.

4.2.4. Saliva samples and hormone assays

On both days participants were instructed to abstain from eating, drinking, smoking, or brushing their teeth for one hour before testing as well as drinking alcohol for twelve hours before the first experimental session Saliva samples were collected using Salimetrics oral swabs (SOS; Salimetrics LLC, State College PA) placed under the tongue, according to vendor usage instructions for testosterone determinations (this location is not recommended for some analytes, such as α-amylase and SIgA, that show differential glandular secretion rates). According to the vendor, the SOS device consists of “an inert food-grade polymer” individually validated for use in specific assays that include salivary testosterone determinations in both men and women. Unlike cotton swabs, SOS devices show high volume recovery and measurement accuracy properties that compare well with passive drool techniques, according to the vendor. Samples were chilled immediately following collection, and then frozen within few hours and held at -20°C until assay. Samples were assayed in duplicate using competitive enzyme immunoassays for testosterone (Salimetrics LLC, State College, PA). The average intra-assay coefficient of variation was 5.24% and the inter-assay coefficients averaged across high and low controls was 9.52%.

It is important to remember that in target tissues, the biologically active fraction of total circulating testosterone consists of the free testosterone plus the albumin-linked testosterone. The large fraction of testosterone bound to the carrier protein sex hormone binding globulin (SHBG) is biologically unavailable as it cannot enter cells and interact with androgen receptors. Salivary testosterone provides a measure of free testosterone that significantly correlates with serum total and free testosterone levels (Shirtcliff et al., 2002).

4.2.5. Statistical analyses

Differences between winners and losers on mood, MRT score and testosterone reactivity (T2-T1 for day1 and T4-T3 on day2) were assessed using independent t-tests and factorial ANOVA. Within group (e.g., winners vs. losers) testosterone reactivity outliers (>3SD) were excluded. The time of day and month of sample collection were recorded, for use as covariates if significantly associated with the hormonal measures.

Hierarchical linear multiple regressions were run to test the effect of Day 1 testosterone reactivity, competition outcome (win vs. lose) and their interaction on Day 2 competition performance (where Tetris score obtained on the first day was subtracted from Tetris score on the second day). Observations associated with standardized residuals greater than 3 SD and/or standardized DFBETAs greater than |1| were excluded (Cohen et al., 2003). Victory and social defeat might influence post-competition spatial ability and that these differences might be mediated by testosterone reactivity, similarly to what observed for other post-encounter behaviors (e.g., aggression; Carré et al., 2013). A mediation analysis, using the bootstrapping approach (Preacher & Hayes, 2008), was run to test this hypothesis. The threshold for statistical significance in all analyses was set at a p value of .05 (two-tailed, in the case of t-tests).

4.3. Results

4.3.1. Competition Effect: Day 1

Three participants (two winners and one loser) indicated suspicion about the competition manipulation on the first day and one winner differed by more than three standard deviations on his group testosterone reactivity mean. These individuals were removed from the analyses. Prior to testing, baseline testosterone did not differ between randomly assigned winners (n=40) and losers (n=40) [t (78) = -.292, p = 0.771], as expected. Following the competition manipulation, a significant difference in testosterone reactivity emerged, with winners (M = .085, SD = 19.44) showing higher levels than losers (M = -11.36, SD = 26.41) [t (78) = 2.208, p = 0.030, d = 0.49] (Table 4.1). Winners also reported more positive mood [t (76) = 2.601, p = 0.011] and scored higher on two basic positive emotion scales than losers [joviality, t (76) = 4.508, p < 0.001; self-assurance, t (76) = 2.412, p = 0.018] as well as surprise [t (76) = 3.529, p = 0.001].

The average MRT score was 22 (M = 21.74, SD = 7.31), with scores ranging from 4 to 42. Surprisingly, losers (M = 23.33, SD = 7.81) scored higher than winners (M = 20.24, SD = 6.54), but this difference did not reach statistical significance [t (72) = -1.852, p = 0.068]. However, testosterone reactivity did not correlate with the MRT performance either in losers (r = -.113, p = .510) or winners (r = .266, p = .107) or both (r

= -.016, p = .890). Further, testosterone reactivity was not found to mediate the effects of Day 1 competition outcome on mental rotation performance [95% CI: -1.2319, .6355]. Similar results emerged when MRT was scored by assigning one point only when, for each item, both comparison stimuli were identified correctly.

Table 4.1. Descriptive statistics for testosterone on Day 1.

	Winners and Losers (n = 80)		Winners (n = 40)		Losers (n = 40)	
	M (SEM)	SD	M (SEM)	SD	M (SEM)	SD
Pre-competition testosterone (pg/mL)	156.46 (4.7)	41.89	155.08 (6.2)	39.14	157.83 (7.1)	44.94
Post-competition testosterone (pg/mL)	150.41 (4.3)	38.34	154.35 (6.2)	38.96	146.47 (6)	37.79
Changes in testosterone (pg/mL)	-5.64 (2.7)	23.75	-.08 (3.1)	19.44	-11.36 (4.2)	26.41

4.3.2. Association between testosterone reactivity on Day 1 and Tetris performance on Day 2

Hierarchical regression analysis was used to examine the association between the Day 1 competition outcome, individual differences in testosterone reactivity, and performance on the competitive task. Performance on Day 2 corrected by Day 1 performance (i.e. score difference) was regressed onto competition outcome (0 = win, 1 = lose) and testosterone reactivity (centered) (Step 1) and competition outcome by testosterone reactivity interaction (Step 2). One individual, who reported practicing Tetris before the experiment, as well as two Step 1 regression outliers (one individual was associated with a standardized DFBETA greater than |1| for the testosterone reactivity regression coefficient, while one individual was associated with a residual equal to 3.5 SD) were excluded from the analyses. Testosterone reactivity was associated with performance on the competitive task [β = .329, p = .005] (Step 1) but there was no outcome by testosterone reactivity interaction [β = -.088, p = .631; β = .026, p = .899, after removing two Step 2 regression outliers] (Step 2). In other words, testosterone changes on Day 1 were positively correlated with performance improvement on Day 2. Figure 4.1 illustrates the positive association between the Tetris score difference

(unstandardized residuals after controlling for competition outcome) and changes in testosterone on Day 1 [R2 = 9.9%, p =.006].

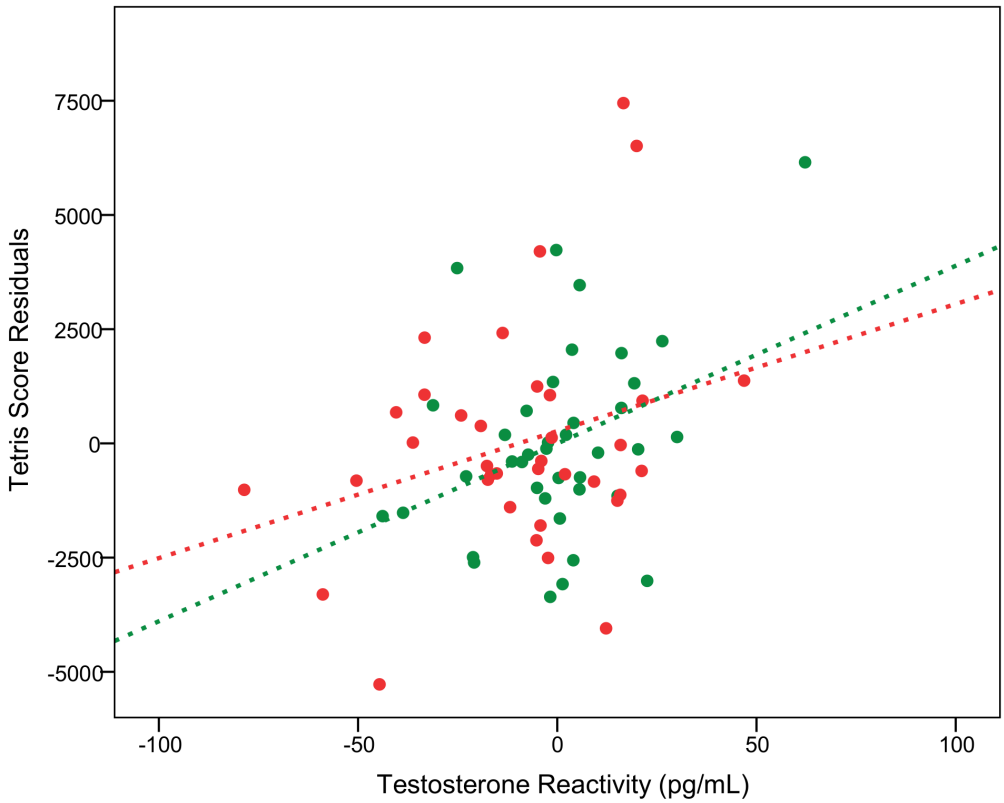

Figure 4.1. The relationship between Day 1 testosterone reactivity and Tetris score difference (unstandardized residuals after controlling for competition outcome) in winners (green circles) and losers (red circles).

4.3.3. Competition Effect: Day 2

Participants' mood was re-evaluated using the PANAS-X at the beginning of the Day 2 testing session. Interestingly, Day 1 losers reported more positive affect [t (78) = -2.393, p = 0.019], attentiveness [t (78) = -2.096, p = 0.039], joviality [t (78) = -2.649, p = 0.010] and self-assurance [t (78) = -2.139, p = 0.036] than Day 1 winners. However, no difference between the winners (M = 141.59, SD = 39.92) and losers (M = 145.03, SD = 42.68) was found for Day 2 baseline testosterone (i.e. T3 testosterone) [t (78) = -.372, p = 0.711]. Three participants (two losers and one winner) indicated suspicion about the competition manipulation on the second day and one loser differed by more than three standard deviations from the group testosterone reactivity mean. These individuals were

removed from analyses involving testosterone reactivity on the second day. Descriptive statistics for pre-competition, post-competition and testosterone changes are reported in Table 4.2.

Table 4.2. Descriptive statistics for testosterone on Day 2.

	Winners and Losers (*n* = 76)		Winners (*n* = 39)		Losers (*n* = 37)	
	M (SEM)	SD	M (SEM)	SD	M (SEM)	SD
Pre-competition testosterone (pg/mL)	141.82 (4.7)	41.12	139.36 (6.5)	40.43	144.42 (6.9)	42.24
Post-competition testosterone (pg/mL)	147.5 (5.2)	45.28	146.95 (7.5)	46.72	148.08 (7.3)	44.35
Changes in testosterone (pg/mL)	-5.67 (2.7)	23.91	7.58 (3.7)	23.21	3.66 (4.1)	24.78

A factorial ANCOVA with Day 1 competition outcome and Day 2 competition outcome as factors, and month of sample collection (i.e. seasonality) as a covariate (p = .007), revealed a significant interaction effect [F (1,67) = 5.241, p = .025, η_p^2= .07], indicating that winning and losing on the first day influenced second day winners and losers differently. Specifically, the same model among Day 2 winners, revealed that testosterone reactivity was not related to Day 1 outcome [F (1,32) = .817, p = .373]; however, the same model among Day 2 losers revealed a greater testosterone elevation in subjects who were Day 1 winners compared to those who were Day 1 losers [F (1,30) = 4.898, p = .035, η_p^2= .14]. These double losers – those who were losers on both Day 1 and Day 2 – evinced a sharp decline in testosterone on Day 2 (Figure 4.2). Testosterone fluctuations on Day 2 were also analyzed considering the type of status hierarchy (stable vs. unstable) that emerged as a result of the combined outcomes of the two competitions. A factorial ANCOVA with type of status hierarchy as factor, and month of sample collection (i.e. seasonality) as a covariate (p = .005), revealed a significant main effect [F (1,69) = 5.261, p = .025, η_p^2= .07], indicating that men in unstable hierarchies (first day winners/second day losers *and* first day losers/second day winners) experienced an increase in testosterone compared to men in the stable hierarchies (double winners *and* double losers).

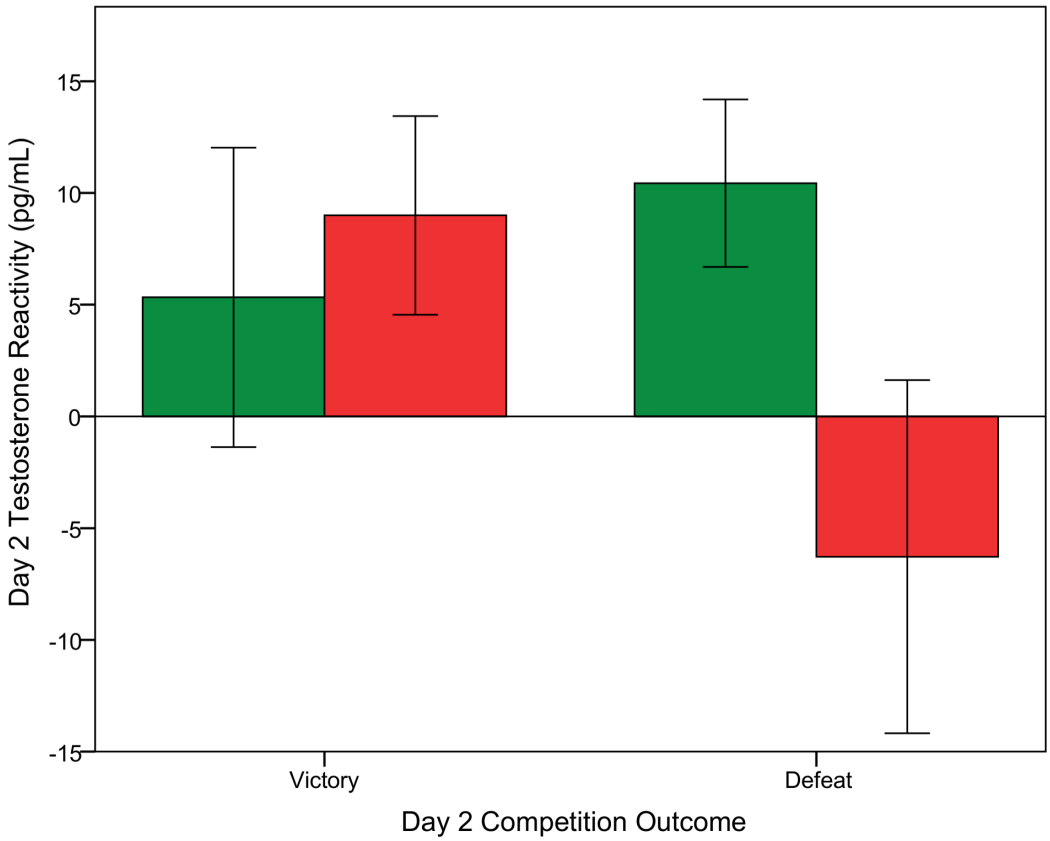

Figure 4.2. Means (SEM) for Day 2 testosterone reactivity as a function of Day 2 competition outcome (victory vs. defeat) and Day 1 competition outcome (green indicates Day 1 winners and red indicates Day 1 losers).

4.4. Discussion

The aims of the current study were: 1) to investigate the longer-term functional consequences of testosterone responses to competition; and, 2) explore how different combinations of successive wins and/or losses influence testosterone secretion in human males.

Confirming previous reports (Carré et al., 2013; Zilioli & Watson, 2012) we found a significant competition effect on the first day of the experiment, with individuals who were randomly assigned to lose the rigged head-to-head Tetris competition showing a decrease in testosterone compared to the randomly assigned winners. Moreover, regardless of the competition outcome, changes in testosterone on the first day

predicted task performance (i.e. the relative change in Tetris score) on the second day. This demonstration of longer-term effects of endogenous fluctuations of testosterone is grounded in both data and theory. Recently, for example, it has been showed that testosterone administration on one day leads to a performance improvement on a perceptual task on the next day (Wright et al., 2012). Similarly, in our study, testosterone changes on Day 1 were positively correlated with performance improvement on Day 2. According to Wright et al. (Wright et al., 2012), testosterone may influence consolidation of procedural memories in the service of acquiring and improving motor and cognitive skills. In particular, it is proposed that testosterone may specifically act on the aspect of consolidation referred to as "off-line" learning: the component of skill improvement that occurs between training sessions (Robertson et al., 2004). This proposal builds on the well-established links that exist between androgens and memory performance (Janowsky, 2006): Endogenous testosterone is related to memory performance in youth (Ackermann et al., 2012) and elderly men (Barrett-Connor, Goodman-Gruen, & Patay, 1999) and women (Barrett-Connor & Goodman-Gruen, 1999), and exogenous testosterone positively affects various dimensions of cognition, including spatial and verbal memory (Postma et al., 2000; Cherrier et al., 2001).

Longer-term effects of testosterone fluctuations following a contest can be interpreted in the context of the Winner Effect: the enhanced competitiveness in future contests conferred by prior winning experiences, which is mediated by androgens in various species (Oliveira et al., 2009; Fuxjager, Montgomery, & Marler, 2011; Gleason et al., 2009). The results of the present study suggest that a similar mechanism may operate in humans. From a comparative perspective, the positive correlation that emerged between testosterone increase on Day 1 and the improvement in the competitive task on Day 2 overlaps with the testosterone-mediated increase in aggression that is observed in some rodents and fishes and leads to winning streaks (Gleason et al., 2009; Oliveira et al., 2009). In these species, because the outcome of a fight depends on the fast display of various attacks (e.g., initiating biting or chasing, Eisenberg, 1962) the Winner Effect has been often framed in terms of social aggression. However, androgens offer a biological substrate for a variety of behaviors and empirical evidence supports the idea that behaviors other than aggression might mediate this phenomenon (Gleason et al., 2009). For example, testosterone surges could reinforce

and/or stimulate learning processes associated with the contest, such as the preference for the competition location, as shown in conditioned place preference experiments (Martinez et al., 1995; Meisel & Joppa, 1994), or the behavioral strategies that led to victory (or defeat) (Marler et al., 2005). Although it is only preliminary evidence, our results are in line with these hypotheses: Net changes in testosterone experienced by our participants might have contributed to a differential learning/acquisition of the task.

In our study, the relationship between individual differences in testosterone responsiveness on Day 1 and task performance on Day 2 was remarkably similar in winners and losers. This observation may reflect differences between our study and previous reports, which found the strength of the Winner Effect seems to be proportionate to the number of wins experienced, with repeated winners (i.e. winners of at least 3 contests) becoming formidable opponents (Oyegbile & Marler, 2005). A similar situation has not yet been modeled in humans; in our study, winners experienced only one prior social victory. Follow-up research, therefore, could further address how multiple consecutive winning (or losing) experiences modulate subsequent hormone-mediated cognitive, affective, and competitive behaviors in humans. A second substantial difference concerns the types of social victory and defeat that characterize human studies compared to studies in nonhumans. In rodents and fishes social victory is achieved through displays of direct physical aggression, recording behaviors such as attack latency, freeze latency (Oyegbile & Marler, 2005) or biting (Eisenberg, 1962). Thus, social confrontations are violent, and losers often pay a substantially higher price for fighting than the winners, in terms of injuries, expenditures of energy, and social withdrawal. In primates, including humans, contests are more often ritualized and often no serious harm comes to defeated individuals (Sapolsky, 2005). This is particularly true in studies with human participants, of course, perhaps explaining why the observed longer-term effect of testosterone on task performance did not differ between winners and losers. In other words, when the cost-benefit ratio for a defeated individual is not so drastically different from a winner, then changes in testosterone might impact behavior similarly in both categories, in agreement with previous reports (Carré et al., 2013).

The second aim of the present study was to examine testosterone responsiveness to multiple competitive interactions. An interaction effect emerged between Day 1 and Day 2 competition outcomes. The average increase in Day 2

winners that had also won on Day 1 (double winners) was not different from the average increase in Day 2 winners that lost on Day 1 (lose-win); however, Day 2 losers that won on Day 1 (win-lose) experienced a statistically significant increase in testosterone compared to those individuals that lost both competitions (double losers). The steep decline in testosterone observed in double losers matches previous findings in rodents (Huhman et al., 1991) and primates (Rose, Berstein, & Gordon, 1975), including humans (Mazur et al., 1992) and can be broadly viewed as an adaptive physiological response subserving behavioral down-regulation, minimizing unnecessary losses and concomitant costs in terms of energy and injury (Lehner, Rutte, & Taborsky, 2011). On the other hand, the observed testosterone increase in Win-Lose participants is in keeping with the Challenge Hypothesis – the idea that testosterone elevation occurs in response to a challenge (i.e. losing the high-status rank obtained after the first competition) thus encouraging further attempts at regaining status (Mehta & Josephs, 2006).

Testosterone fluctuations on Day 2 were also modeled in terms of the stability of the social hierarchy that emerged as a result of the combined outcomes of the two competitions. In stable hierarchies, the social status obtained after Day 1 remains intact after Day 2; thus, participants that either won or lost both competitions –against the same opponent- would belong to this group. In contrast, a mismatch between Day 1 and Day 2 social status would indicate a certain degree of instability in the hierarchy. We found that men in unstable hierarchies (first day winners/second day losers *and* first day losers/second day winners) experienced an increase in testosterone compared to men in the stable hierarchies (double winners *and* double losers). These results are consistent with data from non-human primates (Sapolsky, 1983; Higham, Heistermann, & Maestripieri, 2013) and provide further support for the Challenge Hypothesis.

Previous studies found that rapid changes in testosterone secretion following a challenge tap into behaviors that probably conferred an adaptive advantage with respect to reproductive success in the ancestral environment. Examples thus include aggression (Carré et al., 2013), willingness to compete (Mehta & Josephs, 2006) and risk taking (Apicella et al., 2014). In our study we did not find any effect of Day 1 testosterone reactivity on a mental rotation task that participants performed a few minutes after the conclusion of the competitive interaction. One possible explanation is that spatial cognition is affected by testosterone on a different timescale than those behaviors

mentioned above. This is possible given that previous effects of exogenous testosterone on cognition (spatial memory in (Postma et al., 2000) and visuospatial ability in (Aleman, Bronk, Kessels, Koppeschaar, & van Honk, 2004) were observed after a delay of about four hours. Recent evidence suggests that testosterone, along with its androgenic and estrogenic metabolites, may exert its effect on spatial memory via down-regulation of luteinizing hormone (McConnell et al., 2012). This slow mechanism, acting through negative feedback, is more consistent with the timescale observed in “off-line” learning and therefore potentially responsible for the association we found between testosterone changes and performance improvement between sessions.

In summary, we found a relationship between individual differences in competition-induced testosterone changes and performance on the same competitive task a day later. This finding suggests that the functional significance of testosterone fluctuations in response to competitive challenges might not be restricted to behaviors immediately following a contest but could be extended to behavioral manifestations occurring on a slower timescale. Moreover, when looking at testosterone reactivity on the second day, we found that those individuals that lost both competitions experienced the steepest decline in testosterone compared to those individuals who lost on the second day but won on the first day, suggesting that intricate interconnection between complex situational factors (multiple competitive outcomes) and endogenous testosterone exit.

Chapter 5. GENERAL DISCUSSION

5.1. Summary of the Main Findings

This dissertation had the overarching goal of understanding how evolutionary relevant social contexts and stimuli impact testosterone secretion and what behavioral changes this endocrine response propels. Three specific aims were pursued:

(1) to examine whether facial display of emotions can be considered among those social behavioral systems that modulate endocrine status (Study 1);

(2) to better understand the interplay between motivational, situational and physiological factors shaping androgen profiles in competitive situations (Study 2 and Study 3); and,

(3) to investigate the short-term and longer-term functional consequences of testosterone responses to competition (Study 3).

In Study 1, I employed a between-subject design wherein participants were asked to rate emotional intensity of orthogonal facial displays (i.e. happy or angry faces) of people of either the same sex or the opposite sex. I found that, regardless of the emotional content expressed, extended and uninterrupted exposure to faces of the opposite sex compared to exposure to faces of the same sex was accompanied by an increase in salivary testosterone in both men and women. Moreover, women experienced an additional specific neuroendocrine response, with an increase in testosterone occurring only when viewing faces of angry individuals compared to happy individuals, regardless of the sex. These effects were independent of individual differences in basic motivational systems, as measured through the BIS/BAS scales, as well as mood.

In Study 2, I investigated the possible interaction between the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) axis in predicting transient changes in testosterone after social victory or defeat on a familiar competitive task; a sample of healthy young men provided saliva samples before and after competing for fifteen minutes with a peer on a widely played commercial videogame, Tetris. Unbeknownst to subjects, the videogame outcome was rigged. Subjects, who were randomly assigned to win or lose, reported their perceived locus of control about the competition and their mood. I found a significant interaction between HPG and HPA axes status and the competition effect on testosterone in the randomly assigned videogame winners, such that winners with a pre-competition combination of high baseline testosterone and low baseline cortisol exhibited significantly greater post-competition testosterone concentrations. The randomly assigned videogame losers showed significantly decreased post-competition levels of testosterone. This pattern of results bolsters the notion that the competition effect may be jointly determined by key physiological and motivational characteristics of the competitors. Specifically, the emergence of a competition effect may rely on a combination of an ecologically valid competition task that is believable and engaging (and thus amenable to subjects' attributions of locus of control) and the baseline status of both the HPA and HPG axes of the participants, acting jointly.

The aims of Study 3 were: 1) to explore how different combinations of successive wins and/or losses influence testosterone secretion in human males; and, (2) to investigate the short-term and longer-term functional consequences on cognition of testosterone responses to competition. In other words, Study 3 aimed at exploring how, in a laboratory setting, complex situational factors (i.e. repeated competitive interaction) modulated testosterone release in men; and, testing whether short-term and long-term cognitive abilities can be added among the behavior affected by socially-induced testosterone pulses. Salivary testosterone was collected from pairs of male participants engaging, on two consecutive days, in head-to-head competitions on a previously validated laboratory task (i.e., Tetris). Similarly to Study 2, I found that testosterone reactivity on the first day was congruent with the competition effect. Further, when looking at testosterone reactivity on the second day, those individuals that lost both competitions experienced the steepest decline in testosterone compared to those

individuals who lost on the second day but won on the first day. Testosterone fluctuations on the second day were also analyzed considering the type of status hierarchy (stable vs. unstable) that emerged as a result of the combined outcomes of the two competitions. In accordance with the Challenge Hypothesis, men in unstable hierarchies (first day winners/second day losers *and* first day losers/second day winners) experienced an increase in testosterone compared to men in the stable hierarchies (double winners *and* double losers). In terms of functional significance, testosterone changes on the first day did not predict short-term cognitive performance (i.e. MRT score); however, I found a relationship between individual differences in competition-induced testosterone changes on the first day and performance on the same competitive task on the second day. This finding suggests that the functional significance of testosterone fluctuations in response to competitive challenges might not be restricted to behaviors immediately following a contest but could be extended to behavioral manifestations occurring on a slower timescale.

5.2. Theoretical Implications and Future Directions

The work presented in this dissertation has important theoretical implications for the field of Social Neuroendocrinology and in particular for our understanding of the role of testosterone in shaping social and cognitive behavior.

Study 1 brings the topic of sex differences in testosterone release to the readers' attention. Although the majority of the work on the social endocrinology of testosterone is based on studies employing male participants, recent evidence indicates that context-induced changes in testosterone in women resemble, to a certain extent, what is observed in men. Studies on female athletes have shown pattern of androgenic response congruent with the "Competition Effect" (Jimenez, Aguilar, & Alvero-Cruz, 2012; Oliveira, Gouveia, & Oliveira, 2009). Similar effects have been found also in laboratory experiments, in which the absence of physical exertion allows to better isolate psychosocial variables responsible for the competition effect (Costa & Salvador, 2012; Denson, Mehta, & Ho Tan, 2012). For example, Denson and colleagues (2012) found an increment in testosterone among women who won a reaction-time task competition with a fictitious anger-provoking peer. Likewise, Costa and Salvador (2012), found evidence

in favor of the biosocial model of status in a sample of women engaging in a face-to-face competition on attention. Interestingly, comparative studies also show similarities in testosterone fluctuations (and function) in males and females of some species of birds (Wingfield et al., 2000; Moller, Garamszegi, Gil, Hurtrez-Bousses, & Eens, 2005 Zysling et al., 2006; Cain & Ketterson, 2012). Men and women also respond similarly to attractive members of the opposite sex (van der Meij, Buunk, van de Sande, & Salvador, 2008; Lòpez et al., 2009), another scenario implicated in mating effort and therefore associated with an androgenic response (Archer, 2006).

In line with the idea that the Challenge Hypothesis -although mainly concerned with males- can be extended to females, especially in the case of less sexually-dimorphic species (Ketterson, Nolan, & Sandell, 2005). Study 1 provides preliminary evidence for the suggestion that testosterone dynamics are similar in men and women. However, alternative explanations might account for some of the phenomena observed. In Study 1, I found an increase in testosterone in men and women exposed to faces of the opposite sex, but –likely due to limitations of statistical power- I could not precisely identify how emotions modulates this physiological response. One possibility is that the rise in testosterone observed in men watching faces of women, regardless of their emotional expression, is mainly driven by the increase in testosterone observed in those men exposed to happy females. On the other hand, the opposite could be true in women: The significant increase in testosterone in women exposed to male faces could have been driven by the testosterone response of those women exposed to angry men. Evolutionary perspectives on human sexuality (Trivers, 1972; Buss & Schmitt, 1993) combined with the testosterone-arousal link in men (Stoleru, Ennaji, Cournot, & Spira, 1993; Alexander et al., 1997) may provide some basis for this speculation. According to the parental investment theory (Trivers, 1972), gender differences in sexual motivation and behavior, among which reproductive strategies (Buss & Schmitt, 1993), are determined by biological differences between females and males in the amount of resources invested in the offspring. Because mammalian females produce fewer and larger gametes than males and are responsible for gestation and lactation, they are typically the higher investing sex (high investment in fewer offspring) and exert more sexual selection pressure on males in order to obtain optimal genes, along with protection and resources for the offspring. Conversely, mammalian males with their

cheap gametes are generally predicted to seek maximal mating opportunities and minimize parental investment (thereby making a lower investment in many offspring). Although the effects are often subtle, experimental and cross-cultural evidence is generally consistent with the sex differences in mate preference predicted by this theoretical perspective (Buss & Schmitt, 1993). It is possible that similar sex differences exist with respect to sexual arousal, with men being more easily and indiscriminately sexually aroused than females (Knoth, Boyd, & Singer, 1988), in keeping with a less-selective reproductive strategy. Although we did not measure arousal directly, perhaps testosterone changes were influenced by changes in arousal state (Alexander et al., 1997) and possibly mediated by luteinizing hormone (LH) secretion (Stoleru et al., 1993).

The testosterone-arousal hypothesis might also explain why the same androgenic response might be of smaller magnitude and more likely to be influenced by circumstantial conditions (e.g., menstrual cycle phase). As a result of their higher parental investment, it may be of adaptive benefit for females to be sexually aroused in more selective contexts, such as in the context of a romantic relationship where parental investment is shared with a partner (Knoth et al., 1988). Possibly in line with this account is the observation that the testosterone response in women exposed to sexual and emotional stimuli seems to be more selective (Lòpez, Hay, & Conklin, 2009; Goldey & van Anders, 2011). For example, while investigating cognitive arousal, Goldey and van Anders (2011) found that testosterone increased in those women who imagined a *self-defined* enjoyable sexual encounter with an attractive man. In other words, women increased in testosterone when they were free to imagine the type of person and situation most attractive to them. Similarly, Lopez and collaborators (2009) found that watching a video about a courtship interaction between a highly attractive man and a young woman was necessary to cause testosterone accumulation in her female sample. Interestingly, in both cases, the results were restricted to naturally cycling women. Therefore, happy male faces, which women consider as least attractive when compared to other men's FDEs (Tracy & Beall, 2011), might not be adequate to trigger hormonal responses. This explanation potentially overlaps with research investigating sex differences in neural, cognitive and autonomic responses to positive emotional cues, including FDEs and erotica (Bradley, Codispoti, Cuthbert, & Lang, 2001; Hamann, Herman, Nolan, & Wallen, 2004; Alexander & Charles, 2009).

In summary, Study 1 provides initial evidence for the notion that testosterone dynamics are similar in men and women, but also hints at the possibility that testosterone release might be more prominent in women exposed to threatening stimuli. Although preliminary, these data support both the hypothesis that testosterone dynamics in females represents correlated responses to selection on male (Ketterson et al., 2005) and the hypothesis that testosterone dynamics are a product of selection acting directly on the female phenotype (Ketterson et al., 2005).

The main theoretical implication of Study 2 centres on the moderating role played by cortisol in understanding the androgenic response to competition. Adding the cross-talk between the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes to the list of physiological factors that influence socially-induced testosterone pulses helps shed light on the individual differences ultimately observed in behaviors. In fact, growing evidence supports the idea that testosterone-related behaviors, such as status seeking and aggression, are better explained by considering the interaction between cortisol and testosterone than by evaluating testosterone fluctuations in isolation. Early reports (Dabbs, Jurkovic, & Frady, 1991; Popma et al., 2007; Mehta & Josephs, 2010) showed that testosterone concentrations at rest were positively correlated with dominant behaviors only among individuals with low baseline cortisol, while the same relationship was either reversed (see Study 2; Mehta & Josephs, 2010) or absent among individuals with high cortisol concentrations (Popma et al., 2007). This is in line with the idea that environmental stress – as partially reflected by cortisol concentrations- would buffer or even halt the effect of testosterone on direct (i.e. courtship behavior) and indirect (i.e. competition for mates) reproductive behaviors (Viau, 2002). Later studies supported this interpretation (Pfattheicher, Landhauber, & Keller, 2013), but see (Mazur & Booth, 2014), but also extended it by showing how the testosterone-cortisol interaction might take different forms depending on the specificity of the behavior and context considered (Geniole, Carré, & McCormick, 2011; Denson et al., 2012; Welker, Lozoya, Campbell, Neumann, & Carrè, 2014). For example, Geniole et al. (2011) found that socially excluded men with both high basal testosterone and high basal cortisol showed the highest levels of reactive aggression. A similar finding is reported by Welker et al. (2014), who found that, although both testosterone and cortisol were positively correlated with psychopathic traits in a non-clinical sample of young men,

cortisol moderated the relationship between testosterone and psychopathy in men, such that high-testosterone and high-cortisol men reported the highest levels of psychopathy compared to the high-testosterone low cortisol men. Regardless of the form taken by this interaction, adding cortisol to the list of physiological modulators of testosterone release represents an important step towards a better understand of how androgens ultimately shape social behavior.

Within the same framework, future studies would benefit from investigating the moderating role of other homeostatic/allostatic indicators (e.g., pathogen load or fasting), so as to expand the range of biological factors able to buffer (or enhance) androgen release. For example, it has been showed that receiving influenza vaccination (Simmons & Roney, 2009), illness (Muehlenbein, Hirschtick, Bonner, & Swartz, 2010) and fasting (Trumble, Brindle, Kupsik, & O'Connor, 2010) all lower basal levels of testosterone; however, no studies have investigated how these factors influence not only basal testosterone but also androgen reflexes. It can be hypothesized that individuals with an immune system occupied in fighting off sickness might not be able to mount the same androgen response observed in those individuals that are not undergoing through any pathogen stress. From an evolutionary perspective we can think of this interaction as an advantage for acquiring valued resources and preserving stable social groups. Acquisition and maintenance of high hierarchy position require the ability to sustain appropriate aggressive responses during and after competitive encounters, which is regulated by transient increases in testosterone (Wingfield et al., 1987). Individuals with a particularly active immune system would be temporarily inhibited with regard to metabolically costly and potentially dangerous dominance challenges (even if they had high baseline testosterone).

If Study 2 demonstrates that the effect of winning and losing a competition on testosterone is moderated by the individual physiological state, Study 3 shows how situational factors (i.e. the stability of the social hierarchy that emerges from repeated dominance challenges) should be considered. In line with studies on other primates (Higham, Heistermann, & Maestripieri, 2013), men in unstable hierarchies experienced an increase in testosterone compared to men in the stable hierarchies. It is common in human social neuroendocrinology to think of the BMS, which posits a differential androgenic response between winners and losers, and the Challenge Hypothesis, which

posits an increase in testosterone in response to competition regardless of its outcome, as two opposing theoretical frameworks. It might be possible to reconcile these two perspectives within a common model among psychologists, the biopsychosocial model of challenge and threat, which addresses individuals' psychological and physiological responses to active tasks (Blascovich & Tomaka, 1996).

An active task is a an uncertain, and potentially stressful/ threatening situation characterized by a performance instrumental to reach self-relevant goals such as a face-to-face competition (Blascovich & Mendes, 2000). According to the biopsychosocial model, subjects who appraise task demands (e.g. in terms of required effort, danger, or uncertainty) as exceeding their personal resources (e.g., their disposition, external support, skills) will feel threatened, whereas subjects who evaluate their resources as meeting or exceeding demands will interpret the task as a challenge. Although the model posits that threat/challenge appraisals occur before an active task, it also possible that similar evaluative mechanisms would continue even after the end of the active task (Oliveira & Oliveira, 2014).

In Study 3, I observed a net testosterone increase in winners of the first day. Testosterone accumulation was also observed in Day 2 winners who lost on the first day. In parallel, testosterone declined in losers of the first day but increased in losers of the second day who had won on the first day. One could speculate that the BMS might be a more suitable explanation for those challenges that see two unfamiliar individuals facing each other for the first time. In this context, winning might be associated with higher pleasantness and confidence and, although both men might feel the challenge at the beginning of the competition, they might have different appraisals of the situation once the competition is over (challenge vs. threat). On other hand, the Challenge Hypothesis might become a more likely explanation in those situations, likely after few antagonistic encounters, where both opponents feel that a dominance hierarchy has not been clearly established and both interpret the situation as a challenge (vs. threat). In brief, if corroborated the finding of Study 3 might be help resolve this apparent contrast between two alternative distinct -but complementary- theoretical perspectives.

Study 3 also investigated the functional significance of challenge-induced testosterone release, finding evidence for long-term effects. This novel finding not only

fits with the animal literature on the Winner Effect (Gleason, Fuxjager, Oyegbile, & Marler, 2009), but also corroborates recent experimental evidence on the effect of testosterone administration on between-days learning (Wright, Edwards, Fleming, & Dolan, 2012). It also expands, the handful of human studies that show how acute changes in testosterone map onto behaviors occurring immediately after the contest. These behaviors range from learning (Schultheiss & Rohde, 2002; Schultheiss et al., 2005) to social behaviors, including competitiveness (Mehta & Josephs, 2006; Carré & McCormick, 2008), risk-taking (Apicella, Dreber, & Mollerstrom, 2014), aggression (Carré, Putnam, & McCormick, 2009; Carré, Campbell, Lozoya, Goetz, & Welker, 2013; Carré, Iselin, Welker, Hariri, & Dodge, 2014) and courtship behavior (van der Meij, Almela, Buunk, Fawcett, & Salvador, 2012). For example, pioneering work from Schultheiss and colleagues demonstrated that victory-induced increases in testosterone positively correlated with implicit learning of a visual-motor sequence that was embedded in the competitive task, whereas defeat-induced decreases in testosterone predict impaired implicit learning of the same sequence (Schultheiss & Rohde, 2002; Schultheiss et al., 2005). The authors proposed that testosterone pulses are involved in modulating learning of those behaviors that lead to winning dominance contests -an explanation that fits perfectly with evidence from research with lab animals (Gleason et al., 2009). Building on the contributions of Schultheiss et al., researchers have investigated social behaviors that might be affected by testosterone changes. Two studies (Mehta & Josephs, 2006; Carré & McCormick, 2008) found that contest-induced increases in testosterone predicted willingness to engage in another contest, whereas decreases in testosterone predicted men's behavioral withdrawal from dominance situations. Along the same lines, testosterone increase has been repeatedly associated with reactive aggression (Carré et al., 2009; Carré et al., 2013; Carré et al., 2014). Lastly, two recent reports introduced the idea that natural fluctuation in testosterone might also map onto inclination towards risky behavior (Apicella et al., 2014) as well as courtship behaviors (van der Meij et al., 2012). For instance, Apicella and colleagues found that, regardless of the outcome of the competition, men who experienced an increase in testosterone were more likely to take risks, as measured via an economics decision-making task (Apicella et al., 2014). Van der Meij and colleagues, instead, found that, when given the opportunity to interact with a woman, those men who had experienced a greater testosterone increase after a competition engaged in more self-

presentation, smiled more and made more eye contact with the member of the opposite sex, supporting the idea that testosterone might facilitate direct access to mates (van der Meij et al., 2012).

In conclusion, all these findings clearly support the hypothesis, first presented in the introduction, that testosterone secretion sustains and promotes behaviors associated with mating effort. However, whether these socially-induced testosterone fluctuations promote mating effort at the expense of parental behavior remains unexplored. Future research is needed to shed light on this missing piece of the puzzle.

References

Ackermann, S., Spalek, K., Rasch, B. r., Gschwind, L., Coynel, D., Fastenrath, M., . . . de Quervain, D. J.-F. (2012). Testosterone levels in healthy men are related to amygdala reactivity and memory performance. *Psychoneuroendocrinology, 37*(9), 1417-1424.

Aiken, L. S., & West, S. G. (1991). *Multiple regression: Testing and interpreting interactions*. Thousand Oaks, CA US: Sage Publications, Inc.

Alatalo, R. V., Hoglund, J., Lundberg, A., Rintamaki, P. T., & Silverin, B. (1996). Testosterone and male mating success on the black grouse leks. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 263*(1377), 1697-1702.

Aleman, A., Bronk, E., Kessels, R. P., Koppeschaar, H. P., & van Honk, J. (2004). A single administration of testosterone improves visuospatial ability in young women. *Psychoneuroendocrinology, 29*(5), 612-617.

Alexander, G. M., & Charles, N. (2009). Sex Differences in Adults' Relative Visual Interest in Female and Male Faces, Toys, and Play Styles. *Archives of Sexual Behavior, 38*(3), 434-441. doi: 10.1007/s10508-008-9429-7

Alexander, G. M., Swerdloff, R. S., Wang, C., Davidson, T., McDonald, V., Steiner, B., & Hines, M. (1997). Androgen-behavior correlations in hypogonadal men and eugonadal men .1. Mood and response to auditory sexual stimuli. *Hormones and Behavior, 31*(2), 110-119. doi: 10.1006/hbeh.1997.1372

Alexander, R. D., & Culligan, K. G. (1979). *Darwinism and human affairs* (Vol. 39): University of Washington Press Seattle.

Altmann, J., Sapolsky, R., & Licht, P. (1995). Baboon fertility and social status. *Nature, 377*(6551), 688-689.

Alvergne, A., Faurie, C., & Raymond, M. (2009). Variation in testosterone levels and male reproductive effort: insight from a polygynous human population. *Hormones and Behavior, 56*(5), 491-497.

Amstislavskaya, T. G., & Popova, N. K. (2004). Female-induced sexual arousal in male mice and rats: behavioral and testosterone response. *Hormones and Behavior, 46*(5), 544-550.

Andersson, M. B. (1994). *Sexual selection*: Princeton University Press.

Andrew, R., & Rogers, L. (1972). Testosterone, search behaviour and persistence. *Nature, 237*, 343-345.

Apicella, C. L., Dreber, A., & Mollerstrom, J. (2014). Salivary testosterone change following monetary wins and losses predicts future financial risk-taking. *Psychoneuroendocrinology, 39*, 58-64.

Archer, J. (1991). The influence of testosterone on human aggression. *British Journal of Psychology, 82*(1), 1-28.

Archer, J. (2006). Testosterone and human aggression: an evaluation of the challenge hypothesis. [Proceedings Paper]. *Neuroscience and Biobehavioral Reviews, 30*(3), 319-345. doi: 10.1016/j.neubiorev.2004.12.007

Barinaga, M. (1996). Neurobiology - Social status sculpts activity of crayfish neurons. *Science, 271*(5247), 290-291.

Barrett-Connor, E., & Goodman-Gruen, D. (1999). Cognitive function and endogenous sex hormones in older women. *Journal of the American Geriatrics Society, 47*(11), 1289-1293.

Barrett-Connor, E., Goodman-Gruen, D., & Patay, B. (1999). Endogenous sex hormones and cognitive function in older men. *Journal of Clinical Endocrinology & Metabolism, 84*(10), 3681-3685.

Bateup, H. S., Booth, A., Shirtcliff, E. A., & Granger, D. A. (2002). Testosterone, cortisol, and women's competition. *Evolution and Human Behavior, 23*(3), 181-192. doi: 10.1016/s1090-5138(01)00100-3

Becker, D. V., Anderson, U. S., Mortensen, C. R., Neufeld, S. L., & Neel, R. (2011). The Face in the Crowd Effect Unconfounded: Happy Faces, Not Angry Faces, Are More Efficiently Detected in Single- and Multiple-Target Visual Search Tasks. *Journal of Experimental Psychology-General, 140*(4), 637-659. doi: 10.1037/a0024060

Becker, D. V., Kenrick, D. T., Neuberg, S. L., Blackwell, K. C., & Smith, D. M. (2007). The confounded nature of angry men and happy women. *Journal of Personality and Social Psychology, 92*(2), 179-190. doi: 10.1037/0022-3514.92.2.179

Bernhardt, P. C., Dabbs, J. M., Fielden, J. A., & Lutter, C. D. (1998). Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. *Physiology & Behavior, 65*(1), 59-62.

Bernstein, I. S., Rose, R. M., & Gordon, T. P. (1974). Behavioral and environmental events influencing primate testosterone levels. [Article]. *Journal of Human Evolution, 3*(6), 517-525.

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. *Psychoneuroendocrinology, 20*(2), 117-134.

Blascovich, J., & Mendes, W. B. (2000). Challenge and threat appraisals: The role of affective cues. In J. Forgas (Ed.), *Feeling and Thinking: The role of affect in social cognition* (pp. 59-82). Paris: Cambridge University Press.

Blascovich, J., & Tomaka, J. (1996). The biopsychosocial model of arousal regulation. *Advances in Experimental Social Psychology, 28*, 1-51.

Bonilla-Jaime, H., Vazquez-Palacios, G., Arteaga-Silva, M., & Retana-Marquez, S. (2006). Hormonal responses to different sexually related conditions in male rats. *Hormones and Behavior, 49*(3), 376-382. doi: 10.1016/j.yhbeh.2005.08.005

Booth, A., & Dabbs, J. M. (1993). Testosterone and men's marriages. *Social Forces, 72*(2), 463-477.

Booth, A., Granger, D. A., Mazur, A., & Kivlighan, K. T. (2006). Testosterone and social behavior. *Social Forces, 85*(1), 167-191.

Borg, K. E., Esbenshade, K. L., Johnson, B. H., Lunstra, D. D., & Ford, J. J. (1992). Effects of sexual experience, season, and mating stimuli on endocrine concentrations in the adult ram. *Hormones and Behavior, 26*(1), 87-109. doi: 10.1016/0018-506x(92)90034-s

Bos, P. A., Hermans, E. J., Montoya, E. R., Ramsey, N. F., & van Honk, J. (2010). Testosterone administration modulates neural responses to crying infants in young females. *Psychoneuroendocrinology, 35*(1), 114-121. doi: 10.1016/j.psyneuen.2009.09.013

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and Motivation I: Defensive and Appetitive Reactions in Picture Processing. *Emotion, 1*(3), 276-298. doi: 10.1037//1528-3542.1.3.276

Bradley, M. M., Codispoti, M., Sabatinelli, D., & Lang, P. J. (2001). Emotion and Motivation II: Sex Differences in Picture Processing. *Emotion, 1*(3), 300-319. doi: 10.1037//1528-3542.1.3.300

Bremner, W. J., Vitiello, M. V., & Prinz, P. N. (1983). Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *Journal of Clinical Endocrinology & Metabolism, 56*(6), 1278-1281.

Bronson, F. H., Stetson, M. H., & Stiff, M. E. (1973). Serum FSH and LH in male mice following aggressive and nonaggressive interaction. [Article]. *Physiology & Behavior, 10*(2), 369-372. doi: 10.1016/0031-9384(73)90324-7

Brown, C. R., Brown, M. B., Raouf, S. A., Smith, L. C., & Wingfield, J. C. (2005). Effects of endogenous steroid hormone levels on annual survival in cliff swallows. *Ecology, 86*(4), 1034-1046.

Burger, H. G. (2002). Androgen production in women. *Fertility and sterility, 77*, 3-5.

Burgoon, J. K., Johnson, M. L., & Koch, P. T. (1998). The nature and measurement of interpersonal dominance. *Communication Monographs, 65*(4), 308-335.

Burnstein, K. L., Maiorino, C. A., Dai, J. L., & Cameron, D. J. (1995). Androgen and glucocorticoid regulation of androgen receptor cDNA expression. [Article]. *Molecular and Cellular Endocrinology, 115*(2), 177-186.

Buss, D. M., & Schmitt, D. P. (1993). Sexual strategies theory - An evolutionary perspective on human mating. *Psychological Review, 100*(2), 204-232. doi: 10.1037/0033-295x.100.2.204

Cain, K. E., & Ketterson, E. D. (2012). Competitive females are successful females; phenotype, mechanism, and selection in a common songbird. *Behavioral Ecology and Sociobiology, 66*(2), 241-252.

Campbell, I. T., Walker, R. F., Riadfahmy, D., Wilson, D. W., & Griffiths, K. (1982). Circadian-rhythms of testosterone and cortisol in saliva - effects of activity-phase shifts and continuous daylight. *Chronobiologia, 9*(4), 389-396.

Carani, C., Bancroft, J., Del Rio, G., Granata, A., Facchinetti, F., & Marrama, P. (1990). The endocrine effects of visual erotic stimuli in normal men. *Psychoneuroendocrinology, 15*(3), 207-216.

Carré, J. M., Campbell, J. A., Lozoya, E., Goetz, S. M., & Welker, K. M. (2013). Changes in testosterone mediate the effect of winning on subsequent aggressive behaviour. *Psychoneuroendocrinology, 38*(10), 2034-2041.

Carré, J. M., Iselin, A.-M. R., Welker, K. M., Hariri, A. R., & Dodge, K. A. (2014). Testosterone Reactivity to Provocation Mediates the Effect of Early Intervention on Aggressive Behavior. *Psychological Science*, 0956797614525642.

Carré, J. M., & McCormick, C. M. (2008). Aggressive behavior and change in salivary testosterone concentrations predict willingness to engage in a competitive task. *Hormones and Behavior, 54*(3), 403-409. doi: 10.1016/j.yhbeh.2008.04.008

Carré, J. M., & Putnam, S. K. (2010). Watching a previous victory produces an increase in testosterone among elite hockey players. *Psychoneuroendocrinology, 35*(3), 475-479.

Carré, J. M., Putnam, S. K., & McCormick, C. M. (2009). Testosterone responses to competition predict future aggressive behaviour at a cost to reward in men. [Article]. *Psychoneuroendocrinology, 34*(4), 561-570. doi: 10.1016/j.psyneuen.2008.10.018

Caruso, J. F., Lutz, B. M., Davidson, M. E., Wilson, K., Crane, C. S., Craig, C. E., . . . Sheaff, R. J. (2012). Salivary hormonal values from high-speed resistive exercise workouts. *The Journal of Strength & Conditioning Research, 26*(3), 625-632.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*(2), 319-333. doi: 10.1037//0022-3514.67.2.319

Cerda-Molina, A. L., Hernandez-Lopez, L., Chavira, R., Cardenas, M., Paez-Ponce, D., Cervantes-De la Luz, H., & Mondragon-Ceballos, R. (2006). Endocrine changes in male stumptailed macaques (*Macaca arctoides*) as a response to odor stimulation with vaginal secretions. *Hormones and Behavior, 49*(1), 81-87.

Cerda-Molina, A. L., Hernandez-Lopez, L., Claudio, E., Chavira-Ramirez, R., & Mondragon-Ceballos, R. (2013). Changes in men's salivary testosterone and cortisol levels, and in sexual desire after smelling female axillary and vulvar scents. *Frontiers in endocrinology, 4*.

Chase, I. D., Tovey, C., Spangler-Martin, D., & Manfredonia, M. (2002). Individual differences versus social dynamics in the formation of animal dominance hierarchies. [Article]. *Proceedings of the National Academy of Sciences of the United States of America, 99*(8), 5744-5749.

Chen, S. Y., Wang, J., Yu, G. Q., Liu, W. H., & Pearce, D. (1997). Androgen and glucocorticoid receptor heterodimer formation - A possible mechanism for mutual inhibition of transcriptional activity. [Article]. *Journal of Biological Chemistry, 272*(22), 14087-14092.

Cherrier, M., Asthana, S., Plymate, S., Baker, L., Matsumoto, A., Peskind, E., . . . Petrova, A. (2001). Testosterone supplementation improves spatial and verbal memory in healthy older men. *Neurology, 57*(1), 80-88.

Chichinadze, K., & Chichinadze, N. (2008). Stress-induced increase of testosterone: contributions of social status and sympathetic reactivity. *Physiology & Behavior, 94*(4), 595-603.

Ciechanowska, M., Lapot, M., Mateusiak, K., & Przekop, F. (2010). Neuroendocrine regulation of GnRH release and expression of GnRH and GnRH receptor genes in the hypothalamus-pituitary unit in different physiological states. *Reproductive Biology, 10*(2), 85-124.

Clancy, A., Singer, A., Macrides, F., Bronson, F., & Agosta, W. (1988). Experiential and endocrine dependence of gonadotropin responses in male mice to conspecific urine. *Biology of Reproduction, 38*(1), 183-191.

Clark, M. M., & Galef Jr, B. G. (1999). A testosterone-mediated trade-off between parental and sexual effort in male Mongolian gerbils (*Meriones unguiculatus*). *Journal of Comparative Psychology, 113*(4), 388.

Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2003). *Applied multiple regression/correlation analysis for the behavioral sciences (3rd ed.)*. Mahwah, NJ US: Lawrence Erlbaum Associates Publishers.

Coquelin, A., & Bronson, F. (1979). Release of luteinizing hormone in male mice during exposure to females: habituation of the response. *Science, 206*(4422), 1099-1101.

Coquelin, A., & Bronson, F. (1980). Secretion of Luteinizing Hormone in Male Mice: Factors that Influence Release during Sexual Encounters*. *Endocrinology, 106*(4), 1224-1229.

Coquelin, A., & Desjardins, C. (1982). Luteinizing hormone and testosterone secretion in young and old male mice. *American Journal of Physiology-Endocrinology And Metabolism, 243*(3), E257-E263.

Costa, R., & Salvador, A. (2012). Associations between success and failure in a face-to-face competition and psychobiological parameters in young women. *Psychoneuroendocrinology, 37*(11), 1780-1790.

Crawford, B. A. L., Liu, P. Y., Kean, M. T., Bleasel, J. F., & Handelsman, D. J. (2003). Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. [Article]. *Journal of Clinical Endocrinology & Metabolism, 88*(7), 3167-3176. doi: 10.1210/jc.2002-021827

Crewther, B. T., Sanctuary, C. E., Kilduff, L. P., Carruthers, J. S., Gaviglio, C. M., & Cook, C. J. (2013). The workout responses of salivary-free testosterone and cortisol concentrations and their association with the subsequent competition outcomes in professional rugby league. *The Journal of Strength & Conditioning Research, 27*(2), 471-476.

Cronbach, L. J. (1970). How we should measure change – or should we. [Article]. *Psychological Bulletin, 74*(1), 68-80.

Dabbs, J. M. (1990). Salivary testosterone measurements - reliability across hours, days, and weeks. *Physiology & Behavior, 48*(1), 83-86.

Dabbs, J. M., Jurkovic, G. J., & Frady, R. L. (1991). Salivary testosterone and cortisol among late adolescent male-offenders. *Journal of Abnormal Child Psychology, 19*(4), 469-478.

Dabbs, J. M., & Mohammed, S. (1992). Male and female salivary testosterone concentrations before and after sexual activity. *Physiology & Behavior, 52*(1), 195-197.

Dabbs Jr, J. M., & Mohammed, S. (1992). Male and female salivary testosterone concentrations before and after sexual activity. *Physiology & Behavior, 52*(1), 195-197.

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience & Biobehavioral Reviews, 35*(7), 1562-1592.

Denson, T. F., Mehta, P. H., & Ho Tan, D. (2012). Endogenous testosterone and cortisol jointly influence reactive aggression in women. *Psychoneuroendocrinology*.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. [Review]. *Psychological Bulletin, 130*(3), 355-391. doi: 10.1037/0033-2090.130.3.355

Dimberg, U., & Öhman, A. (1996). Behold the wrath: Psychophysiological responses to facial stimuli. *Motivation and Emotion, 20*(2), 149-182. doi: 10.1007/bf02253869

Dixson, A. F. (1980). Androgens and Aggressive Behavior in Primates: A Review. [Review]. *Aggressive Behavior, 6*(1), 37-67.

Dufty, A. M., Clobert, J., & Moller, A. P. (2002). Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution, 17*(4), 190-196.

Edinger, K. L., & Frye, C. A. (2007). Androgens' effects to enhance learning may be mediated in part through actions at estrogen receptor-β in the hippocampus. *Neurobiology of learning and memory, 87*(1), 78-85.

Edwards, D. A., & Kurlander, L. S. (2010). Women's intercollegiate volleyball and tennis: Effects of warm-up, competition, and practice on saliva levels of cortisol and testosterone. *Hormones and Behavior, 58*(4), 606-613.

Edwards, D. A., Wetzel, K., & Wyner, D. R. (2006). Intercollegiate soccer: Saliva cortisol and testosterone are elevated during competition, and testosterone is related to status and social connectedness with teammates. [Article]. *Physiology & Behavior, 87*(1), 135-143. doi: 10.1016/j.physbeh.2005.09.007

Eisenberg, J. F. (1962). Studies on the behavior of *Peromyscus maniculatus gambelii* and *Peromyscus californicus parasiticus*. *Behaviour*, 177-207.

Ekman, P., & Friesen, W. V. (1971). Constants across cultures in face and emotion. *Journal of Personality and Social Psychology, 17*(2), 124-129. doi: 10.1037/h0030377

Elefther, B. E., & Church, R. L. (1967). Effects of repeated exposure to aggression and defeat on plasma and pituitary levels of luteinizing hormone in C57BL/6J mice. *General and Comparative Endocrinology, 9*(2), 263-266. doi: 10.1016/0016-6480(67)90060-3

Elefther, B. E., & Church, R. L. (1968). Levels of hypothalamic luteinizing hormone-releasing factor after exposure to aggression (defeat) in C57BL/6J mice. [Note]. *Journal of Endocrinology, 42*(2), 347-348. doi: 10.1677/joe.0.0420347

Ellis, L. (1995). Dominance and reproductive success among nonhuman animals - a cross-species comparison. *Ethology and Sociobiology, 16*(4), 257-333.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*(4300), 215-223.

Ethofer, T., Wiethoff, S., Anders, S., Kreifelts, B., Grodd, W., & Wildgruber, D. (2007). The voices of seduction: cross-gender effects in processing of erotic prosody. *Social cognitive and affective neuroscience, 2*(4), 334-337.

Evans, M. R., Goldsmith, A. R., & Norris, S. R. (2000). The effects of testosterone on antibody production and plumage coloration in male house sparrows (Passer domesticus). *Behavioral Ecology and Sociobiology, 47*(3), 156-163.

Exton, M. S., Bindert, A., Kr√¯ºger, T., Scheller, F., Hartmann, U., & Schedlowski, M. (1999). Cardiovascular and endocrine alterations after masturbation-induced orgasm in women. *Psychosomatic Medicine, 61*(3), 280-289.

Fahey, C. P. (2003). Tetris AI. http://www.colinfahey.com.

Filaire, E., Maso, F., Sagnol, M., Ferrand, C., & Lac, G. (2001). Anxiety, hormonal responses, and coping during a judo competition. *Aggressive Behavior, 27*(1), 55-63.

Fine, J. G., Semrud-Clikeman, M., & Zhu, D. C. (2009). Gender differences in BOLD activation to face photographs and video vignettes. *Behavioural Brain Research, 201*(1), 137-146.

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *American Naturalist*, 603-622.

Foradori, C., Weiser, M., & Handa, R. (2008). Non-genomic actions of androgens. *Frontiers in Neuroendocrinology, 29*(2), 169-181.

Foss, J. (1998). Testosterone and the second sex. [Editorial Material]. *Behavioral and Brain Sciences, 21*(3), 374-375. doi: 10.1017/s0140525x98371228

Fox, C., Ismail, A., Love, D., Kirkham, K., & Loraine, J. (1972). Studies on the relationship between plasma testosterone levels and human sexual activity. *Journal of Endocrinology, 52*(1), 51-58.

Frye, C. A. (2007). Some rewarding effects of androgens may be mediated by actions of its 5α-reduced metabolite 3α-androstanediol. *Pharmacology Biochemistry and Behavior, 86*(2), 354-367.

Furman, D., Hejblum, B. P., Simon, N., Jojic, V., Dekker, C. L., Thi√¯©baut, R., . . . Davis, M. M. (2014). Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proceedings of the National Academy of Sciences, 111*(2), 869-874.

Fuxjager, M. J., Forbes-Lorman, R. M., Coss, D. J., Auger, C. J., Auger, A. P., & Marler, C. A. (2010). Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression. *Proceedings of the National Academy of Sciences, 107*(27), 12393-12398. doi: 10.1073/pnas.1001394107

Fuxjager, M. J., & Marler, C. A. (2010). How and why the winner effect forms: influences of contest environment and species differences. *Behavioral Ecology, 21*(1), 37-45.

Fuxjager, M. J., Mast, G., Becker, E. A., & Marler, C. A. (2009). The "home advantage" necessary for a full winner effect and changes in post-encounter testosterone. *Hormones and Behavior, 56*(2), 214-219.

Fuxjager, M. J., Montgomery, J. L., & Marler, C. A. (2011). Species differences in the winner effect disappear in response to post-victory testosterone manipulations. *Proceedings of the Royal Society B: Biological Sciences, 278*(1724), 3497-3503.

Fuxjager, M. J., Oyegbile, T. O., & Marler, C. A. (2011). Independent and additive contributions of postvictory testosterone and social experience to the development of the winner effect. *Endocrinology, 152*(9), 3422-3429.

Gaulin, S. J., & FitzGerald, R. W. (1986). Sex differences in spatial ability: an evolutionary hypothesis and test. *American Naturalist*, 74-88.

Geniole, S. N., Carré, J. M., & McCormick, C. M. (2011). State, not trait, neuroendocrine function predicts costly reactive aggression in men after social exclusion and inclusion. [Article]. *Biological Psychology, 87*(1), 137-145. doi: 10.1016/j.biopsycho.2011.02.020

Gettler, L. T., McDade, T. W., Feranil, A. B., & Kuzawa, C. W. (2011). Longitudinal evidence that fatherhood decreases testosterone in human males. *Proceedings of the National Academy of Sciences, 108*(39), 16194-16199.

Girard-Buttoz, C., Heistermann, M., Krummel, S., & Engelhardt, A. (2009). Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*). *Physiology & Behavior, 98*(1), 168-175.

Gizewski, E. R., Krause, E., Schlamann, M., Happich, F., Ladd, M. E., Forsting, M., & Senf, W. (2009). Specific cerebral activation due to visual erotic stimuli in male-to-female transsexuals compared with male and female controls: an fMRI study. *The Journal of Sexual Medicine, 6*(2), 440-448.

Gladue, B. A., Boechler, M., & McCaul, K. D. (1989). Hormonal response to competition in human males. [Article]. *Aggressive Behavior, 15*(6), 409-422.

Gleason, E. D., Fuxjager, M. J., Oyegbile, T. O., & Marler, C. A. (2009). Testosterone release and social context: when it occurs and why. *Frontiers in Neuroendocrinology, 30*(4), 460-469.

Gleason, E. D., & Marler, C. A. (2010). Testosterone response to courtship predicts future paternal behavior in the California mouse, *Peromyscus californicus*. *Hormones and Behavior, 57*(2), 147-154.

Goldey, K. L., & van Anders, S. M. (2011). Sexy thoughts: Effects of sexual cognitions on testosterone, cortisol, and arousal in women. *Hormones and Behavior, 59*(5), 754-764. doi: 10.1016/j.yhbeh.2010.12.005

Goldey, K. L., & van Anders, S. M. (2012). Sexual thoughts: Links to testosterone and cortisol in men. *Archives of Sexual Behavior, 41*(6), 1461-1470.

Gonzalez-Bono, E., Salvador, A., Ricarte, J., Serrano, M. A., & Arnedo, M. (2000). Testosterone and attribution of successful competition. *Aggressive Behavior, 26*(3), 235-240.

Gonzalez-Bono, E., Salvador, A., Serrano, M. A., & Ricarte, J. (1999). Testosterone, cortisol, and mood in a sports team competition. *Hormones and Behavior, 35*(1), 55-62.

Goos, L. M., & Silverman, I. (2002). Sex related factors in the perception of threatening facial expressions. *Journal of Nonverbal Behavior, 26*(1), 27-41. doi: 10.1023/a:1014418503754

Gouchie, C., & Kimura, D. (1991). The relationship between testosterone levels and cognitive ability patterns. *Psychoneuroendocrinology, 16*(4), 323-334.

Gould, L., & Ziegler, T. (2007). Variation in fecal testosterone levels, inter-male aggression, dominance rank and age during mating and post-mating periods in wild adult male ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology, 69*(12), 1325-1339.

Gray, P. B., Kahlenberg, S. M., Barrett, E. S., Lipson, S. F., & Ellison, P. T. (2002). Marriage and fatherhood are associated with lower testosterone in males. *Evolution and Human Behavior, 23*(3), 193-201.

Greenberg, N., & Crews, D. (1990). Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *General and Comparative Endocrinology, 77*(2), 246-255.

Gyger, M., Karakashian, S. J., Dufty Jr, A. M., & Marler, P. (1988). Alarm signals in birds: the role of testosterone. *Hormones and Behavior, 22*(3), 305-314.

Hamann, S., Herman, R. A., Nolan, C. L., & Wallen, K. (2004). Men and women differ in amygdala response to visual sexual stimuli. *Nature Neuroscience, 7*(4), 411-416. doi: 10.1038/nn1208

Hamilton, L. D., van Anders, S. M., Cox, D. N., & Watson, N. V. (2009). The Effect of Competition on Salivary Testosterone in Elite Female Athletes. [Article]. *International Journal of Sports Physiology and Performance, 4*(4), 538-542.

Hau, M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays, 29*(2), 133-144.

Hawkes, K. (1991). Showing off: tests of an hypothesis about men's foraging goals. *Ethology and Sociobiology, 12*(1), 29-54.

Hayashi, K. T., & Moberg, G. P. (1987). Influence of acute stress and the adrenal axis on regulation of LH and testosterone in the male Rhesus-monkey (Macaca-mulatta). [Article]. *American Journal of Primatology, 12*(3), 263-273. doi: 10.1002/ajp.1350120304

Hellhammer, D. H., Hubert, W., & Schurmeyer, T. (1985). Changes in saliva testosterone after psychological stimulation in men. *Psychoneuroendocrinology, 10*(1), 77-81.

Hermans, E. J., Ramsey, N. F., & van Honk, J. (2008). Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. [Article]. *Biological Psychiatry, 63*(3), 263-270. doi: 10.1016/j.biopsych.2007.05.013

Higham, J. P., Heistermann, M., & Maestripieri, D. (2013). The endocrinology of male rhesus macaque social and reproductive status: a test of the challenge and social stress hypotheses. *Behavioral Ecology and Sociobiology, 67*(1), 19-30.

Hirschenhauser, K., Frigerio, D., Grammer, K., & Magnusson, M. S. (2002). Monthly patterns of testosterone and behavior in prospective fathers. *Hormones and Behavior, 42*(2), 172-181.

Hirschenhauser, K., & Oliveira, R. F. (2006). Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Animal Behaviour, 71*(2), 265-277.

Hirschenhauser, K., Taborsky, M., Oliveira, T., Canario, A., & Oliveira, R. (2004). A test of the "challenge hypothesis" in cichlid fish: simulated partner and territory intruder experiments. *Animal Behaviour, 68*(4), 741-750.

Hong, Y., Gagnon, J., Rice, T., Perusse, L., Leon, A., Skinner, J., . . . Rao, D. (2001). Familial resemblance for free androgens and androgen glucuronides in sedentary black and white individuals: the HERITAGE Family Study. Health, Risk Factors, Exercise Training and Genetics. *Journal of Endocrinology, 170*(2), 485-492.

Horrocks, P. M., Jones, A. F., Ratcliffe, W. A., Holder, G., White, A., Holder, R., . . . London, D. R. (1990). Patterns of ACTH and cortisol pulsatility over 24 hours in normal males and females. [Article]. *Clinical Endocrinology, 32*(1), 127-134.

Houck, L., & Woodley, S. (1995). Field studies of steroid hormones and male reproductive behaviour in amphibians. *Amphibian biology, 2*, 677-703.

Huhman, K. L., Moore, T. O., Ferris, C. F., Mougey, E. H., & Meyerhoff, J. L. (1991). Acute and repeated exposure to social conflict in male golden hamsters: increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Hormones and Behavior, 25*(2), 206-216.

Huovinen, J., Tulppo, M., Nissila, J., Linnamo, V., Hakkinen, K., & Kyrolainen, H. (2009). Relationship between heart rate variability and the serum testosterone-to-cortisol ratio during military service. [Article]. *European Journal of Sport Science, 9*(5), 277-284. doi: 10.1080/17461390902874040

Izard, C. E. (1994). Innate and universal facial expressions - Evidence from developmental and cross-cultural research. *Psychological Bulletin, 115*(2), 288-299. doi: 10.1037/0033-2909.115.2.288

Jacobs, L. F., Gaulin, S., Sherry, D. F., & Hoffman, G. E. (1990). Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. *Proceedings of the National Academy of Sciences, 87*(16), 6349-6352.

Janowsky, J. S. (2006). Thinking with your gonads: testosterone and cognition. *Trends in Cognitive Sciences, 10*(2), 77-82.

Jimenez, M., Aguilar, R., & Alvero-Cruz, J. R. (2012). Effects of victory and defeat on testosterone and cortisol response to competition: Evidence for same response patterns in men and women. *Psychoneuroendocrinology, 37*(9), 1577-1581.

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress - a dynamic overview of hormonal and behavioral homeostasis. [Review]. *Neuroscience and Biobehavioral Reviews, 16*(2), 115-130.

Josephs, R. A., Sellers, J. G., Newman, M. L., & Mehta, P. H. (2006). The mismatch effect: When testosterone and status are at odds. *Journal of Personality and Social Psychology, 90*(6), 999-1013. doi: 10.1037/0022-3514.90.6.999

Kaplan, H. S., & Gangestad, S. W. (2005). Life history theory and evolutionary psychology. *The handbook of evolutionary psychology*, 68-95.

Kasperk, C., Helmboldt, A., B√ ∂ rcs√ ∂ k, I., Heuthe, S., Cloos, O., Niethard, F., & Ziegler, R. (1997). Skeletal site-dependent expression of the androgen receptor in human osteoblastic cell populations. *Calcified tissue international, 61*(6), 464-473.

Katongole, C., Naftolin, F., & Short, R. (1971). Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. *Journal of Endocrinology, 50*(3), 457-466.

Ketterson, E., Nolan Jr, V., & Sandell, M. (2005). Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? *The American Naturalist, 166*(S4), S85-S98.

Ketterson, E. D., & Nolan Jr, V. (1992). Hormones and life histories: an integrative approach. *American Naturalist*, S33-S62.

Ketterson, E. D., Nolan Jr, V., Wolf, L., & Ziegenfus, C. (1992). Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (Junco hyemalis). *American Naturalist*, 980-999.

Killgore, W. D., & Yurgelun-Todd, D. A. (2001). Sex differences in amygdala activation during the perception of facial affect. *Neuroreport, 12*(11), 2543-2547.

Kimura, D. (2000). *Sex and cognition*: The MIT Press.

Kirk, R. E. (1995). *Experimental design: Procedures for the behavioral sciences (3rd ed.)*. Belmont, CA US: Thomson Brooks/Cole Publishing Co.

Knoth, R., Boyd, K., & Singer, B. (1988). Empirical Tests of Sexual Selection Theory: Predictions of Sex Differences in Onset, Intensity, and Time Course of Sexual Arousal. *The Journal of Sex Research, 24*(ArticleType: research-article / Full publication date: 1988 / Copyright ¬© 1988 Taylor & Francis, Ltd.), 73-89.

Knutson, B. (1996). Facial expressions of emotion influence interpersonal trait inferences. *Journal of Nonverbal Behavior, 20*(3), 165-182. doi: 10.1007/bf02281954

Koolhaas, J. M., de Boer, S. F., Buwalda, B., & van Reenen, K. (2007). Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain, behavior and evolution, 70*(4), 218-226.

Kraemer, H. C., Becker, H. B., Brodie, H. K. H., Doering, C. H., Moos, R. H., & Hamburg, D. A. (1976). Orgasmic frequency and plasma testosterone levels in normal human males. *Archives of Sexual Behavior, 5*(2), 125-132.

Kritzer, M. (2004). The distribution of immunoreactivity for intracellular androgen receptors in the cerebral cortex of hormonally intact adult male and female rats: localization in pyramidal neurons making corticocortical connections. *Cerebral Cortex, 14*(3), 268-280.

Kritzer, M. F. (1997). Selective colocalization of immunoreactivity for intracellular gonadal hormone receptors and tyrosine hydroxylase in the ventral tegmental area, substantia nigra, and retrorubral fields in the rat. *Journal of Comparative Neurology, 379*(2), 247-260.

Kruger, T., Exton, M. S., Pawlak, C., Muhlen, A. v. z., Hartmann, U., & Schedlowski, M. (1998). Neuroendocrine and cardiovascular response to sexual arousal and orgasm in men. *Psychoneuroendocrinology, 23*(4), 401-411.

Kuzawa, C. W., Gettler, L. T., Huang, Y.-y., & McDade, T. W. (2010). Mothers have lower testosterone than non-mothers: Evidence from the Philippines. *Hormones and Behavior, 57*(4), 441-447.

LaFerla, J. J., Anderson, D. L., & Schalch, D. S. (1978). Psychoendocrine response to sexual arousal in human males. *Psychosomatic Medicine, 40*(2), 166-172.

Lee, P. A., Jaffe, R. B., Midgley Jr, A., Baker, H., Burger, H., De Kretser, D., . . . Brenner, P. (1974). Lack of alteration of serum gonadotropins in men and women following sexual intercourse. *American journal of obstetrics and gynecology, 120*(2), 985-987.

Lefevre, C., Lewis, G. J., Perrett, D. I., & Penke, L. (2013). Telling facial metrics: facial width is associated with testosterone levels in men. *Evolution and Human Behavior*.

Lehner, S. R., Rutte, C., & Taborsky, M. (2011). Rats benefit from winner and loser effects. *Ethology, 117*(11), 949-960.

Liening, S. H., Stanton, S. J., Saini, E. K., & Schultheiss, O. C. (2010). Salivary testosterone, cortisol, and progesterone: two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels. *Physiology & Behavior, 99*(1), 8-16.

Lloyd, J. A. (1971). Weights of testes, thymi, and accessory reproductive glands in relation to rank in paired and grouped house mice (Mus-musculus). [Article]. *Proceedings of the Society for Experimental Biology and Medicine, 137*(1), 19-22.

Lòpez, H. H., Hay, A. C., & Conklin, P. H. (2009). Attractive men induce testosterone and cortisol release in women. *Hormones and Behavior, 56*(1), 84-92. doi: 10.1016/j.yhbeh.2009.03.004

Lundqvist, D., Flykt, A., & Öhman, A. (1998). The Karolinska Directed Emotional Faces. In K. Institute (Ed.). Stockholm.

Macrides, F., Bartke, A., & Dalterio, S. (1975). Strange females increase plasma testosterone levels in male mice. *Science*.

Maestu, J., Jurimae, J., & Jurimae, T. (2005). Hormonal response to maximal rowing before and after heavy increase in training volume in highly trained male rowers. *The Journal of sports medicine and physical fitness, 45*(1), 121-126.

Magee, J. C., & Galinsky, A. D. (2008). Social Hierarchy: The Self-Reinforcing Nature of Power and Status. [Article]. *Academy of Management Annals, 2*, 351-398. doi: 10.1080/19416520802211628

Mak, A. K. Y., Hu, Z. G., Zhang, J. X. X., Xiao, Z. W., & Lee, T. M. C. (2009). Sex-related differences in neural activity during emotion regulation. *Neuropsychologia, 47*(13), 2900-2908. doi: 10.1016/j.neuropsychologia.2009.06.017

Marler, C. A., Oyegbile, T. O., Plavicki, J., & Trainor, B. C. (2005). Response to Wingfield's commentary on "A continuing saga: the role of testosterone in aggression'. *Hormones and Behavior, 48*(3), 256-258.

Martinez, M., Guillen-Salazar, F., Salvador, A., & Simon, V. M. (1995). Successful intermale aggression and conditioned place preference in mice. *Physiology & Behavior, 58*(2), 323-328.

Mascaro, J. S., Hackett, P. D., & Rilling, J. K. (2013). Testicular volume is inversely correlated with nurturing-related brain activity in human fathers. *Proceedings of the National Academy of Sciences, 110*(39), 15746-15751.

Mayer, M., & Rosen, F. (1977). Interaction of glucocorticoids and androgens with skeletal-muscle. *Metabolism-Clinical and Experimental, 26*(8), 937-962.

Mazur, A., & Booth, A. (1998). Testosterone and dominance in men. [Review]. *Behavioral and Brain Sciences, 21*(3), 353-363.

Mazur, A., & Booth, A. (2014). Testosterone is related to deviance in male army veterans, but relationships are not moderated by cortisol. *Biological Psychology, 96*, 72-76.

Mazur, A., Booth, A., & Dabbs, J. M. (1992). Testosterone and Chess Competition. [Article]. *Social Psychology Quarterly, 55*(1), 70-77.

Mazur, A., & Lamb, T. A. (1980). Testosterone, status, and mood in human males. [Article]. *Hormones and Behavior, 14*(3), 236-246.

Mazur, A., Susman, E. J., & Edelbrock, S. (1997). Sex difference in testosterone response to a video game contest. *Evolution and Human Behavior, 18*(5), 317-326.

Mazurski, E. J., Bond, N. W., Siddle, D. A. T., & Lovibond, P. F. (1996). Conditioning with facial expressions of emotion: Effects of CS sex and age. *Psychophysiology, 33*(4), 416-425. doi: 10.1111/j.1469-8986.1996.tb01067.x

McCaul, K. D., Gladue, B. A., & Joppa, M. (1992). Winning, losing, mood, and testosterone. *Hormones and Behavior, 26*(4), 486-504.

McClure, E. B., Monk, C. S., Nelson, E. E., Zarahn, E., Leibenluft, E., Bilder, R. M., . . . Pine, D. S. (2004). A developmental examination of gender differences in brain engagement during evaluation of threat. *Biological Psychiatry, 55*(11), 1047-1055. doi: 10.1016/j.biopsych.2004.02.013

McConnell, S. E., Alla, J., Wheat, E., Romeo, R. D., McEwen, B., & Thornton, J. E. (2012). The role of testicular hormones and luteinizing hormone in spatial memory in adult male rats. *Hormones and Behavior, 61*(4), 479-486.

McDonald, M. M., Navarrete, C. D., & Van Vugt, M. (2012). Evolution and the psychology of intergroup conflict: the male warrior hypothesis. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1589), 670-679.

McGlothlin, J. W., Jawor, J., Greives, T., Casto, J., Phillips, J., & Ketterson, E. (2008). Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. *Journal of Evolutionary Biology, 21*(1), 39-48.

McGlothlin, J. W., Jawor, J. M., & Ketterson, E. D. (2007). Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *The American Naturalist, 170*(6), 864-875.

McGlothlin, J. W., Whittaker, D. J., Schrock, S. E., Gerlach, N. M., Jawor, J. M., Snajdr, E. A., & Ketterson, E. D. (2010). Natural selection on testosterone production in a wild songbird population. *The American Naturalist, 175*(6), 687-701.

Mehta, P. H., & Beer, J. (2010). Neural Mechanisms of the Testosterone-Aggression Relation: The Role of Orbitofrontal Cortex. *Journal of Cognitive Neuroscience, 22*(10), 2357-2368. doi: 10.1162/jocn.2009.21389

Mehta, P. H., Jones, A. C., & Josephs, R. A. (2008). The social endocrinology of dominance: Basal testosterone predicts cortisol changes and behavior following victory and defeat. *Journal of Personality and Social Psychology, 94*(6), 1078-1093. doi: 10.1037/0022-3514.94.6.1078

Mehta, P. H., & Josephs, R. A. (2006). Testosterone change after losing predicts the decision to compete again. *Hormones and Behavior, 50*(5), 684-692. doi: 10.1016/j.yhbeh.2006.07.001

Mehta, P. H., & Josephs, R. A. (2010). Testosterone and cortisol jointly regulate dominance: Evidence for a dual-hormone hypothesis. *Hormones and Behavior, 58*(5), 898-906. doi: 10.1016/j.yhbeh.2010.08.020

Meisel, R. L., & Joppa, M. A. (1994). Conditioned place preference in female hamsters following aggressive or sexual encounters. *Physiology & Behavior, 56*(5), 1115-1118.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin, 133*(1), 25.

Miller, S. L., & Maner, J. K. (2010). Scent of a woman men's testosterone responses to olfactory ovulation cues. *Psychological Science, 21*(2), 276-283.

Miller, S. L., Maner, J. K., & McNulty, J. K. (2012). Adaptive attunement to the sex of individuals at a competition: the ratio of opposite-to same-sex individuals correlates with changes in competitors' testosterone levels. *Evolution and Human Behavior, 33*(1), 57-63.

Mills, S. C., Grapputo, A., Koskela, E., & Mappes, T. (2007). Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proceedings of the Royal Society B: Biological Sciences, 274*(1606), 143-150.

Moller, A. P., Garamszegi, L. Z., Gil, D., Hurtrez-Bousses, S., & Eens, M. (2005). Correlated evolution of male and female testosterone profiles in birds and its consequences. *Behavioral Ecology and Sociobiology, 58*(6), 534-544.

Mooradian, A. D., Morley, J. E., & Korenman, S. G. (1987). Biological actions of androgens. *Endocrine Reviews, 8*(1), 1-28.

Muehlenbein, M. P., & Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. *American Journal of Human Biology, 17*(5), 527-558.

Muehlenbein, M. P., Hirschtick, J. L., Bonner, J. Z., & Swartz, A. M. (2010). Toward quantifying the usage costs of human immunity: altered metabolic rates and hormone levels during acute immune activation in men. *American Journal of Human Biology, 22*(4), 546-556.

Muller, M. N., Marlowe, F. W., Bugumba, R., & Ellison, P. T. (2009). Testosterone and paternal care in East African foragers and pastoralists. *Proceedings of the Royal Society B: Biological Sciences, 276*(1655), 347-354.

Muroyama, Y., Shimizu, K., & Sugiura, H. (2007). Seasonal variation in fecal testosterone levels in free‚Äêranging male Japanese macaques. *American Journal of Primatology, 69*(6), 603-610.

Neave, N., & Wolfson, S. (2003). Testosterone, territoriality, and the "home advantage". *Physiology & Behavior, 78*(2), 269-275.

Nyby, J. G. (2008). Reflexive testosterone release: a model system for studying the nongenomic effects of testosterone upon male behavior. *Frontiers in Neuroendocrinology, 29*(2), 199-210.

O'Connor, D. B., Archer, J., Hair, W. M., & Wu, F. C. (2001). Activational effects of testosterone on cognitive function in men. *Neuropsychologia, 39*(13), 1385-1394.

Oliveira, G. a. A., & Oliveira, R. F. (2014). Androgen responsiveness to competition in humans: the role of cognitive variables. *Neuroscience & Neuroeconomicss, 3*.

Oliveira, R. F., Carneiro, L. A., & Canàrio, A. V. (2005). Behavioural endocrinology: no hormonal response in tied fights. *Nature, 437*(7056), 207-208.

Oliveira, R. F., Lopes, M., Carneiro, L. A., & Canario, A. V. (2001). Watching fights raises fish hormone levels. *Nature, 409*(6819), 475-475.

Oliveira, R. F., Silva, A., & Canàrio, A. V. (2009). Why do winners keep winning? Androgen mediation of winner but not loser effects in cichlid fish. *Proceedings of the Royal Society B: Biological Sciences, 276*(1665), 2249-2256.

Oliveira, T., Gouveia, M. J., & Oliveira, R. F. (2009). Testosterone responsiveness to winning and losing experiences in female soccer players. [Article]. *Psychoneuroendocrinology, 34*(7), 1056-1064. doi: 10.1016/j.psyneuen.2009.02.006

Oxford, J., Ponzi, D., & Geary, D. C. (2010). Hormonal responses differ when playing violent video games against an ingroup and outgroup. *Evolution and Human Behavior, 31*(3), 201-209. doi: 10.1016/j.evolhumbehav.2009.07.002

Oyegbile, T. O., & Marler, C. A. (2005). Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Hormones and Behavior, 48*(3), 259-267.

Penton-Voak, I. S., & Chang, H. Y. (2008). Attractiveness judgements of individuals vary across emotional expression and movement conditions. *Journal of Evolutionary Psychology, 6*(2), 89-100.

Peters, M., Laeng, B., Latham, K., Jackson, M., Zaiyouna, R., & Richardson, C. (1995). A redrawn Vandenberg and Kuse mental rotations test - Different versions and factors that affect performance. *Brain and Cognition, 28*(1), 39-58. doi: 10.1006/brcg.1995.1032

Pfattheicher, S., Landhauber, A., & Keller, J. (2013). Individual differences in antisocial punishment in public goods situations: The interplay of cortisol with testosterone and dominance. *Journal of Behavioral Decision Making*.

Pirke, K. M., Kockott, G. t., & Dittmar, F. (1974). Psychosexual stimulation and plasma testosterone in man. *Archives of Sexual Behavior, 3*(6), 577-584.

Pollet, T. V., Cobey, K. D., & van der Meij, L. (2013). Testosterone levels are negatively associated with childlessness in males, but positively related to offspring count in fathers. *Plos One, 8*(4), e60018.

Popma, A., Vermeiren, R., Geluk, C., Rinne, T., van den Brink, W., Knol, D. L., . . . Doreleijers, T. A. H. (2007). Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. *Biological Psychiatry, 61*(3), 405-411. doi: 10.1016/j.biopsych.2006.06.006

Postma, A., Meyer, G., Tuiten, A., van Honk, J., Kessels, R. P., & Thijssen, J. (2000). Effects of testosterone administration on selective aspects of object-location memory in healthy young women. *Psychoneuroendocrinology, 25*(6), 563-575.

Preacher, K. J., & Hayes, A. F. (2008). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods, 40*(3), 879-891. doi: 10.3758/brm.40.3.879

Purvis, K., Landgren, B.-M., Cekan, Z., & Diczfalusy, E. (1976). Endocrine effects of masturbation in men. *Journal of Endocrinology, 70*(3), 439-444.

Puts, D. A., Apicella, C. L., & Cardenas, R. A. (2012). Masculine voices signal men's threat potential in forager and industrial societies. *Proceedings of the Royal Society B: Biological Sciences, 279*(1728), 601-609.

Rantala, M. J., Moore, F. R., Skrinda, I., Krama, T., Kivleniece, I., Kecko, S., & Krams, I. (2012). Evidence for the stress-linked immunocompetence handicap hypothesis in humans. *Nature communications, 3*, 694.

Redoute, J., Stoleru, S., Gregoire, M.-C., Costes, N., Cinotti, L., Lavenne, F., . . . Pujol, J.-F. (2000). Brain processing of visual sexual stimuli in human males. *Human Brain Mapping, 11*(3), 162-177.

Riadfahmy, D., Read, G. F., Walker, R. F., & Griffiths, K. (1982). Steroids in saliva for assessing endocrine function. *Endocrine Reviews, 3*(4), 367-395.

Riadfahmy, D., Read, G. F., Walker, R. F., Walker, S. M., & Griffiths, K. (1987). Determination of ovarian-steroid hormone levels in saliva - an overview. *Journal of Reproductive Medicine, 32*(4), 254-272.

Richardson, H. N., Nelson, A. L., Ahmed, E. I., Parfitt, D. B., Romeo, R. D., & Sisk, C. L. (2004). Female pheromones stimulate release of luteinizing hormone and testosterone without altering GnRH mRNA in adult male Syrian hamsters (< i> Mesocricetus auratus</i>). *General and Comparative Endocrinology, 138*(3), 211-217.

Roberts, M. L., Buchanan, K. L., & Evans, M. (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour, 68*(2), 227-239.

Robertson, E. M., Pascual-Leone, A., & Miall, R. C. (2004). Current concepts in procedural consolidation. *Nature Reviews Neuroscience, 5*(7), 576-582.

Ronay, R., & von Hippel, W. (2010). The presence of an attractive woman elevates testosterone and physical risk taking in young men. *Social Psychological and Personality Science, 1*(1), 57-64.

Roney, J. R., Lukaszewski, A. W., & Simmons, Z. L. (2007). Rapid endocrine responses of young men to social interactions with young women. *Hormones and Behavior, 52*(3), 326-333. doi: 10.1016/j.yhbeh.2007.05.008

Roney, J. R., Mahler, S. V., & Maestripieri, D. (2003). Behavioral and hormonal responses of men to brief interactions with women. *Evolution and Human Behavior, 24*(6), 365-375. doi: 10.1016/s1090-5138(03)00053-9

Roney, J. R., & Simmons, Z. L. (2012). Men Smelling Women: Null Effects of Exposure to Ovulatory Sweat on Me's Testosterone. *Evolutionary Psychology, 10*(4).

Roney, J. R., Simmons, Z. L., & Lukaszewski, A. W. (2010). Androgen receptor gene sequence and basal cortisol concentrations predict men's hormonal responses to potential mates. *Proceedings of the Royal Society B: Biological Sciences, 277*(1678), 57-63.

Rose, R. M., Berstein, I. S., & Gordon, T. P. (1975). Consequences of social conflict on plasma testosterone levels in rhesus monkeys. *Psychosomatic Medicine, 37*(1), 50-61.

Rosvall, K., Burns, C. B., Barske, J., Goodson, J., Schlinger, B., Sengelaub, D., & Ketterson, E. (2012). Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. *Proceedings of the Royal Society B: Biological Sciences, 279*(1742), 3547-3555.

Rowland, D. L., Heiman, J. R., Gladue, B. A., Hatch, J. P., Doering, C. H., & Weiler, S. J. (1987). Endocrine, psychological and genital response to sexual arousal in men. *Psychoneuroendocrinology, 12*(2), 149-158.

Sabatinelli, D., Flaisch, T., Bradley, M. M., Fitzsimmons, J. R., & Lang, P. J. (2004). Affective picture perception: gender differences in visual cortex? *Neuroreport, 15*(7), 1109-1112.

Salvador, A. (2005). Coping with competitive situations in humans. *Neuroscience and Biobehavioral Reviews, 29*(1), 195-205. doi: 10.1016/j.neubiorev.2004.07.004

Salvador, A., & Costa, R. (2009). Coping with competition: Neuroendocrine responses and cognitive variables. *Neuroscience and Biobehavioral Reviews, 33*(2), 160-170. doi: 10.1016/j.neubiorev.2008.09.005

Salvador, A., Simon, V., Suay, F., & Llorens, L. (1987). Testosterone and cortisol responses to competitive fighting in human males - a pilot-study. *Aggressive Behavior, 13*(1), 9-13.

Sapolsky, R. M. (1983). Endocrine aspects of social instability in the olive baboon (*Papio anubis*). *American Journal of Primatology, 5*(4), 365-379.

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science, 308*(5722), 648-652.

Schienle, A., Schafer, A., Stark, R., Walter, B., & Vaitl, D. (2005). Gender differences in the processing of disgust-and fear-inducing pictures: an fMRI study. *Neuroreport, 16*(3), 277.

Schneider, S., Peters, J., Bromberg, U., Brassen, S., Menz, M., Miedl, S., . . . Barker, G. (2011). Boys do it the right way: Sex-dependent amygdala lateralization during face processing in adolescents. *NeuroImage, 56*(3), 1847-1853.

Schultheiss, O. C., Campbell, K. L., & McClelland, D. C. (1999). Implicit power motivation moderates men's testosterone responses to imagined and real dominance success. *Hormones and Behavior, 36*(3), 234-241.

Schultheiss, O. C., & Rohde, W. (2002). Implicit power motivation predicts men's testosterone changes and implicit learning in a contest situation. *Hormones and Behavior, 41*(2), 195-202. doi: 10.1006/hbeh.2001.1745

Schultheiss, O. C., Wirth, M. M., Torges, C. M., Pang, J. S., Villacorta, M. A., & Welsh, K. M. (2005). Effects of implicit power motivation on men's and women's implicit learning and testosterone changes after social victory or defeat. *Journal of Personality and Social Psychology, 88*(1), 174-188.

Schwabl, H. (1996). Maternal testosterone in the avian egg enhances postnatal growth. *Comparative Biochemistry and Physiology Part A: Physiology, 114*(3), 271-276.

Scott, M. P. (2006). Resource defense and juvenile hormone: the "challenge hypothesis" extended to insects. *Hormones and Behavior, 49*(3), 276-281.

Sell, A., Tooby, J., & Cosmides, L. (2009). Formidability and the logic of human anger. *Proceedings of the National Academy of Sciences, 106*(35), 15073-15078.

Sellers, J. G., Mehl, M. R., & Josephs, R. A. (2007). Hormones and personality: Testosterone as a marker of individual differences. *Journal of Research in Personality, 41*(1), 126-138. doi: 10.1016/j.jrp.2006.02.004

Sherry, D. F., & Hampson, E. (1997). Evolution and the hormonal control of sexually-dimorphic spatial abilities in humans. *Trends in Cognitive Sciences, 1*(2), 50-56.

Sherwin, B. B., Gelfand, M. M., & Brender, W. (1985). Androgen enhances sexual motivation in females: a prospective, crossover study of sex steroid administration in the surgical menopause. *Psychosomatic Medicine, 47*(4), 339-351.

Shirtcliff, E. A., Granger, D. A., & Likos, A. (2002). Gender differences in the validity of testosterone measured in saliva by immunoassay. *Hormones and Behavior, 42*(1), 62-69. doi: 10.1006/hbeh.2002.1798

Silverin, B. (1980). Effects of long-acting testosterone treatment on freeliving pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Animal Behaviour, 28*(3), 906-912.

Simerly, R., Swanson, L., Chang, C., & Muramatsu, M. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *Journal of Comparative Neurology, 294*(1), 76-95.

Simmons, Z. L., & Roney, J. R. (2009). Androgens and energy allocation: Quasi-experimental evidence for effects of influenza vaccination on men's testosterone. *American Journal of Human Biology, 21*(1), 133-135.

Slatcher, R. B., Mehta, P. H., & Josephs, R. A. (2011). Testosterone and self-reported dominance interact to influence human mating behavior. *Social Psychological and Personality Science, 2*(5), 531-539.

Stanton, S. J., Beehner, J. C., Saini, E. K., Kuhn, C. M., & LaBar, K. S. (2009). Dominance, Politics, and Physiology: Voters' Testosterone Changes on the Night of the 2008 United States Presidential Election. *Plos One, 4*(10), e7543. doi:7510.1371/journal.pone.0007543. doi: e7543

Stanton, S. J., Mullette-Gillman, O., & Huettel, S. A. (2011). Seasonal variation of salivary testosterone in men, normally cycling women, and women using hormonal contraceptives. *Physiology & Behavior, 104*(5), 804-808.

Stanton, S. J., & Schultheiss, O. C. (2009). The hormonal correlates of implicit power motivation. *Journal of Research in Personality, 43*(5), 942-949. doi: 10.1016/j.jrp.2009.04.001

Stanton, S. J., Wirth, M. M., Waugh, C. E., & Schultheiss, O. C. (2009). Endogenous testosterone levels are associated with amygdala and ventromedial prefrontal cortex responses to anger faces in men but not women. *Biological Psychology, 81*(2), 118-122. doi: 10.1016/j.biopsycho.2009.03.004

Stearns, E., Winter, J., & Faiman, C. (1973). Effects of Coitus on Gonadotropin, Prolactin and Sex Steroid Levels in Man. *The Journal of Clinical Endocrinology & Metabolism, 37*(5), 687-691.

Stoleru, S. G., Ennaji, A., Cournot, A., & Spira, A. (1993). LH pulsatile secretion and testosterone blood-levels are influenced by sexual arousal in human males. *Psychoneuroendocrinology, 18*(3), 205-218. doi: 10.1016/0306-4530(93)90005-6

Suay, F., Salvador, A., Gonzalez-Bono, E., Sanchis, C., Martinez, M., Martinez-Sanchis, S., . . . Montoro, J. B. (1999). Effects of competition and its outcome on serum testosterone, cortisol and prolactin. *Psychoneuroendocrinology, 24*(5), 551-566.

Surbeck, M., Deschner, T., Schubert, G., Weltring, A., & Hohmann, G. (2012). Mate competition, testosterone and intersexual relationships in bonobos, *Pan paniscus*. *Animal Behaviour, 83*(3), 659-669.

Terburg, D., Morgan, B., & van Honk, J. (2009). The testosterone-cortisol ratio: A hormonal marker for proneness to social aggression. *International Journal of Law and Psychiatry, 32*(4), 216-223. doi: 10.1016/j.ijlp.2009.04.008

Terlecki, M. S., & Newcombe, N. S. (2005). How important is the digital divide? The relation of computer and videogame usage to gender differences in mental rotation ability. *Sex Roles, 53*(5-6), 433-441. doi: 10.1007/s11199-005-6765-0

Thompson, C. W., & Moore, M. C. (1992). Behavioral and hormonal correlates of alternative reproductive strategies in a polygynous lizard: tests of the relative plasticity and challenge hypotheses. *Hormones and Behavior, 26*(4), 568-585.

Tilbrook, A. J., Turner, A. I., & Clarke, I. J. (2000). Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. [Review]. *Reviews of Reproduction, 5*(2), 105-113.

Tracy, J. L., & Beall, A. T. (2011). Happy Guys Finish Last: The Impact of Emotion Expressions on Sexual Attraction. *Emotion, 11*(6), 1379-1387. doi: 10.1037/a0022902

Trainor, B. C., Bird, I. M., & Marler, C. A. (2004). Opposing hormonal mechanisms of aggression revealed through short-lived testosterone manipulations and multiple winning experiences. *Hormones and Behavior, 45*(2), 115-121.

Trivers, R. L. (1972). Parental investment and sexual selection. *Sexual Selection and the Descent of Man, 1871–1971* (pp. 136-179). Chicago: Aldine–Atherton.

Trumble, B. C., Brindle, E., Kupsik, M., & O'Connor, K. A. (2010). Responsiveness of the reproductive axis to a single missed evening meal in young adult males. *American Journal of Human Biology, 22*(6), 775-781.

Valentine, K. A., Li, N. P., Penke, L., & Perrett, D. I. (2014). Judging a Man by the Width of His Face The Role of Facial Ratios and Dominance in Mate Choice at Speed-Dating Events. *Psychological Science*, 0956797613511823.

Van Anders, S. M., Brotto, L., Farrell, J., & Yule, M. (2009). Associations among physiological and subjective sexual response, sexual desire, and salivary steroid hormones in healthy premenopausal women. *The Journal of Sexual Medicine, 6*(3), 739-751.

van Anders, S. M., & Goldey, K. L. (2010). Testosterone and partnering are linked via relationship status for women and 'relationship orientation' for men. *Hormones and Behavior, 58*(5), 820-826. doi: 10.1016/j.yhbeh.2010.08.005

van Anders, S. M., Hamilton, L. D., Schmidt, N., & Watson, N. V. (2007). Associations between testosterone secretion and sexual activity in women. *Hormones and Behavior, 51*(4), 477-482.

Van Anders, S. M., Hampson, E., & Watson, N. V. (2006). Seasonality, waist-to-hip ratio, and salivary testosterone. *Psychoneuroendocrinology, 31*(7), 895-899.

van Anders, S. M., Tolman, R. M., & Volling, B. L. (2012). Baby cries and nurturance affect testosterone in men. *Hormones and Behavior, 61*(1), 31-36.

van Anders, S. M., & Watson, N. V. (2006a). Menstrual cycle irregularities are associated with testosterone levels in healthy premenopausal women. *American Journal of Human Biology, 18*(6), 841-844. doi: 10.1002/ajhb.20555

van Anders, S. M., & Watson, N. V. (2006b). Social neuroendocrinology - Effects of social contexts and behaviors on sex steroids in humans. *Human Nature-an Interdisciplinary Biosocial Perspective, 17*(2), 212-237.

van Anders, S. M., & Watson, N. V. (2007). Effects of ability- and chance-determined competition outcome on testosterone. *Physiology & Behavior, 90*(4), 634-642. doi: 10.1016/j.physbeh.2006.11.017

van der Meij, L., Almela, M., Buunk, A. P., Fawcett, T. W., & Salvador, A. (2012). Men with elevated testosterone levels show more affiliative behaviours during interactions with women. *Proceedings of the Royal Society B: Biological Sciences, 279*(1726), 202-208. doi: 10.1098/rspb.2011.0764

van der Meij, L., Buunk, A. P., Almela, M., & Salvador, A. (2010). Testosterone responses to competition: The opponent's psychological state makes it challenging. *Biological Psychology, 84*(2), 330-335. doi: 10.1016/j.biopsycho.2010.03.017

van der Meij, L., Buunk, A. P., van de Sande, J. P., & Salvador, A. (2008). The presence of a woman increases testosterone in aggressive dominant men. *Hormones and Behavior, 54*(5), 640-644. doi: 10.1016/j.yhbeh.2008.07.001

Van Duyse, E., Pinxten, R., & Eens, M. (2002). Effects of Testosterone on Song, Aggression, and Nestling Feeding Behavior in Male Great Tits, *Parus major*. *Hormones and Behavior, 41*(2), 178-186.

van Honk, J., Tuiten, A., Hermans, E., Putman, P., Koppeschaar, H., Thijssen, J., . . . van Doornen, L. (2001). A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. *Behavioral Neuroscience, 115*(1), 238-242. doi: 10.1037/0735-7044.115.1.238

van Honk, J., Tuiten, A., van den Hout, M., Koppeschaar, H., Thijssen, J., de Haan, E., & Verbaten, R. (2000). Conscious and preconscious selective attention to social threat: different neuroendocrine response patterns. *Psychoneuroendocrinology, 25*(6), 577-591. doi: 10.1016/s0306-4530(00)00011-1

Van Roo, B. L. (2004). Exogenous testosterone inhibits several forms of male parental behavior and stimulates song in a monogamous songbird: the blue-headed vireo (*Vireo solitarius*). *Hormones and Behavior, 46*(5), 678-683.

Veldhuis, J. D., King, J. C., Urban, R. J., Rogol, A. D., Evans, W. S., Kolp, V. A., & Johnson, M. L. (1987). Operating Characteristics of the Male Hypothalamo-Pituitary-Gonadal Axis: Pulsatile Release of Testosterone and Follicle-Stimulating Hormone and Their Temporal Coupling with Luteinizing Hormone*. *The Journal of Clinical Endocrinology & Metabolism, 65*(5), 929-941.

Vermeersch, H., T'Sjoen, G., Kaufman, J.-M., & Vincke, J. (2009). The relationship between sex steroid hormones and behavioural inhibition (BIS) and behavioural activation (BAS) in adolescent boys and girls. *Personality and Individual Differences, 47*(1), 3-7.

Viau, V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. [Article]. *Journal of Neuroendocrinology, 14*(6), 506-513.

Viau, V., & Meaney, M. J. (1996). The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *Journal of Neuroscience, 16*(5), 1866-1876.

Watson, D., & Clark, L. A. (1994). *The PANAS-X: manual for the positive and negative affect schedule – expanded form.* Department of Psychology. University of Iowa. Iowa City.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology, 54*(6), 1063-1070. doi: 10.1037//0022-3514.54.6.1063

Watson, N. V. (2001). Sex differences in throwing: Monkeys having a fling. *Trends in Cognitive Sciences, 5*(3), 98-99.

Weatherhead, P. J., & Robertson, R. J. (1979). Offspring quality and the polygyny threshold:" the sexy son hypothesis". *American Naturalist*, 201-208.

Welker, K. M., Lozoya, E., Campbell, J. A., Neumann, C. S., & Carrè, J. M. (2014). Testosterone, cortisol, and psychopathic traits in men and women. *Physiology & Behavior, 129*, 230-236.

Whittle, S., Yücel, M., Yap, M. B. H., & Allen, N. B. (2011). Sex differences in the neural correlates of emotion: Evidence from neuroimaging. *Biological Psychology, 87*(3), 319-333. doi: 10.1016/j.biopsycho.2011.05.003

Williams, L. M., Barton, M. J., Kemp, A. H., Liddell, B. J., Peduto, A., Gordon, E., & Bryant, R. A. (2005). Distinct amygdala-autonomic arousal profiles in response to fear signals in healthy males and females. *NeuroImage, 28*(3), 618-626. doi: 10.1016/j.neuroimage.2005.06.035

Wilson, E. O. (1975). *Sociobiology*. Cambridge, MA: Belknap Press of Harvard University Press.

Wingfield, J., Jacobs, J., Tramontin, A., Perfito, N., Meddle, S., Maney, D., & Soma, K. (2000). Toward an ecological basis of hormone-behavior interactions in reproduction of birds. *Reproduction in context*, 85-128.

Wingfield, J., & Wada, M. (1989). Changes in plasma levels of testosterone during male-male interactions in the song sparrow, Melospiza melodia: time course and specificity of response. *Journal of Comparative Physiology A, 166*(2), 189-194.

Wingfield, J. C. (1994). Control of territorial aggression in a changing environment. *Psychoneuroendocrinology, 19*(5), 709-721.

Wingfield, J. C., Ball, G. F., Dufty, A. M., Hegner, R. E., & Ramenofsky, M. (1987). Testosterone and aggression in birds. [Article]. *American Scientist, 75*(6), 602-608.

Wingfield, J. C., Hegner, R. E., Dufty, A. M., & Ball, G. F. (1990). The challenge hypothesis - theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. [Article]. *American Naturalist, 136*(6), 829-846.

Wingfield, J. C., Lynn, S., & Soma, K. K. (2001). Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *Brain, behavior and evolution, 57*(5), 239-251.

Wirth, M. M., & Schultheiss, O. C. (2007). Basal testosterone moderates responses to anger faces in humans. [Article]. *Physiology & Behavior, 90*(2-3), 496-505. doi: 10.1016/j.physbeh.2006.10.016

Wirth, M. M., & Schultheiss, O. C. (2007). Basal testosterone moderates responses to anger faces in humans. *Physiology & Behavior, 90*(2), 496-505.

Wirth, M. M., Welsh, K. M., & Schultheiss, O. C. (2006). Salivary cortisol changes in humans after winning or losing a dominance contest depend on implicit power motivation. *Hormones and Behavior, 49*(3), 346-352. doi: 10.1016/j.yhbeh.2005.08.013

Wrase, J., Klein, S., Gruesser, S. M., Hermann, D., Flor, H., Mann, K., . . . Heinz, A. (2003). Gender differences in the processing of standardized emotional visual stimuli in humans: a functional magnetic resonance imaging study. *Neuroscience Letters, 348*(1), 41-45. doi: 10.1016/s0304-3940(03)00565-2

Wright, N. D., Edwards, T., Fleming, S. M., & Dolan, R. J. (2012). Testosterone induces off-line perceptual learning. *Psychopharmacology, 224*(3), 451-457.

Ziegler, T. E., Schultz-Darken, N. J., Scott, J. J., Snowdon, C. T., & Ferris, C. F. (2005). Neuroendocrine response to female ovulatory odors depends upon social condition in male common marmosets,< i> Callithrix jacchus</i>. *Hormones and Behavior, 47*(1), 56-64.

Zilioli, S., & Watson, N. V. (2012). The hidden dimensions of the competition effect: Basal cortisol and basal testosterone jointly predict changes in salivary testosterone after social victory in men. *Psychoneuroendocrinology, 37*, 1855-1865. doi: 10.1016/j.psyneuen.2012.03.022

Zilioli, S., & Watson, N. V. (2014). *Transient impairment of spatial cognition in men following exposure to happy females faces: the mediating role of cortisol*. Paper presented at the 14th Annual Meeting of The Society for Personality and Social Psychology, New Orleans, Louisiana.

Zysling, D. A., Greives, T. J., Breuner, C. W., Casto, J. M., Demas, G. E., & Ketterson, E. D. (2006). Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Hormones and Behavior, 50*(2), 200-207.