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SOCIAL MODULATION OF ANDROGENS IN HUMANS: PSYCHOLOGICAL MECHANISMS AND ADAPTIVE FUNCTION

Gonçalo Aires de Oliveira

Tese submetida como requisito parcial para obtenção do grau de
Doutoramento em Psicologia
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Dissertação orientada por Professor Doutor Rui Filipe Pais de Oliveira
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Palavras-chave

Competição; Testosterona; Hipótese do desafio; Modelo biosocial do estatuto; Avaliação cognitiva

Key words:

Competition; Testosterone; Challenge hypothesis; Biosocial model of status; Appraisal

Categorias de Classificação da tese

2300 Human Experimental Psychology

2400 Animal Experimental & Comparative Psychology

    2440 Social & Instinctive Behavior

RESUMO

Esta tese procura clarificar os processos subjacentes às discrepâncias entre a direcção da resposta de androgénios à competição encontrada em estudos empíricos e as predicções das teorias para a modulação social de androgénios. Sugerimos que estes resultados imprevistos podem resultar de interacções com variáveis cognitivas e elegemos especificamente a avaliação cognitiva como um forte candidato a moderador da resposta de testosterona (T) aos desafios sociais. Várias experiências foram realizadas para testar esta hipótese. No Capítulo II e III, testou-se o efeito da familiaridade do oponente e da avaliação do resultado da competição como ameaça/desafio, na resposta de T a uma competição contra um membro do mesmo sexo. Nas mulheres foi encontrado um maior aumento dos níveis de T quando eram derrotadas por um oponente não familiar e quando o resultado era avaliado como ameaçador. Este efeito de moderação não foi detectado para os homens. Continuou-se a investigação sobre os efeitos da familiaridade do oponente e avaliação de ameaça no Capítulo IV, mas com um ciclídeo. Num paradigma de repetidas invasões territoriais por machos estranhos e familiares, encontrou-se uma maior resposta de androgénios no macho residente para as intrusões realizadas por um estranho, comparada com as de um macho familiar. O efeito do componente de expectativas da avaliação cognitiva, na resposta de T à competição em mulheres, foi testado através da manipulação das expectativas dos participantes em relação ao resultado da competição antes da tarefa competitiva (Capítulo V). Os vencedores inesperados baixaram os níveis de T depois da competição, mostrando uma inversão da resposta predicta pelos modelos teóricos. No Capítulo VI, testou-se o efeito directo das alterações afectivas nos níveis de T usando excertos de filmes emocionais. Um decréscimo significativo de T foi observado nos participantes da condição de tristeza, numa direcção congruente com as predicções da literatura. Finalmente, no Capítulo VII, abordou-se a função adaptiva das mudanças de androgénios induzidas pela competição proposta pelos modelos teóricos. Especificamente, testou-se o efeito do resultado da competição e dos níveis pós-competitivos de T na capacidade do individuo detectar faces emocionais ameaçadoras. Os nossos resultados sugerem que os vencedores foram mais rápidos e melhores a discriminar faces de raiva do que os perdedores. A discriminação de raiva foi também melhorada quando os níveis de T pós-competição eram elevados. No geral, estes resultados apoiam a hipótese de uma moderação cognitiva da resposta de T em mulheres. As implicações destes resultados para as teorias de modulação social de andrógenios são discutidas numa perspectiva comparada e integrativa.

ABSTRACT

This thesis aims to clarify the processes underlying the discrepancies between the direction of the androgen response to competition found in empirical studies and predictions of the theories for the social modulation of androgens. We suggest that these unpredicted results could result from interactions with cognitive variables and specifically select appraisal as a strong candidate to moderate the testosterone (T) response to social challenges. Several experiments were conducted to test this hypothesis. On Chapter II and III, we have tested the effect of opponent familiarity and the evaluation of the competition outcome as a threat/challenge on the T response to a competition with a member of the same sex. We have found that women show greater increases in T levels when they were defeated by an unfamiliar opponent and evaluated the outcome as threat. This moderation effect was not detected for men. We have continued the research on the effects of opponent familiarity and threat assessment on Chapter IV, but this time using a cichlid fish. In a paradigm of repeated territorial intrusions by stranger and familiar males, the resident male's androgen response was higher for the intrusions performed by a stranger compared to those performed by a familiar male. The effect of the expectations component of appraisal on the T response to competition in women was tested by manipulating the expectations of the participants on the outcome of the competition before the competitive task (Chapter V). We have found that the unexpected winners decreased their T levels, showing a reversal of response predicted by the theoretical models. On Chapter VI, we have tested the direct effect of affective changes on T levels using emotional film clips. T significantly decreased for those participants assigned to the sadness condition, a direction that is congruent with predictions of the literature. Finally, on Chapter VII, we have addressed the adaptive function of the androgen changes elicited by the competition, as proposed by the theoretical models. Specifically, we have tested the effect of the competition outcome and post-competition T levels on the individual's capacity to detect threatening emotional faces. Our findings suggest that winners were faster and better in discriminating angry faces than losers. Anger discrimination was also enhanced when post-competition T levels were high. Together these findings support the hypothesis of a cognitive moderation of the T response to competition in women. Results are discussed in terms of their implication to the theories for the social modulation of androgens in a comparative and integrative perspective.

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Chapter I

Social Modulation of Androgens: theories, mechanisms and functions.

Introduction

Over the last decades, accumulated evidence has revealed a reciprocal relationship between androgen levels and the social environment. As a result, androgens are no longer seen exclusively as sex steroids involved in reproduction, but rather as modulators of social behaviour. Early models for the interaction between hormones and behavior (Leshner, 1975, 1979; Mazur, 1976), already presented the core ideas that would be further developed in subsequent formal explanations, namely that androgen levels influence the behavioral response to social stimuli and that changes in androgens can be elicited by the social environment, thus creating a reciprocal interaction between androgens and behavior.

Theoretical Models for the Social Modulation of Androgens

The reciprocal model of androgens and social behavior has been formalized in two different hypotheses, each presenting different theoretical constraints and generating its own predictions. The biosocial model of status (BMS), initially proposed by Mazur (Mazur & Booth, 1998; Mazur, 1985), establishes a dynamic and mutual reinforcing relationship between androgens and social dominance. According to this model, androgens promote status seeking behaviors, and the achievement of higher status through dominance contests feeds back on the individuals' androgen levels, according to the individual's new position in the social hierarchy. Therefore, the BMS predicts that dominant individuals should have higher baseline levels of androgens than subordinates and while it is expected that winning an agonistic interaction results in increased androgen levels, establishing a positive feedback loop between status and androgens, losing such an interaction should result in decreased androgens and an inhibition of the individuals' engagement in further dominance contests (Mazur & Booth, 1998).

While the BMS focused essentially on androgens and social dominance, Wingfield and co-workers proposed the “challenge hypothesis” with the goal of providing an explanation for the interspecific seasonal variation of androgen levels, linking fluctuations in androgen levels with its functions in reproductive and aggressive contexts (Wingfield, Hegner, Dufty, Jr., & Ball, 1990). The “challenge hypothesis” (Figure 1) predicts that androgen levels increase from a non-breeding constitutive baseline (level A) to breeding season levels (level B) to allow for the expression of secondary sex characters and reproductive behaviors; short term further increases in androgen levels up to a maximum physiological level (level C) may occur in response to agonistic encounters (e.g. territorial intrusions). Recent revisions of the “challenge hypothesis” have shown that B to C increases do not reflect the effect of social challenges and in fact, across species, no correlation was found between seasonal androgen responsiveness and the androgen response to an experimental territorial challenge (Goymann, Landys, & Wingfield, 2007). These two time scales of the androgen response to the social environment are expected to rely on different mechanisms (e.g., non-genomic and genomic steroid action: (Baker, 2003; Balthazart, Baillien, & Ball, 2006), and thus should be seen as separate phenomena. For example, while the dynamic reciprocal changes of the BMS and of the acute response to a territorial intrusion in the “challenge hypothesis,” are acute and short-lived and therefore are expected to rely on either non-genomic or on transient changes in gene expression, seasonal changes in androgen responses are gradual and long-lasting, and therefore are expected to rely on genomic and epigenetic mechanisms.

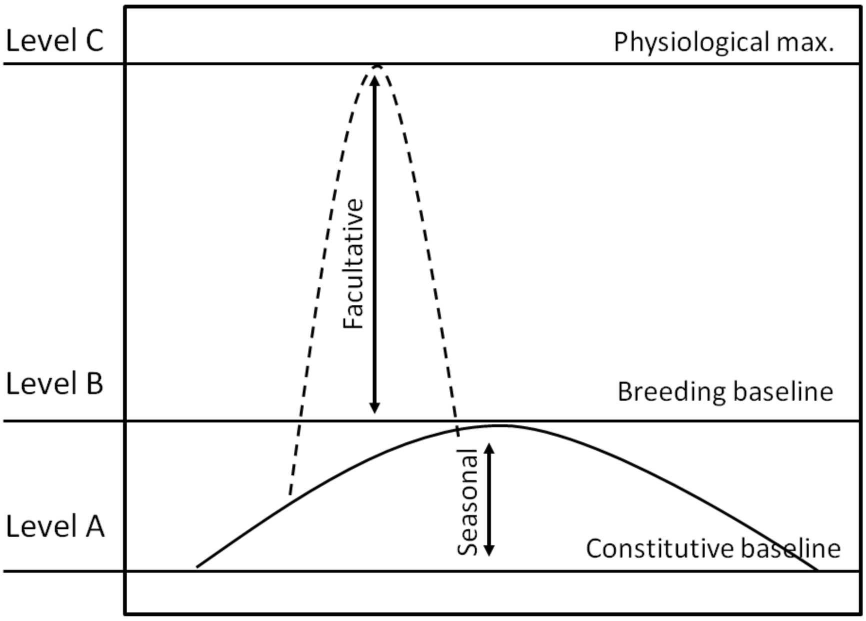

Figure 1. Representation of the androgen changes proposed by the challenge hypothesis: (A) constitutive androgen levels; (B) breeding baseline levels needed for successful reproduction; and (C) maximum physiological levels.

In conclusion, the BMS and the "challenge hypothesis" generate different predictions for the T response to a social challenge. While the BMS explicitly predicts outcome-dependent T changes for winners and losers, the "challenge hypothesis" predicts an increase in T levels for the participants involved in an agonistic encounter, without specifying the direction of the T changes when the interaction reaches a resolution. However, in both models, the T response to a social challenge is expected to be acute, short-lived and contribute to the adjustment of behavioral and cognitive processes to the changes in the social context and influence future social interactions.

Mechanisms of Androgen Response to Social Challenges

In males, most androgen production results from the activation of the hypothalamic-pituitary-gonadal (HPG) axis in which a sequential pulsatile hormonal cascade targets the Leydig cells in male gonads, to elicit testosterone (T) production and its release into circulation (Gleason, Fuxjager, Oyegbile, & Marler, 2009). In human males, a small part of the circulating levels of T originates from the adrenal glands (Laue & Cutler, 1997). In contrast, in premenopausal women, the adrenal *zona fasciculata* and the ovarian stroma contributes in equal parts to the circulating levels of T, with the remaining 50% resulting from the conversion of androstenedione (Burger, 2002; Longcope, 1986). Androgens can also be produced *de novo* in the brain from cholesterol and converted into other hormones (Schmidt et al., 2008) and both processes can be modulated by social context (Cornil, Ball, & Balthazart, 2012; Pradhan et al., 2010). In fact, studies in several taxa (fish, birds, mammals) suggest that the effects of androgens on agonistic behavior are mediated by their rapid aromatization into estrogens in the brain (Charlier et al., 2011; Huffman, O'Connell, & Hofmann, 2013; Soma, Schlinger, Wingfield, & Saldanha, 2003; Trainor, Kyomen, & Marler, 2006). Additionally, tissue sensitivity to androgens can also be socially modulated through rapid changes in the expression of androgen receptors (Burmeister, Kailasanath, & Fernald, 2007; Fuxjager et al., 2010).

The adjustment of androgen levels according to the social environment requires mechanisms that can translate and integrate multi-modal social information relevant to the organism and modulate neuroendocrine activity responsible for the production of androgens. Cichlid fish have been a very successful study model in this respect. Experiments with cichlid fish have shown how changes in social status can induce rapid changes in HPG axis activity leading to changes in circulating androgens (for comprehensive reviews see (Maruska & Fernald, 2013; R. F. Oliveira, 2009). When opportunities to ascend in social status arise subordinates can rapidly exhibit the traits of dominant fish (e.g., coloration and aggressive behavior), and sequentially increase the expression of GnRH1 in the preoptic area, pituitary gonadotropins and androgen levels (Maruska, Zhang, Neboori, & Fernald, 2013). Conversely, dominant males experiencing a decrease in social status present a reduced expression of GnRH1 and pituitary gonadotropins, and a decrease of androgen levels (Maruska et al., 2013). Furthermore, the social information signaling social opportunity seems to be conveyed by changes in the expression of the immediate early gene *egr-1* in high density GnRH1 neuron areas of the anterior preoptic area, indicating that *egr-1* is interfacing social information with the activity of the HPG (Burmeister, Jarvis, & Fernald, 2005).

Testosterone Response to Competition in Humans

In humans, sports competition and laboratory tasks have been used as a proxy for the agonistic encounters studied in non-human animals. Early research on this topic was conducted almost exclusively with men (see Appendix A) and allowed the extension of the theories for the social modulation of androgens from non-human animals to human males. On one notable exception (Mazur, Susman, & Edelbrock, 1997), women were also tested in a competition using a videogame and the general decrease of T throughout the experiment, led the authors to conclude that the T dynamics to competition could be different between the sexes. These findings along with the need to control for additional sources of T variation in women (e.g. phase of the menstrual cycle, use of oral contraceptives) and the higher levels of circulating T in men, may have contributed to a lack of investment in researching women's T response to competition. More recent studies have shown that T levels are relatively stable across the phases of the menstrual cycle (Dabbs & Rue, 1991; Liening, Stanton, Saini, & Schultheiss, 2010) and although the use of oral contraceptives may decrease the baseline T levels, they do not impair the reactivity of this hormone or change the direction of the T response to a social challenge (Edwards & O'Neal, 2009). As a consequence, the number of studies with female samples has significantly increased. Overall, these studies have shown an inconsistent pattern in both sexes, with T levels increasing in winners and decreasing in losers (as predicted by the BMS), increasing both in winners and losers, or not showing significant changes in response to the competitive event (Appendix A). It has been suggested that these heterogeneous androgen responses to competition may reflect a mediation/moderation of the androgen response by cognitive variables, such as perceived threat/challenge, mood changes, etc. (Salvador & Costa, 2009; Salvador, 2005). This interaction between cognitive processes and the androgen response is valid for both sexes and may help to explain some of the null findings reported in some female studies that had previously been attributed to differential androgen effects in women (Kemper, 1998; van Anders & Watson, 2006). Unexpected results have also been reported in male studies (Filaire, Maso, Sagnol, Ferrand, & Lac, 2001; van der Meij, Buunk, Almela, & Salvador, 2010) and therefore, it is premature to downplay the role of T in women based on competition studies that did not find results according to the predictions of the theoretical models, especially since in women, T has also been linked to status and dominance, (Cashdan, 2003; Edwards, Wetzel, & Wyner, 2006; Grant & France, 2001; Wirth & Schultheiss, 2007) and has been shown to predict the reaction to winning and losing (Mehta, Jones, & Josephs, 2008; Mehta & Josephs, 2006). Moreover, recent studies with females have shown a clear T response to competition in the direction predicted by the BMS (Jiménez, Aguilar, & Alvero-Cruz, 2012; T. F. Oliveira, Gouveia, & Oliveira, 2009), with no observed sex difference in the direction of the T response for winners and losers (Jiménez et al., 2012).

The suggested interaction between cognitive processes and the triggering of the endocrine response to competition should be seen as bidirectional, that is, not only do cognitive processes modulate the androgen response to competition but also, competition-driven changes in androgens should affect subsequent cognitive processes that are relevant to competition for status (Edwards, 2006; Mazur & Booth, 1998; Mazur, 1985). For example, the T increase after a social challenge predicted dominance and willingness to engage in competitive interactions even after losing a previous competition, and has also been linked to choosing aggression instead of behaviors that lead to economic rewards (Carré, Gilchrist, Morrissey, & McCormick, 2010; Carré, Putnam, & McCormick, 2009; Carré & McCormick, 2008; Klinesmith, Kasser, & McAndrew, 2006; Mehta & Josephs, 2006). Interestingly the behavioral effects of these heightened T levels are not necessarily associated with winning the interaction, which is contrary to what would be predicted by the BMS (ie, losers increase T, Mehta & Josephs, 2006; no effect of the perceived outcome, Carré & McCormick, 2008).

Psychological moderators and mediators of androgen response to competition

The proposed interaction between the physiological response and cognitive variables can already be found in the BMS (Edwards, 2006; Mazur, 1985). Among the suggested modulators of the T response to competition, appraisal has been mentioned as a major candidate since it is known to be a key mechanism in the activation of the physiological response to challenges in animals and humans (Edwards, 2006; Salvador & Costa, 2009) (Figure 2). According to this hypothesis, it is not only the objective characteristics of the social interaction that triggers a physiological response but rather, the evaluation of what that event means to that organism at that moment in time. As a consequence, the same exact event may elicit different responses, depending on the way it is appraised by different individuals or by the same individual at different moments in time (e.g, in different social contexts).

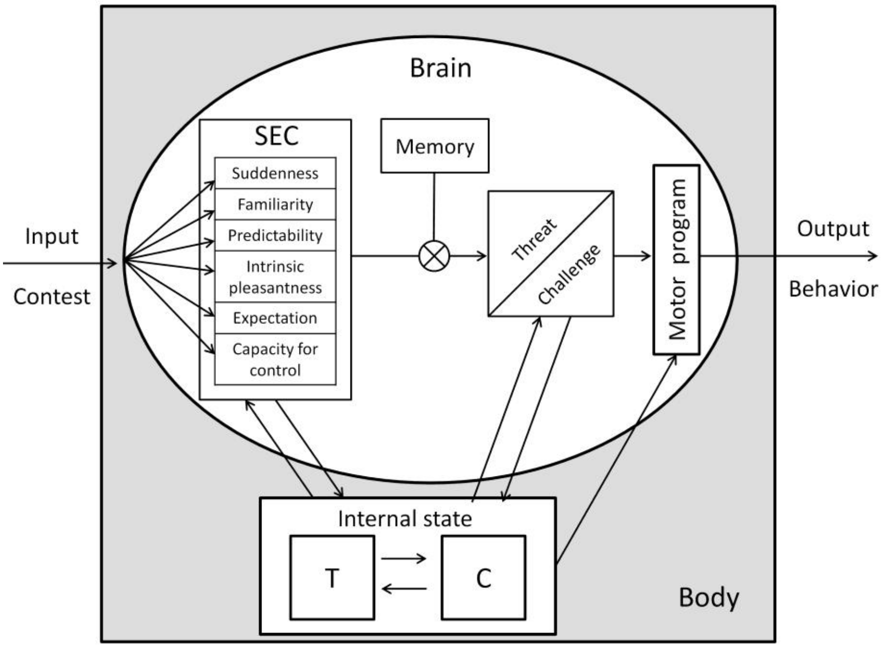

Figure 2. Hypothetical representation of the interaction between hormones and cognition, from contest appraisal to behavioral response. Abbreviations: C, cortisol; SEC, stimulus evaluation checks; T, testosterone.

Definition and models of appraisal

Currently, appraisal is defined as a transactional process between the individual and the environment, in which the individual must detect and assess the significance of an event (Moors, Ellsworth, Scherer, & Frijda, 2013; Smith & Kirby, 2009). In this interactive process, the objective characteristics of the stimuli are combined with the individual's motivational and physiological state, resulting in a circumstantial evaluation that is permeable to the individual's needs, goals and resources (Scherer, 2009a, Smith & Kirby, 2009, Roseman & Smith, 2001). Moreover, in order to generate adaptive responses, appraisal must be understood as a fundamentally dynamic and recursive process in which previous evaluations are monitored and updated based on the constant inflow of sensorial information originating from the environment and/or from changes in the internal state (K. R. Scherer, 2001, 2009b).

Historically, the first appraisal theory was proposed by Magda Arnold (1960) and according to this author an event must be evaluated across three appraisal dimensions: the evaluation of the object *per se* (beneficial, harmful), its presence or absence and the difficulty of attaining or rejecting the object. The combined outcome of these appraisal dimensions would lead to action tendencies and physiological reactions (not simple reflexes), experienced by the individual as emotions, that should not be (Arnold, 1960). Perhaps the most influential of the appraisal theorists, Lazarus (1966, 1991) proposed a cognitive-motivational-relational theory composed by primary and secondary appraisal. In primary appraisal, the individual must evaluate the event on a set of three components (goal relevance, goal congruence and type of ego-involvement) in order to assess the relevance of the event for its well-being (Lazarus, 1966). Secondary appraisal consists of an evaluation of the resources and coping options available to deal with the motive-relevant event. Blame or credit, coping potential and future expectations are the components proposed for secondary appraisal (Lazarus, 1991). It has been suggested (Reisenzein, 2006) that the structural differences between these theories are mostly terminological, since Lazarus's secondary appraisal matches Arnold's third dimension of appraisal and primary appraisal could be understood as a combination of Arnold's first two appraisal dimensions (evaluation and presence-absence).

Later theories of appraisal (Frijda, 1987; Roseman, Spindel, & Jose, 1990; Scherer, 1984; Smith & Ellsworth, 1985) substantially agree on the structure of appraisal, although the number of components, terminology (e.g. predictability - Scherer, 1984; certainty - Smith & Ellsworth, 1985) and operationalization (e.g. categorical vs. dimensional) of these variables may differ. Most appraisal theories include a form of novelty, intrinsic pleasantness, predictability, goal significance, agency, coping potential and compatibility with personal and social standards as dimensions of appraisal (Ellsworth & Scherer, 2003). Other theorists, besides the breaking down of appraisal into components, postulate a supra-ordinate dimension that emerges from a set of appraisal dimensions as an integrated synthesis and therefore cannot be reduced to its' composing parts (Smith & Lazarus, 1993).

Although the models mentioned above recognize a bi-directional mutual feedback relationship between components, the order in which these components are activated for information processing is still debated. A fixed order of activation from the preliminary closure of simpler appraisals, to more complex appraisals has been justified from a perspective of system economy and logical dependency (K. R. Scherer, 1984, 2009b), while other authors argue that processing efficiency requires a flexible order of evaluations, to avoid structural looping and to focus on the more ambiguous components (Smith & Lazarus, 1993). Ellsworth (1991) proposed a middle ground theory according to which the early components must be activated first to start the appraisal process and all subsequent evaluations can take place in flexible order.

In parallel with the structural specifications of appraisal, a theory of appraisal cannot be complete without satisfying the requirement of identifying the cognitive and physiological mechanisms that underlie the evaluations taking place in each appraisal component. Given the abundance of structural models of appraisal, it is perhaps surprising that much less research has been devoted to this aspect. For example, it has been suggested that priming and spreading of activation have been suggested as the mechanisms enabling fast, automatic and memory based forms of appraisal (Smith & Kirby, 2000). The component process model (K. R. Scherer, 2009a, 2009b) updates a previous theoretical proposal aiming to establish a phylogenetic and ontogenetic continuity of appraisal process (Leventhal & Scherer, 1987), making it particularly innovative and relevant to comparative research. Along with the structural components already discussed, in the component process model the stimulus evaluation checks (SEC) taking place in the appraisal components can be performed with different degrees of complexity and processing requirements (sensorimotor, associative and conceptual), facilitating the operationalization and research of appraisal processes in non-human animals (a proposal for the study of appraisal in non-human animals can be found in Faustino, Oliveira, & Oliveira, 2015). This proposal, along with other models already discussed, heavily contrasts with the misconception of appraisal as an exclusively deliberate and controlled process. It has been suggested that given the widespread influence of Lazarus's work the term "cognitive appraisal" might have led to a misunderstanding of the process of appraisal (e.g. Kappas, 2006). Notably, starting from Arnold's theory (Arnold, 1960) to the current models (reviewed in Moors et al., 2013), appraisal has been conceived as encompassing automatic and deliberate processes. While Lazarus's (1991) nomenclature of "cognitive appraisal" emphasizes a research focus on the more controlled processes of appraisal, but should not be interpreted as a denial of the existence of automatic processes in appraisal (Lazarus, 2001).

Evidence for appraisal in competition

The influence of appraisal on the activation of the androgen response to agonistic interactions was highlighted in an experiment with cichlid fish using mirror elicited fights, allowing the decoupling of the effects of expressing aggressive behavior from those related to the assessment of the fight outcome. In this experiment, males engaged in mirror fights and therefore with no information on the outcome of the interaction did not exhibit the androgen response observed in males fighting real opponents (R. F. Oliveira, Carneiro, & Canário, 2005; see also R. F. Oliveira & Canário, 2011). Other experiments are congruent with these findings, suggesting that the expression of aggression is not sufficient, per se, to increase androgen levels, and that animals need to assess social information about the interaction outcome and/or social context in order to trigger an androgen response (Hirschenhauser, Taborsky, Oliveira, Canário, & Oliveira, 2004; Hirschenhauser, Wittek, Johnston, & Möstl, 2008). In human competition, an experiment using a cognitive laboratory task found a connection between increases of T after the competition and opponent self-efficacy, indicating that the information gathered about the opponent was used to adjust the endocrine response (van der Meij et al., 2010). These results are compatible with the abovementioned role of appraisal and opponent assessment in animal agonistic encounters (Hirschenhauser et al., 2004, 2008; R. F. Oliveira et al., 2005). In sports, there is also correlational evidence for the effects of cognitive appraisal on T response, mostly related to the causal attribution of the competition outcome, which can be interpreted as part of the implication-assessment component of cognitive appraisal (Lazarus, 1991a). The association between post-match T and external attribution of the competition outcome has been reported as being negative for winners and positive for losers, (González-Bono, Salvador, Ricarte, Serrano, & Arnedo, 2000;

González-Bono, Salvador, Serrano, & Ricarte, 1999), while another study found no hormonal differences when the sample was split by appraisal of performance and satisfaction with the outcome (Suay et al., 1999).

Effect of territoriality and group membership on the testosterone response

Some results from human experiments suggest that men have higher T levels before matches taking place at their home venue than at away venues and also that these higher T levels are associated with higher team rivalry (Carré, Muir, Belanger, & Putnam, 2006; Neave & Wolfson, 2003). Yet another study showed that a home field victory led to higher postgame T than when victory was achieved at the opponent's venue, but the aforementioned effect of game location on pre-game T levels was not found (Carré, 2009). The same authors proposed that the absence of this effect could be due to the sample, which consisted of amateur rather than elite players.

The identified connection between territorial behavior and team rivalry also brings into question what role is played by the individual group membership when facing a dominance contest. Early evidence of the moderating effect of group membership was found in a domino competition between neighboring Caribbean villages. In this study, the teams competed against familiar men and also against strangers, and although the effect did not reach statistical significance, T tended to increase more before matches against neighboring villages than against teams of their own village (Wagner, Flinn, & England, 2002). Trumble et al organized a soccer tournament among the Amazonian Tsimané and attributed the lack of a winning effect on T response to the interference of in-group factors arising from a relative acquaintance between players of the opposing teams (Trumble et al., 2012). Perhaps the clearest effect of group membership on the T response to competition can be found in a study by Oxford et al that used a video game tournament, with teams competing against each other (between groups) and also, team members competing against their own team mates (within group) (Oxford, Ponzi, & Geary, 2010). Among the findings of this experiment, it was shown that men who contributed more to the team score, and thus was considered as high ranking, had a T response that was different between in-group and out-group competition (Oxford et al., 2010). The high-ranking winners had a higher T after the match when the between-groups competition was played first, but high-ranking players showed a lower T and higher cortisol

(C), independently of winning or losing the match, in the within-group competition (Oxford et al., 2010).

Effect of individual characteristics on the testosterone response

Along with the social and cognitive variables, individual characteristics have also been proposed to play some role in the endocrine response to competition. Power-motivated individuals use assertiveness to achieve an impact on others, while obtaining reward and reinforcement from those actions. Thus, implicit power motivation predicts many of the dominance behaviors usually associated with high T (Stanton & Schultheiss, 2009). Experiments using a contrived competitive task showed that individuals with high power motivation had the highest T levels after winning, but no association was found between personalized power and high T for losers (Schultheiss, Campbell, & McClelland, 1999). Furthermore, high implicit power motivation predicted stronger increases of T post-contest for male winners with low activity inhibition (used as a measure of impulse control) and also enhanced implicit learning (Schultheiss & Rohde, 2002). Sex differences and similarities for the effect of implicit power motivation on T have also been reported (Schultheiss et al., 2005). In these experiments, high implicit power motivation predicted increases of T after the contest for men and women in the winner condition, but the T response in the loser condition was moderated by sex, where power motive was a negative predictor of T for men and a positive predictor of T for women (Schultheiss et al., 2005). The increased T levels found in women in the loser condition were interpreted as readiness to reengage in competition, after the power goal was not achieved (Schultheiss et al., 2005).

The Dual-hormone Hypothesis of Neuroendocrine Response to Social Challenges

Besides the role of psychological variables in the androgen response to social challenges, there is growing evidence that both dominance behavior and T levels change after a contest and that both could be moderated by baseline levels of C and T, reflecting an endocrine interaction between the hypothalamic–pituitary–gonadal and the hypothalamic–pituitary–adrenal axis (Viau, 2002). For example, basal T levels have been shown to predict the C response to winning or losing a competition (Mehta et al., 2008), positive correlations between T and overt aggression have only been found when C levels are low (Popma et al., 2007) and a T/C ratio has been proposed as a marker for the propensity for aggressive behavior (Terburg, Morgan, & Van Honk, 2009). This set of results led to the recent proposal of a dual-hormone hypothesis for the regulation of dominance (Mehta & Josephs, 2010). The proposers of this hypothesis found evidence that the association between T and dominance was moderated by basal C levels in both sexes, with higher T predicting higher dominance scores only when baseline C levels were low. Furthermore, high dominance after losing a competition was predicted by high pre-competition T and low pre-competition C and this relationship was reversed when individuals showed high C levels before the competition (Mehta & Josephs, 2010). This hypothesis received further support in a recent study that found the same pattern of response in the winners of a video game contest; in this study, an increased post-competition T was found when the winning participants presented a high baseline T and low baseline C (Zilioli & Watson, 2012). Together, these results support the idea that the promotion of status-seeking behaviors by high T only occurs when C levels are low. It should be noted here that the dual-hormone hypothesis establishes the interaction between T and C based on acute responses to social challenges, and this mutual regulatory pattern may not account for the changes in hormone levels occurring under chronic events (Gettler, McDade, & Kuzawa, 2011).

Function of the Androgen Response to Social Challenges

The fact that androgen levels change in response to the perceived outcome of an interaction, and not merely by experiencing an agonistic interaction raises the hypothesis that socially driven changes in androgen levels will not directly affect the current interaction, for which the outcome has already been established, but should rather modulate behavioral expression in subsequent social interactions (R. F. Oliveira, 2009). Interestingly, Leshner's (1975) proposal for the reciprocal model had already hinted that the hormone response should modify future behavior when the individuals are facing a similar challenge, and both the BMS and the challenge hypothesis have also implicitly assumed that the adaptive function of the social modulation of androgen levels is to fine tune the expression of androgen-dependent behavior according to the perceived social environment.

More recently, this view has been formalized as the Winning hypothesis (Oyegbile & Marler, 2005) according to which changes in the probability of winning future interactions driven by the success in previous ones (i.e., winner/loser effect; Hsu, Earley, & Wolf, 2006), could be mediated by post-contest transient changes in androgen levels. This hypothesis is currently supported by several lines of evidence. In cichlid fish winner effects can be blocked (i.e., reduction of the winning probability of previous winners from ca. 90% back to chance levels) by the exogeneous administration of the anti-androgen cyproterone acetate to the winners of the first interaction between the agonistic encounters (R. F. Oliveira, Silva, & Canário, 2009). In California mice (*Peromyscus californicus*), in the emergence of the winner effect during successive social interactions is paralleled by increased levels of androgens after cumulative winning experience (Oyegbile & Marler, 2005). Furthermore, unlike the California mice, the white-footed mouse (*Peromyscus leucopus*) does not form a winner effect or respond to a contest with increased T, but a robust winner effect can be induced in this species via a post-contest administration of T (Fuxjager, Montgomery, & Marler, 2011). As it has been previously suggested, it is possible that these effects could result from the aromatization of T in the brain (Trainor et al., 2006). In humans, there is preliminary evidence showing that the winner effect is also present in human males (Zilioli & Watson, 2014) and it is known that increased androgen levels after a competition predict the willingness to engage in further contests, even after losing the first interaction (Carré & McCormick, 2008; Mehta & Josephs, 2006).

Effects of androgens on psychological parameters relevant to performance in competition

One assumption of the Winning hypothesis is that socially driven changes in androgen levels modulate the expression of variables relevant for success in subsequent social contests. Given the time frame of this response these variables are expected to be of the cognitive (i.e., information-processing) domain. Most of the evidence for the effects of androgens on cognitive variables comes from research using paradigms that involve the administration of exogenous T to animals and humans (for a review see Bos, Panksepp, Bluthé, & Van Honk, 2012). The effects of T on social cognition have also been investigated, using endogenous baseline measures of T or via environmental manipulations that induced a change in androgen levels within the physiological range of the individual. Results from these experiments should be compared with those arising from paradigms involving exogenous administration of T – with exogenous administration of T, the dose-response curve follows an inverted U-function, and this procedure may lead to pharmacologically induced supraphysiological hormone levels (Adkins-Regan, 2005).

Given the importance of T in social challenges, experiments have been planned to investigate the relationship between T and the variables involved in threat detection. In a social environment, the rapid detection of threatening stimuli is critical for survival, and the presence of an efficient threat detection system can be seen as an evolutionary adaptive advantage since it allows the appropriate selection of a fight-or-flight response. In humans, facial expressions of fear and anger have been used as signals of threat and are known to elicit an adaptive response on the observer (Parkinson, 2005). Research shows that there is an attentional bias toward threat stimuli and that anxiety and vigilant behavior play a role in this effect (Bradley, Mogg, Falla, & Hamilton, 1998; Bradley, Mogg, & Millar, 2000). The fear-reducing properties of T have already been demonstrated in animals (Aikey, Nyby, Anmuth, & James, 2002; Frye & Seliga, 2001) and the convergence of experiments using different paradigms suggests that this T effect on fear is also present for humans. Participants who had their levels of T experimentally increased showed a decreased unconscious vigilant emotional response to masked fearful faces when compared with a placebo group, but T had no effect on self-reported measures of anxiety (Van Honk, Peper, & Schutter, 2005). Furthermore, exogenous T reduced the fear-potentiated startle reflex and lowered the electrodermal response to negative stimuli, which can be interpreted as an attenuation of the sympathetic components of the stress response (Hermans, Putman, Baas, Koppeschaar, & van Honk, 2006). This effect was also enhanced in participants with initial high-anxiety and high-reactivity to affective startle modulation (Hermans et al., 2007). Moreover, in an experiment using a video morphing a neutral to angry facial expression, anger was consciously detected at a later stage for the participants on the T administration condition compared to placebo (van Honk & Schutter, 2007). Overall, these experiments suggest that the impaired unconscious threat perception, as measured by decreased selective attention to threatening faces after administration of T, might be mediated by the fear reduction properties of T (Hermans, Putman, Baas, et al., 2006; Hermans et al., 2007; Van Honk et al., 2005). Social aggression may be also be facilitated by T, for example, it has been found to increase the cardiac response in participants exposed to angry faces, which can be seen as readiness to aggressively engage in status contests (Van Honk, Tuiten, & Hermans, 2001) and to increase risk-taking behavior by increasing reward and lowering punishment sensitivity (Van Honk et al., 2004).

In all the studies mentioned above, T was increased to supraphysiological levels and only female participants were included. However, Wirth and Schultheiss found similar patterns with endogenous T for men and women. In this study (Wirth & Schultheiss, 2007), basal morning T levels were related to a greater interference with supraliminal angry faces, in an emotional Stroop task, congruent with previous research (Van Honk et al., 1999; van Honk et al., 2000). T also predicted an attentional bias away from angry faces in a dot-probe task (Wirth & Schultheiss, 2007), consistent with the anxiolytic effect of T (Hermans, Putman, Baas, et al., 2006; Hermans et al., 2007; Van Honk et al., 2005). Raising androgens to supraphysiological levels has also been shown to affect interpersonal factors. In fact, T down-regulated interpersonal trust in over-trusting individuals, preparing them for possible competition for status and resources (Bos, Terburg, & van Honk, 2010), and reduced facial mimicry, a critical function in communicating empathy toward conspecifics (Hermans, Putman, & Van Honk, 2006). Furthermore, sublingual T administration induced a marked impairment on the Reading the Mind in the Eyes Task (a test that has been used as a measure of social intelligence and cognitive empathy), but this effect was only found in individuals with high fetal exposure to T, as measured using the ratio of the length of the second and fourth finger of the right hand (2D:4D) (van Honk et al., 2011). These results should be noted with caution since it is still questionable whether the 2D:4D finger index is a valid biomarker of prenatal androgen exposure. For example, the 2D:4D ratios of women with complete androgen insensitivity syndrome are not significantly difference from a control group with normal women, despite the ineffective androgen exposure *in utero* (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009).

Effects of androgens on economic behavior

The effect of T in the context of economic behavior and decision making has been studied, mostly using the “ultimatum game” (UG). In this game, a proposer makes an offer to a responder on how to divide an endowment and the receiver has to decide whether to accept or reject the offer. Acceptance implies the division of the sum as suggested by the proposer, whereas rejection implies that none of the participants will receive any money. In this paradigm, offers of less than 20% of the total sum are considered unfair and are frequently rejected by the receiver (Camerer, 1997). In men, a positive associations was found between baseline T and the rejection of low game offers, suggesting that in settings with repeated interactions, punishment may enhance the reputation of the punisher and alter the behavior of the punished (Burnham, 2007). Likewise, another study found a positive correlation between T and unfair offer rejection, and this effect of T was similar in men and women (Mehta & Beer, 2010).

To clarify the effects of T on fair offers, experiments involving the administration of T have been conducted, with mixed results. An experiment by Zak et al. (2009), used a gel carrying 1% of T and found an effect of this androgen on offer generosity, wherein proposers who received T made offers 27% lower than those who received placebo. However, this difference between groups disappeared with repeated play. In contrast, another experiment with female participants who were given a sublingual administration of T, reported that T had no effect on rejection behavior but also, that the group given T presented higher offers to the receiver than did the placebo group (Eisenegger, Naef, Snozzi, Heinrichs, & Fehr, 2010). This study also found an interference of the participants' beliefs about the effects of T, since lower offers in the UG were presented by individuals in the placebo condition who believed they were given T. A recent paper analyzing the dynamics of T absorption when administrated by a gel (Eisenegger, von Eckardstein, Fehr, & von Eckardstein, 2012), suggested that the study by Zak et al. (2009) tested subjects 13 hours after the peak of T levels. Although these results reveals a mishap in the sampling time of the Zak et al. (2009) experiment, it cannot fully explain the contradictory results concerning the effects of T on fair bargaining, since the experimental subjects in this experiment still presented free T levels that were 97% higher than their baseline measure.

The prosocial dimensions of T hinted in a previous experiment (Eisenegger et al., 2010), that suggested that T could enable the individual to secure important resources and a high status through cooperation, found supporting evidence in a recent study using the “public goods game” as an experimental task (van Honk, Montoya, Bos, van Vugt, & Terburg, 2012). Using this economic game, the authors removed the possibility that the fair behavior found in the UG could be due to the threat of financial punishment. This experiment showed that the effect of T on cooperation was moderated by the 2D:4D ratio, since only participants with a high 2D:4D ratio (hypothetically with low fetal androgen exposure) contributed more monetary units after receiving exogenous T (van Honk et al., 2012).

As mentioned in a previous section, it has previously been shown that T administration elicited increased risk taking and that this was associated with changes in punishment and reward sensitivity (Van Honk et al., 2004). Risk-taking has also been positively correlated with endogenous salivary T levels, in men playing an investment game (Apicella et al., 2008), and risk-aversion has been negatively correlated with T levels, in women (Sapienza, Zingales, & Maestripieri, 2009; for a debate on these results see also Joel & Tarrasch, 2010). Recently, a study with a sample of both sexes, also found a nonlinear U-shaped association of endogenous T with risk taking and with ambiguity preference in economic decision-making (Stanton et al., 2011). In this experiment, a similar pattern of response was found for men and women – individuals presenting low and high T (ie, below and above 1.5 standard deviations from their sex mean, respectively) were neutral to risk and ambiguity, whereas risk and ambiguity aversion were found in those with mid levels of endogenous T (Stanton et al., 2011).

Functional neuroimaging evidence for the action of androgens in psychological parameters

Recently, studies that have employed functional magnetic resonance imaging (fMRI) helped to shed light on: 1) how the endocrine system interacts with target brain areas when individuals are presented with threatening stimuli; 2) how the changes in neural activity may explain the relation between hormones and behavior. For example, female participants with high T and C have a stronger subcortical response to social threat and that after T administration, there is a greater activation of the amygdala and the hypothalamus compared to placebo (Hermans, Ramsey, & Van Honk, 2008). Moreover, the administration of T to middle-aged women with an age-related decrease in androgen levels restored the amygdala activation in response to threatening stimuli to the levels found in younger women, supporting the hypothesis of T regulating amygdala activity (van Wingen et al., 2009).

Studies with endogenous T are congruent with the findings described above and have also found a positive association between T and amygdala activation, specifically for angry and fearful faces (Derntl et al., 2009; Manuck, Marsland, Flory, & Gorka, 2010). However, differences in amygdala reactivity have been found to depend on the variation in length of the trinucleotide cytosine-adenine-guanine (CAG) in the androgen receptor gene. Activation of the dorsal amygdala was not affected by the number of CAG repeats, but a high number of CAG repeats was associated with low ventral amygdala reactivity (when corrected for salivary T levels), suggesting that the androgen effect on the activation of this area of the amygdala may be moderated by variations in the length of CAG in the androgen receptor gene (Manuck et al., 2010).

The orbitofrontal cortex (OFC), a brain region involved in impulse control and emotional regulation that is functionally and anatomically connected with the amygdala, has also been identified as a possible moderator of the effect of T in amygdala reactivity (Coccaro, McCloskey, Fitzgerald, & Phan, 2007; Ochsner & Gross, 2005). After T administration to women, participants showed a reduced functional connectivity between the amygdala and the OFC, suggesting that T may reduce the inhibitory control of the OFC over the amygdala (van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010). This finding is also congruent with a previous experiment, that measured endogenous T in both sexes, reporting that the effect of T on aggression is mediated by the activity of the OFC, with T increasing the propensity for aggressive behavior, due to reduced activation of the OFC (Mehta & Beer, 2010). Evidence has also been found for the effects of T in activating the mesolimbic dopaminergic circuits involved in reinforcement regulation and incentive processing. In one study, female participants with low intrinsic motivation showed an increased activation of the ventral striatum (a target area of the mesolimbic dopaminergic system) in anticipation of a reward after T administration, while those with high motivation showed no further enhancement by T (Hermans et al., 2010).

Modulation of Social Decision-Making Mechanisms in the Brain by Peripheral Hormones

One major question to the adaptive value of the peripheral (e.g. gonadal) changes in androgens induced by social challenges is why should the central nervous system, that controls the production of these hormones through the HPG axis, be open to the influence of the peripheral hormones in order to regulate its social-decision making processes.

If one considers that the social environment is sensed by the brain and that the androgen response to it is a top-down process conveyed by the HPG axis, then, under classical models of cognition, the involvement of peripheral androgens in the modulation of a central decision-making process seems redundant, since the decision-making mechanism already has the relevant information on the social environment and could provide a faster and more economic response per se. However, if one shifts perspective toward embodiment as an essential component of cognition, then neuroendocrine axes can be seen as an example of brain-body-environmental coupling, in which upstream and downstream information relevant for the expression of appropriate social behavior are integrated, and therefore can function as a pathway for coordinated convergent adaptive responses to social change (Adkins-Regan, 2012; R. F. Oliveira, 2009). This view follows a soft definition of embodiment, since it still assumes the brain as a central processor that is merely permeable to bodily as well as environmental raw inputs. A more stringent definition of embodiment goes further, by proposing a distributed cognitive system that goes beyond the brain to include the body (therefore spreading the computational load) in an interacting goal-oriented, problem-solving system, that can be exploited by the agent replacing the need for complex internal mental representations (Beer, 2009; Wilson & Golonka, 2013).

However, just as the brain is embedded in a body, the body is embedded in an environment. This implies a connection between the behavioral agent and the physical or social environment (situatedness) and therefore the characteristics of the environment and the properties arising from this interaction can also be used by the agent to solve adaptive problems (Beer, 2009; Nolfi, 2011). What arises from this situated-embodied-dynamic framework (Figure 3) is a multi-level complex system in which adaptive behavior and cognition cannot be inferred from any of the elements in isolation as it emerges from the non-linear, dynamic interactions between and within these three foundational elements (Chiel & Beer, 1997; Nolfi, 2011; Williams & Beer, 2013). Examples of this multi-level coupling can be seen in animals, in which adequate locomotion depends not on simple neural commands, but on a multimodal integration of information that must include body and environment feedback (Dickinson, Farley, Full, & Koehl, 2000). Also supporting this idea, the body and the morphological characteristics of artificial agents do not simply feed the control center (e.g., brain) with sensory inputs; instead they allow the agent to create or elicit appropriate inputs by actively self-structuring flows of multimodal and temporally specific environmental information into sensorimotor networks, linking information structure from motor activity and information processing in the brain (Lungarella & Sporns, 2005, 2006).

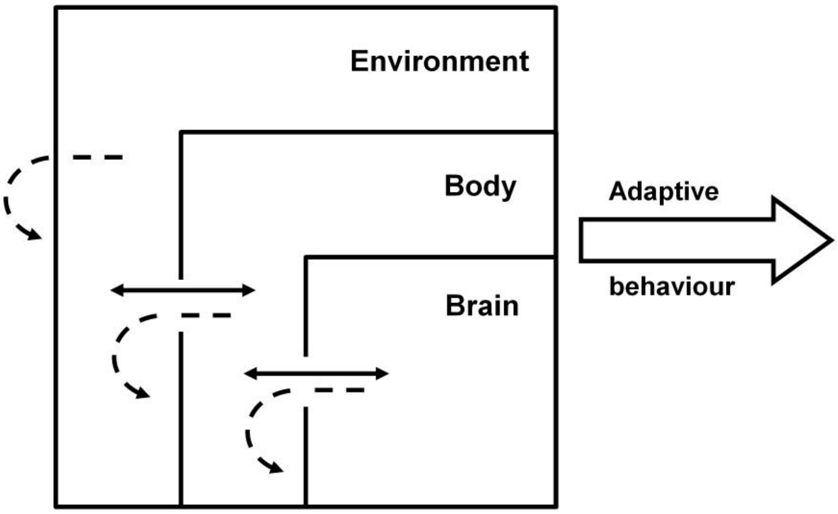

Figure 3. Schematic representation of the situated-dynamic-embodied framework with adaptive behavior resulting from the emergent characteristics of brain-body-environment coupling and not from singular contribution of the components. Full arrows represent dynamic processes between the components. Dashed arrows represent the dynamic processes within the components.

Therefore in embodied agents, a neuromodulatory system, such as the androgen reciprocal model discussed here, must be able to continually guide plasticity, while stabilizing and maintaining previously acquired adaptive structures, and to adapt the agent to variation in behavior, physiology, and external stimuli (Alexander & Sporns, 2002). This definition is compatible with the current hypothesis for the role of androgens on social decision-making mechanisms that has lost the assumptions of causality to focus more on a systems perspective. Empirical evidence for this process can be found in the examples described above (section IV) referring to the effects of T administration, which within a situated-embodied-dynamical framework, can be seen as an experimental manipulation of the information carried by the peripheral signaling of T that is being translated into systemic changes in the brain-body-environment coupling.

Although the neuromodulatory effects of peripheral androgens are well documented, a challenging puzzle arises when one has to account for the dynamics of evolution and the function that peripheral androgens have in this process. If adaptive behavior emerges from brain-body-environment continuous and dynamical interaction, evolution should not select individual components but variations of systemic couplings responsible for the emergent characteristics that originated behavioral efficacy (Beer, 2009). Androgens may play a role in this process by stabilizing the system via pleiotropic effects on neural-dynamics and on relevant body components that could be rapidly enhanced by transient increases in androgens (R. F. Oliveira, 2009). Evidence for non-genomic effects on bodily components can be found in the literature (e.g., review by Rahman & Christian, 2007). For example, acute increases of T enhanced 2-deoxyglucose uptake in cultured myotubules within 1 min (Tsai & Sapolsky, 1996) and increased the intracellular concentration of calcium suggesting the existence of a G protein-linked membrane receptor in skeletal muscle cells (Estrada, Espinosa, Müller, & Jaimovich, 2003). Also, rapid effects of T on vasorelaxation at micromolar concentrations have been reported in several species (Jones, Hugh Jones, & Channer, 2004).

In conclusion, the apparent paradox of social challenges eliciting increases in peripheral androgen levels at a greater cost (Wingfield, Lynn, & Soma, 2001) when brain androgen synthesis is available to the organism, may be better understood by integrating its' action both on neural circuits and on bodily parameters relevant to behavioral performance, influencing the emergent characteristics of the brain-body-environment coupling itself and thus reducing the fitness variability of the expressed phenotypes. Although further research is required to support these claims, previous work (Johnson & Whalen, 1988) proposed that in male mice the signaling of gonadal hormones on brain areas is required to regulate and reduce the inter-individual differences in aggressive behavior observed in gonadectomized animals, that are not present in gonadally-intact or castrated mice treated with T. In our view, these experiments can be seen as an example of how the characteristics of the systemic coupling can be skewed into more variable behavioral outputs when body signaling is disrupted, and restored to a finer context dependent response by restituting the signal to congruent levels. This suggests that body signaling is necessary for effective couplings that generate more adaptive patterns of response and this goal could be achieved by narrowing the degrees of freedom for possible fitness outcomes that could be obtained from the multiple combinations of the components involved in the dynamical system. Although most of the research presented here focused on males, this conceptual framework is expectable to also apply to females, at least for humans where recent studies suggest the similar patterns of androgen responsiveness to social competition in both sexes (Jiménez et al., 2012). However, given the possible sex differences in androgen modulation and signaling integration in central systems across different taxa, further research is needed to fully establish this approach in both sexes.

Summary

The androgen response to social challenges is present in a wide range of animals, including humans. Overall, the literature reviewed here suggests that the T response to competition in humans displays a high degree of variability and violates the patterns of response predicted either by the BMS or by the challenge hypothesis. It has been suggested that this large scope of variation in androgen responsiveness could be explained by the interaction between androgens and other modulators of the social decision-making network in the brain (e.g. dual-hormone hypothesis, Mehta & Josephs, 2010) and by psychological variables (e.g. Salvador & Costa, 2009). The possible interaction between cognition and T was first hypothesized in the BMS (Mazur, 1985), nevertheless, it is interesting to note that the recent revisions of the challenge hypothesis, partly motivated by the differences in the direction of T response to territorial intrusions, also contemplates an interaction between T and intra-individual processes (eg. cognitive variables, Kempenaers, Peters, & Foerster, 2008). Experimental testing of these assumptions should be addressed in future studies. Focusing on the relative contributions of these psychological and physiological moderators and on the interactions between them, should provide new perspectives on current contradictory results.

Objectives and Description of the Empirical Chapters

The empirical work presented in this thesis aims to test and clarify processes that are relevant to the core predictions of both theories for the social modulation of androgens. Based on previous correlational work, we suggest that cognition is a key modulator of the androgen response elicited by social challenges, which could explain the contradictory results reported for the T response to competition. To test this hypothesis, we have conducted a series of experiments in which the contribution of specific appraisal components and other psychological variables are explicitly tested. Moreover, the hypothesis that the competition induced changes in T levels are adaptive and allow the adjustment of behavioral and cognitive processes to the social environment, is still neglected in the literature compared to the amount of work testing the T response to social challenges. An experiment has also been designed in order to test this prediction. Finally, since the theoretical models were first developed based on experiments with male samples and most of the work included in this thesis was conducted with female participants, this thesis may contribute to clarify to which degree the predictions of the BMS and the Challenge hypothesis can be extended to women.

Specifically, on Chapter II we have tested the effects of opponent familiarity and the appraisal of the competition outcome as a threat/challenge on women's T response to competition, using a laboratory task in a face-to-face competitive setting. The experiment reported on Chapter III uses the same paradigm and measures in an attempt to extend to males the findings reported on Chapter II. Moreover, on Chapter IV, we continue the research on the effects of opponent familiarity and threat assessment, but this time using a cichlid fish. In territorial species, like the Mozambique tilapia (*Oreochromis mossambicus*), resident males respond with higher levels of aggression to territorial intrusions by stranger males than by neighbors. This phenomenon is known as the "dear enemy" effect. Using a paradigm of multiple territorial intrusions by neighbors and strange males, we hypothesize that the "dear enemy" effect also modulates the androgen response to territorial intrusions.

In a recent experiment, losers increased T levels after the competition when they were more surprised to have been defeated (Zilioli, Mehta, & Watson, 2014). Based on appraisal theory, we have reinterpreted the emotional state of surprise as the endpoint of an appraisal process characterized by a violation of expectations (K. R. Scherer, Zentner, & Stern, 2004). On Chapter V, we have experimentally manipulated the participant's expectations on winning or losing a competition, in order to test the effect of this appraisal component on the T response to competition. Furthermore, by modeling an unstable hierarchy with unexpected changes in high and low status (e.g. violation of expectations condition), this experiment will also allow us to compare the changes in T levels driven by status instability with the classic predictions of the BMS.

Changes in the emotional state of winners and losers was one of the first proposed mediators of the T response to competition (Mazur & Lamb, 1980) and this hypothesis was supported in a recent review (Chichinadze, Lazarashvili, Chichinadze, & Gachechiladze, 2012). One implication of this hypothesis is the existence of a direct effect of emotional changes on T levels that should be observed even in the absence of a competitive setting. The experiment reported on Chapter VI, tests this hypothesis using an emotion induction paradigm in order to experimentally manipulate the participant's levels.

Finally, on Chapter VII we test the adaptive function of the competition induced changes in T levels on threat detection. In this experiment, after a competitive task, the participants completed an emotion identification task composed by neutral, happy, fearful and angry facial expressions. Signal detection theory was used to identify the cognitive process modulated by the competition outcome and post-competitive androgen levels.

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Chapter II

Threat perception and familiarity moderate the androgen response to competition in women

Abstract

Social interactions elicit androgen responses whose function has been posited to be the adjustment of androgen-dependent behaviors to social context. The activation of this androgen response is known to be mediated and moderated by psychological factors. In this study we tested the hypothesis that the testosterone (T) changes after a competition are not simply related to its outcome, but rather to the way the subject evaluates the event. In particular we tested two evaluative dimensions of a social interaction: familiarity with the opponent and the subjective evaluation of the outcome as threat or challenge. Challenge/threat occurs in goal relevant situations and represents different motivational states arising from the individuals' subjective evaluation of the interplay between the task demands and coping resources possessed. For challenge the coping resources exceed the task demands, while threat represents a state where coping resources are insufficient to meet the task demands. In this experiment women competed in pairs, against a same sex opponent using the number tracking test as a competitive task. Losers appraised the competition outcome as more threatening than winners, and displayed higher post-competition T levels than winners. No differences were found either for cortisol (C) or for dehydroepiandrosterone. Threat, familiarity with the opponent and T response were associated only in the loser condition. Moderation analysis suggests that for the women that lost the competition the effect of threat on T is moderated by familiarity with the opponent.

Keywords: cognitive appraisal, threat, challenge, familiarity, testosterone, competition

Introduction

The responsiveness of androgens to social interactions has been established in behavioral endocrinology for many years (R. F. Oliveira, 2004; Wingfield, Hegner, Dufty, Jr., & Ball, 1990). Testosterone (T) is known to respond in anticipation to a social challenge and as a function of its outcome, and this response is moderated by social context (Hsu, Earley, & Wolf, 2006; R. F. Oliveira, 2009). According to the "challenge hypothesis" (Wingfield et al., 1990), these changes in T levels have the function of adjusting the expression of T-dependent aggressive behavior to social context, thus avoiding the costs associated with keeping chronically elevated T levels when no social challenges are present or anticipated. The challenge hypothesis was originally proposed in birds to explain inter-specific variation in androgen response to social challenges (Wingfield et al., 1990) and it has been subsequently extended to other taxa from invertebrates to humans (Archer, 2006; Hirschenhauser & Oliveira, 2006; Scott, 2006). Although most studies investigating the androgen response to social interactions have focused on T, recent studies have shown that in species that exhibit aggressive behavior outside the breeding season, when gonadal steroids are low, adrenal androgens such as dehydroepiandrosterone (DHEA) may also regulate aggressive behavior (Soma, Scotti, Newman, Charlier, & Demas, 2008; Wingfield, Lynn, & Soma, 2001). In humans, DHEA is also a major circulating androgen, mostly produced in the adrenal cortex and has known effects on aggressive behavior, emotion processing, and cognitive functions (Soma et al., 2008; Wolf & Kirschbaum, 1999). A negative correlation between changes in DHEA and avoidance behavior has been reported (Rasmusson et al., 2004) and adolescent girls with aggressive conduct disorders show lower cortisol to DHEA ratios when compared with girls with non-aggressive conduct disorder (Pajer et al., 2006).

Despite the findings that in women, T predicts the reaction to winning or losing (Josephs, Sellers, Newman, & Mehta, 2006; Mehta, Jones, & Josephs, 2008) and that this androgen is associated with status and dominance (Cashdan, 2003; Edwards, Wetzel, & Wyner, 2006; Grant & France, 2001; Wirth & Schultheiss, 2007), only recently a winner/loser effect in the direction predicted by the challenge hypothesis was reported in a female soccer competition (T. F. Oliveira, Gouveia, & Oliveira, 2009). Further evidence for the relevance of investigating the responsiveness of T in competing women was recently provided by Jiménez et al. (Jiménez, Aguilar, & Alvero-Cruz, 2012), who showed that men and women present the same pattern of T variation (a winning/losing effect) in response to a competitive event. Previous research had shown post-competitive increases of T both in winners and losers

(Bateup, Booth, Shirtcliff, & Granger, 2002; Edwards & O’Neal, 2009; Edwards et al., 2006; Hamilton, van Anders, Cox, & Watson, 2009). These contradictory results can be due to a modulatory role of psychological variables that have not been accounted for in previous studies. For instance, van der Meij et al. (van der Meij, Buunk, Almela, & Salvador, 2010) in an all male sample found a post-competitive increase of T for both winners and losers that was moderated by opponent self-efficacy (i.e., higher T response when the opponent had higher self-efficacy). Thus, the within-species variation in the androgen responsiveness to competition that has been documented both in females and males across different studies may be due to a moderator role of conditional and contextual variables (Archer, 2006; Salvador & Costa, 2009). This view has prompted the quest for the identification of psychological moderator and mediator variables between competition and androgen responsiveness in human research, where sports competition or vicarious competition laboratory tasks are used as proxy for dominance contests (for reviews see Archer, 2006; Carré, McCormick, & Hariri, 2011; Salvador & Costa, 2009; Salvador, 2005; van Anders & Watson, 2006). Personality traits (e.g., implicit power motivation and coping styles) and affective and cognitive variables (e.g., causal attribution, mood, and perceived self-efficacy of the opponent) have been shown to have an effect on the androgen response (Salvador & Costa, 2009; Salvador, 2005; Stanton & Schultheiss, 2009; van der Meij et al., 2010).

One key set of moderator variables of the androgen response is the cognitive appraisal of the competition (i.e., the significance of competition to the individual), such that rather than the objective structure of the competition it is the subject’s perception of the event that triggers the androgen response (R. F. Oliveira, Carneiro, & Canário, 2005). Within this hypothesis, psychological variables that are central for the appraisal of the competition consequences to the subject, such as perception of the outcome as threat vs. challenge and the familiarity with the opponent, have not been investigated in humans so far. Although rooted in the classic appraisal theory (Lazarus, 1991; Scherer, 2001) the processes investigated here are less conscious and more automatic than the ones usually labeled as “cognitive appraisal” in the appraisal psychology literature. Therefore, following Blascovich (Blascovich, 2008) hereafter we will use the term “evaluation” to refer to these processes.

Challenge and threat represent person-situation evoked motivational states, that can drive behavior and increase performance, involving the interplay of affective (feelings and emotions) and cognitive processes (attention and appraisal). Challenge and threat occur in goal relevant situations; they present different patterns of psychological and physiological response, and depend of the balance between the event demands and the perceived coping capacity of the individual (Blascovich & Mendes, 2000; Tomaka, Blascovich, Kelsey, & Leitten, 1993; Tomaka, Blascovich, Kibler, & Ernst, 1997). Evaluation of an event as a threat can occur when the resources of the individual are insufficient to meet the demands (Tomaka et al., 1993, 1997). Individuals with a threat evaluation report higher subjective stress and display lower cardiac reactivity (i.e., heart rate, pre-ejection period and cardiac output) and increased vascular resistance (i.e., vasoconstriction).

The evaluation of an event as challenge appears when the individual experiences sufficient resources to meet the event demands (Tomaka et al., 1993, 1997). There is lower subjective stress when compared to the threat response and it is accompanied by high cardiac reactivity and low vascular resistance, which have been interpreted as a marker of the individual effort to cope with the task demands and mobilize resources to remain in control of the situation (Tomaka et al., 1993, 1997). There is also some evidence that the physiological response is not causally antecedent to the evaluation reported by the individual, as the manipulation of the specific pattern of physiological activity of threat and challenge did not produce an evaluation of a stressor consistent with the physiological activation (Tomaka et al., 1997). Furthermore, since appraisal is also a continuous evaluation process that is updated by the constant flow of information that the organism receives from the environment, the appraisal process implies a subjective selection of relevant information to serve as a basis for the evaluation of the event (Scherer, 2001). Together appraisal theory suggests that the evaluation depends more on how it is experienced by the individual than on the event itself. Specifically in this experiment, we have investigated how winning and losing is evaluated by the participants and in what manner that evaluation of the outcome may affect the endocrine response to competition. Given that familiarity serves as a primary criteria for the selection of relevant information in the appraisal process (Scherer, 2001), this variable was also accounted for in our experiment.

The effects of familiarity on competition have been extensively studied in animals, where the aggressive response depends on the relative threat posed by familiar vs. stranger opponents. In social systems with aggregated stable territories territorial neighbors (familiar opponent) pose less threat than floaters (unfamiliar opponent) that could be looking for a territorial take-over and therefore and elicit less aggression (“dear enemy effect,” e.g. Temeles, 1994; Ydenberg, Giraldeau, & Falls, 1988). There is also some evidence that in other species, familiar opponents heighten the aggressive response. In these groups, neighbors pose a more significant threat for territorial usurpation or mating competition than roaming strangers that are commonly outnumbered by their same sex rivals in the established social groups (Müller & Manser, 2007). A pilot study in our lab has shown that in cichlid fish territorial intrusions by a familiar opponent elicit lower androgen responses than intrusions by strangers (R. F. Oliveira, R. Aires, T. Oliveira, and A. Ros, unpublished data). In human research the moderator effect of familiarity on the androgen response to competition has seldom been considered, but in two studies with coalitional competition in domino (Wagner, Flinn, & England, 2002) and in video-game tournaments testosterone increased in response to out-group but not to in-group contests (Oxford, Ponzi, & Geary, 2010). Other previous work has either ignored this variable or excluded participants with some degree of familiarity by asking contestants that knew each other to sign up for different experimental sessions (e.g., Mehta, Wuehrmann, & Josephs, 2009).

In this study we aim at investigating the effects of opponent familiarity and the evaluation of the competition outcome as threat or challenge on the T response to competition. We have also measured the levels of cortisol (C), since it is known to respond and interact with T when individuals are facing a social challenge (e.g. Mehta & Josephs, 2010; Viau, 2002), and of DHEA since it is the most prevalent androgen for women (Labrie, 2010) and is involved in the regulation of aggressive behavior (e.g. Soma et al., 2008).

Materials and methods

Participants and experimental protocol

Thirty-four undergraduate psychology female students (21.29±3.41 years), signed up to participate in experimental sessions of approximately 1 h, scheduled to 12:30 and 17:30 to control for circadian variation of hormone levels. Participants were tested in pairs (17 dyads) and were rewarded with one course credit and 12 Euros, depending of their competitive task outcome (winners: one course credit and 12 Euros; losers: one course credit). All experimental sessions were conducted by a male and a female experimenter. This experiment was performed in accordance to national regulations and with the approval of the ethics committee of ISPA’s Research Centre. Written consent was given by all participants.

Data collection and psychological variables

Participants were asked to sit face to face across a table, in which a vertical barrier had been placed. This barrier allowed the participants to see their opponent, but restricted the view of the opposite side of the table in such a way that they were unable to see what the opponent was doing during all stages of the competition. Upon arrival the participants provided a baseline saliva sample and filled in the demographic questions, including use of oral contraceptives (OCs) and the date of the last menstruation. Pairs were asked to rate from 1 to 5 how familiar they were with each other prior to this experiment (1 = not familiar; 5 = very familiar). Familiarity was conceptualized as a continuous signal-detection process (e.g. Yonelinas, 1997) and therefore we have avoided a dichotomic classification of “familiar vs. unfamiliar” that would create artificial groups and would not reflect the nature of this variable. For the competitive task we have used the number tracking test (NTT) and this task was introduced to the competitors after completing the first set of questionnaires. The NTT has been used before in competition experiments (e.g., Carré, Putnam, & McCormick, 2009; Schultheiss & Rohde, 2002) and requires participants to connect a sequence of consecutive ascending numbers (1-2-3-4-...) arranged in a matrix and surrounded by distracting numbers. Instructions focused on the competitive nature of the task, by stressing that participants will compete against one another for 12 Euros on a set of trials each associated with a specific NTT matrix. Feedback about who was the first to reach the highlighted end number on each NTT matrix characterized a trial as a “Win” or a “Loss” to the participant. Easy and difficult matrices were created by manipulating the distance between the start and the end number. This procedure allowed an undetectable experimental manipulation of the outcome (winning or losing the competition) and has been used in previous research (e.g., Carré et al., 2009; Schultheiss & Rohde, 2002; Wirth, Welsh, & Schultheiss, 2006). Participants were also unaware of the relative difficulty of the matrices since they had no access to their opponent matrices. Before the competitive NTT trials, participants were allowed to complete a NTT matrix for training purposes. For the competition the NTT was arranged in three sets of four NTT matrices. The first and second NTT sets were manipulated in such a way that the participants would have equal number of victories and defeats (four wins, four losses) before entering the third set. On the third NTT set, the participant in the winner condition would win the four NTT duels and the participant in the loser condition would lose the four NTT duels. The outcome of two pairs violated the expectation (i.e., participant in the winner treatment lost the competition). These participants were coded to their actual competition outcome and included in the sample (see Preliminary analysis for testing). It was tested if the removal of these participants from the sample would affect the results and it was found that the main results remain the same. After the competition outcome was announced, payment was given to the participants according to their task outcome. At this point evaluation of the competition outcome was individually assessed by scoring it as a threat and as a challenge using two items with a four points scale (e.g., I consider my participation in this study as: 1 = not threatening; 4 = very threatening; I consider my participation in this study as: 1 = not challenging; 4 = very challenging) inspired by Tomaka et al. (1993, 1997).Personality questionnaires unrelated to this experiment were then distributed to occupy the participants until the collection of a post-competition saliva sample 20 min after the end of the competition, which ended the experimental session (as in T. F. Oliveira et al., 2009).

Hormone assays

Saliva samples were collected on 5 ml polypropylene vials and stored at−20◦Cimmediately after the end of the experimental session. Samples were thawed, centrifuged at 3600 r.p.m. (2245 × g) for 10 min and the supernatant stored at −20◦C until the assay. Hormone assays were conducted using IBL (Hamburg, Germany) LIA kits for T, C and DHEA. The intra-assay and inter-assay coefficients of variance were respectively, 6.1 and 8.6% for T, 8.3 and 12.4% for C, and 4 and 11.9% for DHEA.

Preliminary analysis

All hormone values were log-transformed for statistical analysis due to skewness and violation of the parametric test assumptions (see Table 1 for absolute values). This transformation is a common procedure for the analysis of hormonal data (e.g., (Mehta et al., 2009; Wirth et al., 2006). All sampling points of the measured hormones were scanned for outliers (three standard deviations) and no participants were excluded based on this criterium. Degrees of freedom vary for the statistical analysis of DHEA, due to an insufficient volume of saliva to carry on the hormone assay for the baseline measurement of two participants. Participants were controlled for the phase of the menstrual cycle and for the use of OCs. Phase of the menstrual cycle was excluded from the analysis, since the number of participants in each category was insufficient for testing (number of winners per phase of the menstrual cycle: follicular=2, ovulation=1, luteal=2; number of losers per phase of the menstrual cycle: follicular = 1, ovulation= 2, luteal= 4). Furthermore, previous research has failed to find an effect of menstrual cycle over the patterns of variation in T and C (e.g., Dabbs & Rue, 1991; Liening, Stanton, Saini, & Schultheiss, 2010).

Table 1

Absolute values for all sampling points of the measured hormones

		Baseline	Post-competition
		Mean (±SE)	Mean (±SE)
Winner	T (pg/ml)	85.546 (±44.940)	55.345 (±10.604)
	C (ng/ml)	2.181 (±.257)	2.597 (±.354)
	DHEA (pg/ml)	335.121 (±75.862)	335.746 (±71.113)
Loser	T (pg/ml)	57.209 (±13.991)	160.322 (±58.388)
	C (ng/ml)	2.703 (±.408)	3.304 (±.393)
	DHEA (pg/ml)	402.359 (±102.635)	476.731 (±85.374)

A repeated measures analysis of variance (ANOVA) was used to check for effects and interactions of OC on hormone levels. Previous research has shown that the use of OC does not affect the androgen response to competition (Edwards & O'Neal, 2009) and we have not found an effect of OC on hormones either for winners (T: Main effect: F(1, 15)=1.637, p= .220, Interaction: F(1, 15)=.957, p=.343; C: Main effect: F(1, 15)=.700, p=.416, Interaction: F(1, 15)=.163, p=.691; DHEA: Main effect: F(1,14)=.284, p=.602, Interaction: F(1, 14)= 1.050, p =.322) or for losers (T: Main effect: F(1, 14)=.040, p=.845, Interaction: F(1, 14)= 2.09, p=.170; C: Main effect: F(1, 14)=1.470, p=.245, Interaction: F(1, 14)=.658, p=.430; DHEA: Main effect: F(1, 13)=.243, p=.630, Interaction: F(1, 13)=.286, p=.601), therefore this factor was also excluded from further testing. We have checked if the patterns of endocrine response for winners and losers were different when the competition outcome was the one predicted by the NTT matrices manipulation or not, and neither test reached statistical significance [T: F(1, 15)=.543, p=.472; C: F(1, 15)=.043, p=.837; DHEA: F(1, 13)=.092, p=.767]. Familiarity was measured but not manipulated. Familiarity ratings between participants ranged from 1 to 5 (mean=3.13 ± 1.61).

Statistical analysis

We have used a mixed model analysis of covariance (ANCOVA) with outcome (winner, loser) as a within variable since we are comparing pairs of participants, familiarity as a covariate and each dependent variable as a repeated measures factor. Dependent variables that were tested in separate ANCOVA were: evaluation (threat, challenge), and the steroid hormones T, C, and DHEA (pre-, post-competition). All comparisons were performed using planned contrasts within the ANCOVA, therefore the degrees of freedom match those of the model.

Moderation analysis followed the procedure outlined by Aiken and West (1991). The unstandardized residuals scores from regressing the pre-competition T on post-competition T, were used as an index of T response (Allison, 1990; Mehta et al., 2008) and inserted as the dependent variable on the moderation model. Threat was centered and used as a predictor and familiarity was also centered and used as the candidate moderator. The interaction term was composed by the product of threat and familiarity. To control for abnormal contributions to the regression model from any individual observation, residuals were scanned for outliers (3 standard deviations). Using this criteria one case was excluded and the linear regression model was adjusted without the outlier observation. Simple slope tests for high and low levels of familiarity were also calculated as suggested by Aiken and West (1991). Similar moderation procedures have been used by Mehta et al. (2008) and van der Meij et al. (2010).

Results

Evaluation of the outcome

The competition outcome was differently evaluated by winners and losers [F(1, 14) = 36.369, p<.001; Figure 1]. Participants in the loser condition evaluated the competition outcome as more threatening than winners [contrast: t(14)=3.621, p=.002], while winners tended to evaluate the outcome more as a challenge than losers, although this difference was not significant [contrast: t(14)=1.893, p=.079].

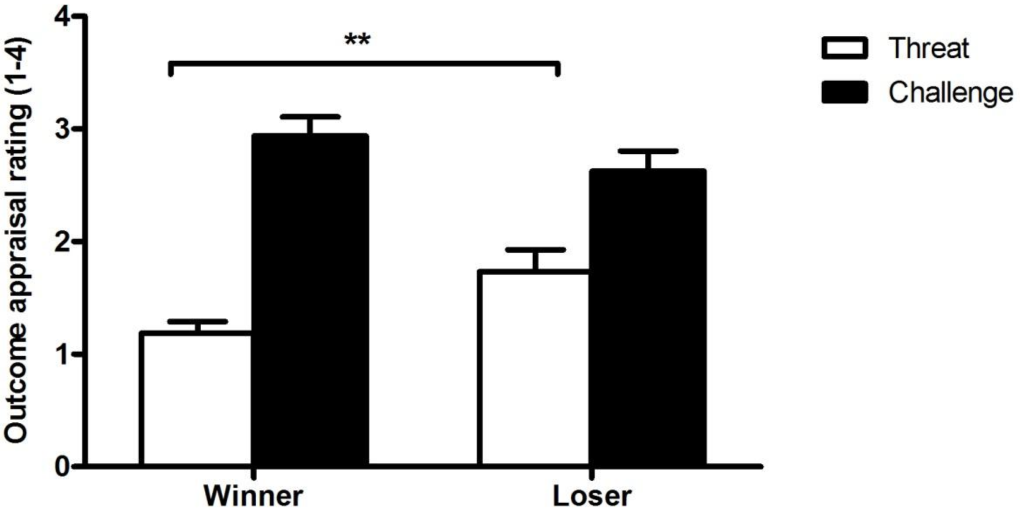

Figure 1. Competition outcome appraisal rating as a Threat/Challenge (Mean±SEM) for participants in the winner and loser condition with familiarity of the opponent as a covariate. (**) indicates significant differences at p≤.01.

Hormonal variables

Testosterone (Figure 2A): A main effect of the competition outcome was found suggesting that losers have overall higher T than winners [F(1, 15)=8.452, p=.010]. Subsequent contrast analysis showed that there were no baseline differences in T levels between winners and losers [contrast: t(15)=.186, p=.854] and that only losers significantly increased their levels of T from pre- to post-competition [contrast: t(15)=2.488, p=.025]. The difference between the winner and loser condition after the competition did not reach statistical significance [contrast: t(15)=1.769, p=.097].

Cortisol (Figure 2B): Statistical analysis for C suggests that there was no overall variation of C levels throughout the competition [F(1, 15) = 1.035, p=0.325] and that C levels were not different in both experimental conditions [F(1, 15) = 1.970, p = 0.180].

Dehydroepiandrosterone (Figure 2C): A non-significant trend was found for DHEA levels to be higher at the end of the competition [F(1, 13) = 3.317, p = 0.091]. DHEA levels were not different between winners and losers neither at the baseline nor at the post-competition measure [Winner contrast: t(13)=0.613, p=0.550; Loser contrast: t(13)=1.300, p=0.216], but losers showed a non-significant trend to have higher DHEA after the competition [t(13) = 1.845, p = 0.088]. Winners show no changes in DHEA levels from pre- to post-competition [t(13) = 0.326, p = 0.749].

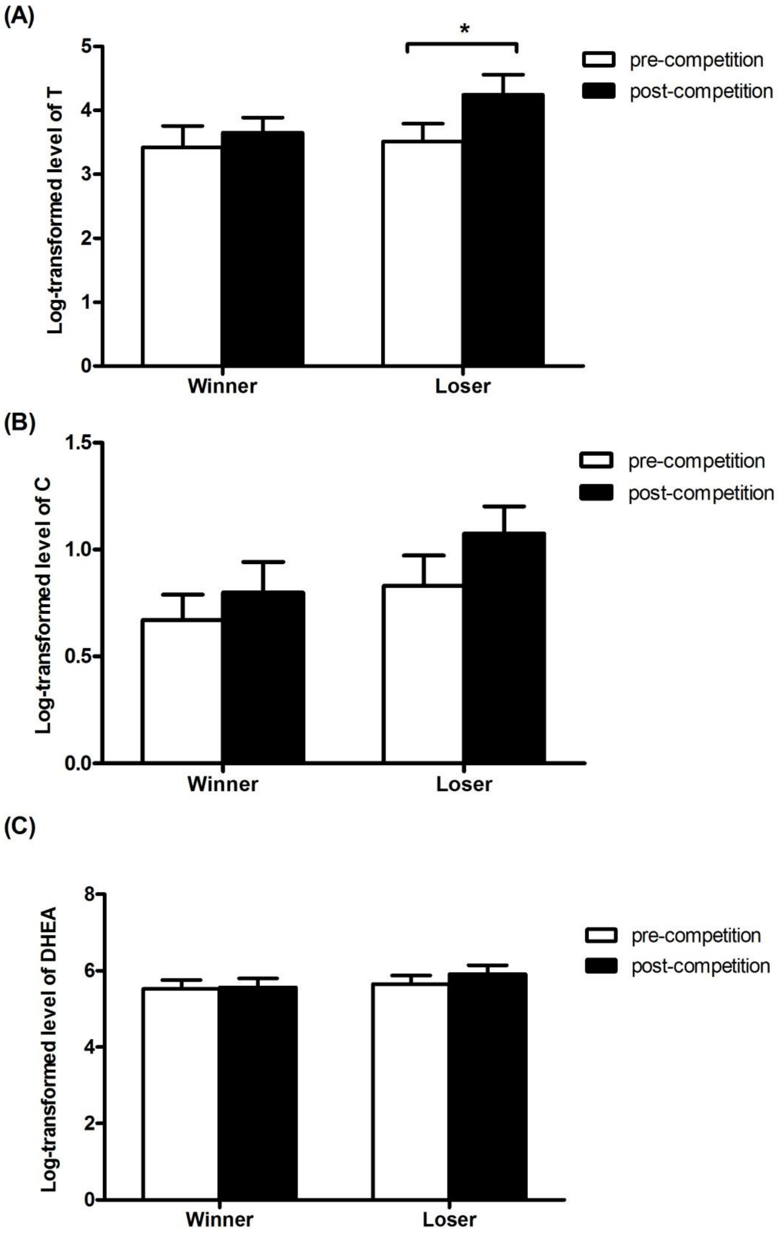

Figure 2. Log-transformed hormone levels (Mean±SEM) measured at baseline level, and 20 minutes after the competition for participants in the winner and loser condition with familiarity of the opponent as a covariate. (A) Testosterone, (B) Cortisol and (C) DHEA; (*) indicates significant differences at p≤.05.

Association between hormones and psychological variables

No association was found between the ratings of the competition as challenge and any of the measured hormones for winners (all p>.292) and losers (all p>.641). Familiarity, Threat and T were only significantly correlated in the loser condition (see Table 2). Post-competitive levels of C and DHEA did not correlate either with threat or with familiarity.

Table 2

Pearson correlation coefficients between threat, familiarity and hormone levels 20 minutes after the competition for Winners (n=17) and Losers (n=17)

		Threat	Familiarity	T2	C2	DHEA2
Winner	Threat	1	.359	.073	-.037	.256
	Familiarity	.359	1	.424	.054	.235
Loser	Threat	1	-.541*	.630**	.101	.338
	Familiarity	-.541*	1	-.506*	-.462	-.218

*significant for p<0.05

**significant for p<0.01

3.4. Moderation analysis of threat perception and familiarity on T levels for the loser condition

T response for participants in the loser condition was calculated as the unstandardized residuals of regressing baseline logT on logT 20 min after the competition (R^2 =.496, p=.002). The regression equation used to test the moderation effect with T response as the dependent variable, threat as predictor, familiarity as the moderator and the interaction between threat and familiarity was significant (R^2=.762, p<.001). The predictor threat (β=.278, p=.149) and familiarity (β=−.287, p=.114) were not significant, however, the interaction term threat × familiarity was highly significant (β =−0.613, p = 0.002). The inclusion of the interaction term also increased the explained variance of the regression model (ΔR^2=.317, p=.002).

Since the interaction of threat × familiarity was significant, we have conducted simple slopes analysis (Aiken & West, 1991; Mehta et al., 2008) for the relationship between T changes and Threat, one standard deviation above and below the mean of familiarity. Slope testing (Figure 5) shows that when the opponent is not familiar, higher threat leads to increases of T (b=1.102, t(12)=4.935, p=.0003), but no significant effect was found for familiar opponents (b =–.458, t(12)=1.360, p=.198).

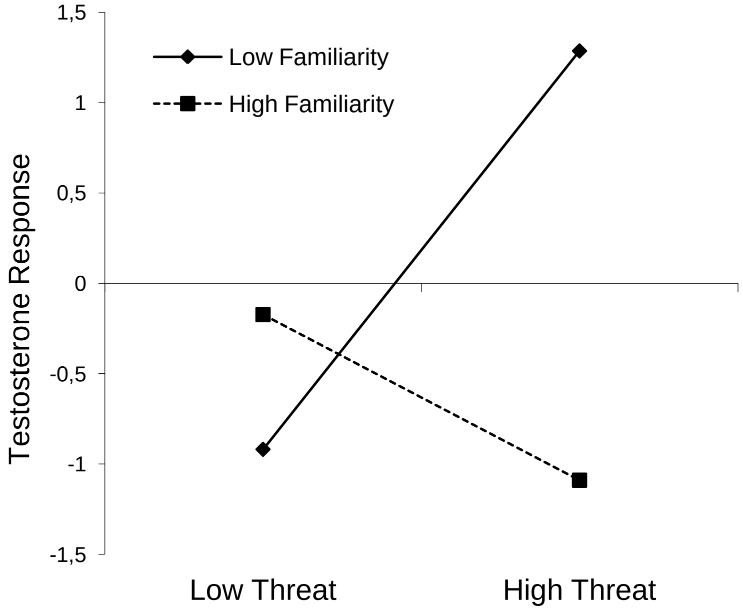

Figure 3. Regression slopes predicting testosterone response (unstandardized residuals) in function of threat and familiarity for women that lost the competition. High familiarity=1 SD above mean, Low familiarity=1 SD below mean. Low threat=minimum observed value, High threat=maximum observed value.

Discussion

In this experiment we investigated how opponent familiarity and evaluation of the competition outcome could modulate the hormonal response to competition. Contrary to the predictions of the challenge hypothesis (Wingfield et al., 1990) and previous findings in our lab (T. F. Oliveira et al., 2009) we did not find a clear winning/losing effect with higher post-competition T levels for winners and a decrease in T levels in losers. In fact, a significant hormonal response could only be found for the participants that were assigned to the loser condition. This group responded with increased post-competitive T levels, whereas the post-competition levels of the other measured hormones (C and DHEA) did not differ from pre-competitive values.

It could be argued that the rising T levels in losers but not in winners could be stress related in losers. Indeed ovaries and adrenals produce approximately the same percentage (25%) of circulating T in women (Burger, 2002), and adrenal androgens respond to stress (Oberbeck et al., 1998). However, in this study neither C nor DHEA, both of adrenal origin, were found to have a similar response to that of T, and previous studies have reported opposite effects of competition on T and C levels (Jiménez et al., 2012), which together suggest an independent response of the hormones to competition. Moreover, simulated competitive team matches failed to increase T levels (Filaire & Lac, 2000), whereas real matches activate a T response in women, thus suggesting that it is the meaning of the competition that triggers the response rather than the physical stress involved in the competition (Edwards & O'Neal, 2009).

It was also in the loser condition that the highest threat ratings were found and for which there was an association between post-competitive T, threat and opponent familiarity. As the moderation analysis has shown, the significant changes in T levels that were detected in this group in response to competition resulted from a moderator effect of the familiarity with the opponent on the evaluation of the outcome as a threat. When these participants lost the competition against an unfamiliar opponent, T levels increased when the evaluation of the task as threat was high. If the competition was lost against a familiar opponent, variations of threat intensity did not lead to changes in T levels. These findings are congruent with evidence from non-human experiments in which familiarity with the opponent moderates the level of elicited aggression as a function of the threat imposed by the opponent (e.g., less aggression elicited by neighbors than by strangers in territorial systems where neighbors, that are also territory owners, impose a lower threat than floaters that are looking for territory take-overs, Temeles, 1994; Ydenberg et al., 1988). Accordingly, a recent study in our lab using a cichlid fish also found that the androgen response to a territory intrusion in a cichlid fish was moderated by the familiarity with the intruder (R. F. Oliveira, R. Aires, T. Oliveira, and A. Ros, unpublished data). The link between higher threat and losing the competition is also coherent with appraisal theory. A threat evaluation may occur when the demands exceed the resources mobilized by the individual to respond to a social challenge (Blascovich & Mendes, 2000). Since the competition outcome was experimentally manipulated, if the participants are motivated and engage in competition a higher threat evaluation is to be expected in the loser condition where participants will always perceive their resources to be insufficient to reverse the score and win the competition. Likewise, it would be possible that the task outcome exerted a suppressing effect over the threat evaluation of the competition for participants in the winner condition, as the resources possessed by the individual were sufficient to resolve the interaction in their favor (Blascovich & Mendes, 2000). In this context the lack of T response in winners can be seen has having an economical and adaptive value, while an increase in losers can be interpreted as a physiological response that prepares the individual for future encounters to regain lost status or to buffer the individual in case of an extended contest (Mehta & Josephs, 2006; Schultheiss et al., 2005). Interestingly the effects of T administration on relevant psychological processes (e.g., perception of threatening faces) for future competition are also moderated by contextual and personal factors (see review by (Bos, Panksepp, Bluthé, & Van Honk, 2012).

The hypothesis that the endocrine response to competition is triggered by the individuals' evaluation instead of by the objective structure of the competitive task is a possible explanation for the divergences in T response patterns to competition (R. F. Oliveira et al., 2005; Salvador & Costa, 2009; Salvador, 2005). In fact, the range of reported androgen responses to competition in the literature varies from T increases in winners, no significant response or even T increases in losers (Hamilton et al., 2009; Salvador & Costa, 2009; van Anders & Watson, 2007). In this respect, the evaluation of threat/challenge (Blascovich & Mendes, 2000) posed by the competition outcome is a good candidate for moderating the T response. In summary, the results presented here support the view that the subjects' evaluation of the event plays a key role in the activation of a T response to competition in women and could partly account for intersexual differences in the endocrine response to competition (e.g., (e.g., Salvador & Costa, 2009), illustrating the need for further studies in which the moderator role of different appraisal dimensions of the competitive event on hormonal responses to competition is formally tested.

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Chapter III

Testosterone response to competition in male losers is unrelated to opponent familiarity and threat appraisal

Abstract

It has been proposed in the literature that the testosterone (T) response to competition in humans may be modulated by cognitive variables. In a previous experiment with a female sample we have reported that opponent familiarity and threat appraisal moderated the T response to competition in women. With this experiment we aim to investigate if these variables have the same impact on males T response to competition, extending the previous findings in our lab. Forty male participants (20 dyads) were recruited to engage in a same sex, face to face competition using the Number Tracking Test as a competitive task. Levels of T, cortisol (C) and dehydroepiandrosterone (DHEA) were measured before and 20 min after the competition. Results show that losers report higher levels of threat than winners and increased their T levels after the competition, however this T change was not predicted by opponent familiarity or threat appraisal. No variation was detected for C and DHEA levels. These findings suggest that there could be sex differences for the moderators/mediators of the T response to competition in humans.

Keywords: androgens, testosterone, challenge hypothesis, sex factors, cognition

Introduction

Androgen responses to social challenges are present in several taxa and have been interpreted as a mechanism to adjust the internal state and the output of androgen dependent behaviors to changes in the social environment (R. F. Oliveira, 2009). Early explanations for this response stressed the reciprocal relationship between androgens and behavior (e.g. Leshner 1975) and culminated in the formalization of two independent hypotheses for the social modulation of androgens: the biosocial model (Mazur, 1985) and the challenge hypothesis (Wingfield, Hegner, Dufty, Jr., & Ball, 1990).

The biosocial model (Mazur & Booth, 1998; Mazur, 1985) postulates a mutual reinforcing relationship between androgens and dominance. Androgens, testosterone (T) in particular, promote status-seeking behaviors and when high status is achieved, in an agonistic interaction, the individual’s androgen levels increase to match the new position in the social hierarchy. On the contrary, after losing a competition T levels are expected to decrease, to avoid the possible status costs of further contests. In the ‘challenge hypothesis’ (Wingfield et al., 1990), transient changes in androgen levels adjusts the expression of androgen-dependent aggressive behaviors to the social context, thus avoiding the costs associated with keeping chronically elevated T levels. Although initially proposed in birds, the “challenge hypothesis” has been extended to other taxa including humans (Archer 2006; Hirschenhauser and Oliveira 2006). In response to an agonistic interaction, the “challenge hypothesis” predicts an increase in T levels without specifically defining if this effect is valid for winners and losers. However, neither of the these two hypotheses explain the diversity of T responses to competition in humans found in the literature (G. A. Oliveira & Oliveira, 2014b). In recent reviews, this variety of androgen responses to social competition has been interpreted as a consequence of the moderation of the androgen response by cognitive variables, with appraisal emerging as the strongest candidate for this effect (G. A. Oliveira & Oliveira, 2014b; Salvador & Costa, 2009).

Appraisal can be defined as a continuous evaluation process of the transactions between the individual and the environment, in which the individual assesses the significance and the implications of an event (Scherer, 2001, 2009). Therefore, the appraisal of an event results from an interaction in which the objective structure of the event is contrasted with the goals, resources and abilities of the individual. In goal relevant situations (e.g. competitive contexts), appraisal can be understood within a demands/ resources continuum (Blascovich &

Mendes, 2000; Tomaka, Blascovich, Kibler, & Ernst, 1997). When an individual evaluates the demands of a task as exceeding the available coping resources, the situation will be appraised as a threat. In contrast, if the perceived resources exceed the task demands, the event will be evaluated as a challenge. In addition to affective and cognitive differences between threats and challenges, patterns of cardiovascular response activated in states of task engagement are specific to each type of appraisal (e.g. threat: lower cardiac reactivity, increased vasoconstriction; challenge: high cardiac reactivity, lower vasoconstriction; Blascovich, Vanman, Mendes, & Dickerson, 2011). Some studies have already provided data that supports the role of appraisal in the androgen responsiveness to social competition. For example, male cichlid fish (Mozambique tilapia, *Oreochromis mossambicus*) fighting unsolved fights against their own image on a mirror (i.e. where they do not experience either a victory or a defeat) fail to exhibit an androgen respond, despite expressing similar levels of aggressive behavior to those of males fighting a real opponent (Hirschenhauser, Wittek, Johnston, & Möstl, 2008; R. F. Oliveira, Carneiro, & Canário, 2005). This dissociation between behavior and androgen response can be explained by the different evaluations the subject makes of unsolved fight and of fights with perceived positive or negative outcomes (Oliveira 2009). Human studies have also provided evidence for the role of appraisal on the androgen response. For example, a laboratory experiment reported that the T response to a face-to-face competition was higher when the opponent was evaluated as having high self-efficacy (van der Meij, Buunk, Almela, & Salvador, 2010).

Familiarity is one of the first components to be evaluated in the appraisal process (Scherer, 2001) and this variable has been extensively studied in the context of agonistic encounters with non-human animals. It has been described, for several territorial species of different taxa, that in aggregated stable territories familiar opponents (e.g. neighbors) pose less threat and elicit less aggression than unfamiliar individuals (dear enemy effect; (Temeles, 1994; Ydenberg, Giraldeau, & Falls, 1988), while in other species neighbors are more likely to compete for territory and mates and thus elicit a higher aggressive response than roaming strangers (Müller & Manser, 2007). In humans, effects of familiarity in social challenges have also been described in the literature. In a domino team competition, T tended to increase more when players were facing teams that were not from their own village (Wagner, Flinn, & England, 2002). Also, in-group membership has been suggested as an explanation for the lack of T response in a sports competition (Trumble et al., 2012) and elicited different T responses for high ranked players in a video game competition (Oxford, Ponzi, & Geary, 2010).

In a previous experiment with women, we provide stronger evidence that familiarity moderated T responses to a face-to-face competition event appraised as a threat (Oliveira et al. 2013). In this study, T increased more in losers that evaluated the outcome as a threat while competing against unfamiliar opponents, while Cortisol (C) and Dehydroepiandrosterone (DHEA) levels remained at their pre-competition levels (Oliveira et al. 2013). On the other hand, winners appraised the competition outcome as less threatening than losers and no significant changes were detected for any of the measured hormones (T, C and DHEA). Because men and women tend to exhibit differences in appraisal tendencies towards competition (Niederle & Vesterlund, 2011) and it has been previously suggested that there may be sex differences for the T response to competition (Josephs, Mehta, & Carré, 2011; Kivlighan, Granger, & Booth, 2005), we decided to investigate if the previous findings described above would also be valid for males, or if there was a sex difference in the cognitive moderation of the T response to competition in humans. Therefore, in this study we tested if males display the same pattern of endocrine response as females to a face-to-face contest, and if opponent familiarity and threat vs. challenge appraisal of the outcome (winner/loser) moderates males T response to competition, using the same experimental paradigm as in Oliveira et al. (2013). Although we have not found changes in C and DHEA for women, these hormones were also monitored in this experiment since it is established that C responds to social stress, influences cognitive variables (McEwen & Sapolsky, 1995) and is known to interact with T in case of social contests (Mehta & Josephs, 2010). On the other hand, DHEA is an important androgen involved in the regulation of aggressive behavior (Soma, Rendon, Boonstra, Albers, & Demas, 2014) and on the processing of signals of threat in humans (Sripada et al., 2013).

Materials and Methods

Participants and experimental protocol

Forty undergraduate psychology male students (mean: 24.00 ± 6.99 years) voluntarily signed up to participate in experimental sessions that lasted for approximately one hour. To control for circadian variation of hormone levels all sessions were scheduled for the afternoon (12:30 to 17:30). Participants were tested in dyads (n=20). One participant presented a pre-competition level of T above 3 standard deviations and therefore its pair was excluded from the sample, bringing the total number of participants to 38 (19 dyads). All participants were rewarded with one course credit and received a monetary payment depending of their condition (winners: 8€, losers: 4€). A male and a female experimenter were present in all the experimental sessions. This experiment was performed in accordance to Portuguese regulations, the declaration of Helsinki and with the approval of the ethics committee of ISPA's Research Centre. Written consent was given by all participants.

Data collection and psychological variables

Participants were asked to sit face-to-face across a table. An opaque vertical barrier was placed on the top of the table between the participants, such that it enabled the participants to establish eye contact but blocked the view of the opponent task and questionnaires during the experiment.

At the beginning of the experiment participants were asked to provide a pre-competition saliva sample and filled in a questionnaire that controls for possible sources of hormone variation. After completing this questionnaire, pairs were asked to rate from 1 to 5 how familiar they were with each other prior to this experiment (1= not familiar; 5= very familiar). Instead of classifying the pairs as "familiar" vs. "not familiar", we have used a continuous measure since it better matches familiarity as a signal-detection component of appraisal (Scherer, 2001).

As in previous experiments (Carré, Putnam, & McCormick, 2009; G. A. Oliveira et al., 2013; Schultheiss, Campbell, & McClelland, 1999), the Number Tracking Test (NTT) was used for the competitive task. The NTT requires participants to connect a sequence of consecutive ascending numbers (1-2-3-4-…) arranged in a matrix and surrounded by distracting numbers, until a highlighted number is reached. To experimentally assign participants to the winner or loser condition, the length of the NTT matrices was manipulated (i.e. winners had shorter NTT matrices than losers). This procedure has been previously used in NTT competition (Carré et al., 2009; G. A. Oliveira et al., 2013; Schultheiss et al., 1999) and allows an undetectable manipulation of the outcome, since participants have no access to their opponent matrices and therefore cannot assess the relative difficulty of their matrices. Experimental conditions associated with a side of the table were randomized and pre-determined before the experiment and participants were free to choose their position. Instructions were focused on the competitive nature of the task and it was also highlighted that the participants would compete against one another over 12 NTT trials and receive 1€ for each trial they had won up to a maximum of 12€. Feedback about who was the first to reach the highlighted end number on each NTT matrix characterized a trial as a “Win” or a “Loss” to the participant. The outcome was confirmed by the experimenter on each trial and 1€ was immediately given to the winner. This was done in order to reinforce the authenticity of the result and the competitive nature of the task.

After the completion of a NTT matrix for training, participants competed over three sets, each one composed by four NTT matrices. The matrices on the first and second NTT sets were manipulated to create a draw between the participants (four wins, four losses). The third NTT set defined the outcome of the competition with the participant in the winner condition winning the four NTT trials and the participant in the loser condition losing the four NTT trials. The outcome of the competition for two pairs was not congruent with the assigned condition (i.e. participant assigned the winner matrix lost the competition) and since their exclusion did not alter the main results reported here, they were coded to their real outcome and included in the sample.

After the competition, participants were asked to evaluate the outcome as a threat and as a challenge using two items with a 4 points scale as in our previous study (Oliveira et al. 2013). Personality questionnaires unrelated to this experiment were given to the participants as a filler task for 20 minutes, until they were asked for a second saliva sample.

Hormone assays

Participants were instructed to abstain from smoking, eating, drinking, physical exercise, brushing their teeth or consuming pH altering substances (several examples for this option were included) for 1 hour before the experiment**.** Saliva samples were collected by passive drool in 5ml polypropylene vials and stored at -20ºC right after the end of the experiment. Samples were thawed, centrifuged at 2245 *g* for 10 min and the supernatant stored at -20 Cº until the assay. Luminescence Immunoassay kits (IBL, Hamburg, Germany) were used to determine concentrations of free T, C and DHEA. The intra-assay and inter-assay coefficients of variance were respectively 6.1% and 8.6% for T, 8.3% and 12.4% for C, and 4% and 11.9% for DHEA. Absolute values for all measured hormones are presented in Table 1.

Table 1

Baseline and post-competitive hormone levels for Winners and Losers

		Baseline	Post-competition
		Mean (±SE)	Mean (±SE)
Winner	T (pg/ml)	146.875 (± 13.020)	157.400 (±16.299)
	C (ng/ml)	3.892 (±0.609)	4.646 (±0.729)
	DHEA (pg/ml)	739.355 (±107.281)	563.953 (±49.523)
Loser	T (pg/ml)	120.167 (±15.027)	150.814 (±16.054)
	C (ng/ml)	2.952 (±0.466)	3.631 (±0.406)
	DHEA (pg/ml)	512.576 (±68.289)	633.585 (±55.824)

Preliminary analysis

A skewed distribution was found for the C levels and therefore these measures were log-transformed before statistical analysis. No transformation was required for T or DHEA levels. All measures were scanned for 3 standard deviation outliers and as reported before, one pair was excluded from the sample. Familiarity between opponents was measured and not manipulated. Ratings for familiarity ranged from 1 to 5 [mean ± standard error of the mean (SEM) = 2.68±1.45].

Statistical analysis

Pairs of competitors were compared using a mixed model analysis of covariance (ANCOVA) with Outcome (winner, loser) as a within subjects factor, Familiarity as a covariate and each dependent variable as a repeated measures factor. The repeated measures factor tested in different ANCOVA models were: Appraisal (threat, challenge) and the measures for T, C and DHEA (pre-, post-competition). Planned contrasts were used for *a priori* comparisons and therefore the reported degrees of freedom match those of the ANCOVA model. Degrees of freedom vary for the DHEA statistical analysis due to an insufficient volume of saliva to run this hormone assay for two of the participants. Partial eta squared (η_p^2) effect sizes are provided for main effects and interactions. Effect sizes for contrasts were calculated using Cohen's *d* with the average of standard deviations as the standardizer and converted to Hedge's *g* corrected for sample size bias (Lakens, 2013). For the moderation analysis (Aiken & West, 1991), the unstandardized residuals from regressing the pre-competition T on post-competition T, were used as an index of T response and inserted as the dependent variable. The variables threat and familiarity were used as predictors and the interaction term was calculated as the product of threat by familiarity.

Results

Appraisal of the competition outcome as threat and challenge

Overall, participants rated the outcome as more of a challenge than a threat [Figure 1; F(1, 15)=48.856, p<.0001, η_p^2=.765]. Losers appraised the competition outcome as more threatening than winners [t(15)=2.114, p=.051, *g*=.781]. For challenge appraisal, no differences were found between the conditions [t(15)=.404 p=.691, *g*=.147]. No familiarity effects were detected on the evaluations as threat and challenge (all β n.s.; Threat/Challenge x Outcome x Familiarity: F(1, 15)=.845, p=.372, η_p^2=.053).

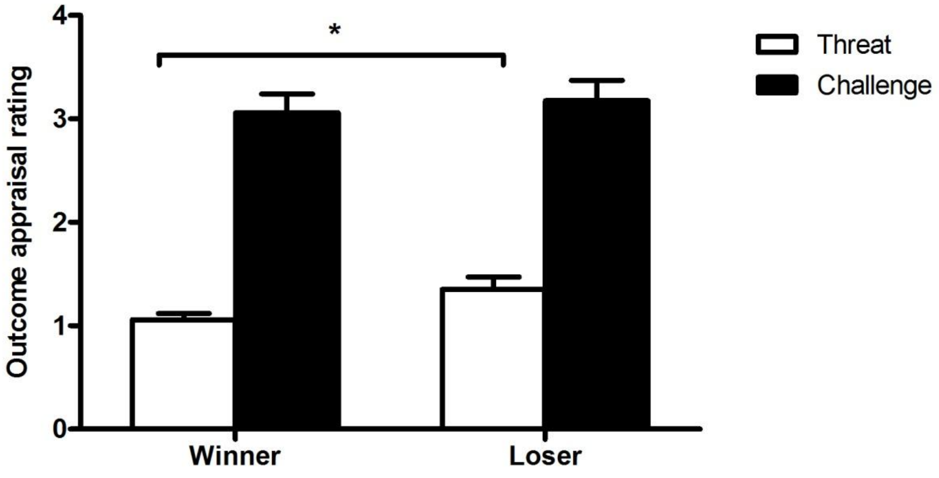

Figure 1. Competition outcome appraisal rating as a Threat/Challenge (Mean±SEM) for participants in the winner and loser condition with familiarity of the opponent as a covariate. (*) indicates significant differences at p≤.05.

Hormonal variables

Testosterone (Figure 2A) – No overall variation of T levels was detected over the competition [F(1, 17)=.004, p=.946, η_p^2<.001]. The two treatments showed different responses to the competition, with a significant increase in T in losers [t(17)=2.601, p=.018, *g*=.442] and no significant change detected in winners [t(17)=.853, p=.405, *g*=.060]. Winners and losers showed different pre-competition levels of T, with subsequent winners exhibiting higher levels than subsequent losers [t(17)=2.609, p=.018, *g*=.427]. However, no differences in T levels were found between winners and losers at the end of the competition [t(17)=.498, p=.624, *g*=.091]. No effects were detected for the covariate familiarity on the T levels [all β n.s; T x Outcome x Familiarity: F(1,17)=.232, p=.636, η_p^2=.013].

Cortisol (Figure 2B) – There was no overall variation of C levels over the competition [F(1, 17)=1.951, p=.180, η_p^2=.102] and there were no differences between the two treatments either before [t(17)=1.220, p=.239, *g*=.373] or after the competition [t(17)=.785, p=.443, *g*=.249]. Within each treatment, there was no C variation over the competition in winners [t(17)=1.106, p=.283, *g*=.246] and a only a marginal increase was observed in the losers [t(17)=1.906, p=.073, *g*=.461]. Furthermore, no significant effects of familiarity were found [all β n.s; C x Outcome x Familiarity: F(1,17)=.579, p=.456, η_p^2=.032].

Dehydroepiandrosterone (Figure 2C) **-** No overall changes of DHEA levels over the competition were detected [F(1, 15)=1.685, p=.213, η_p^2=.101] and DHEA levels were not different between the two treatments either before [t(15)=1.698, p=.109, *g*=.530] or after the competition [t(15)=.670, p=.512, *g*=.247]. Winners marginally decreased their levels of DHEA after the competition [t(15)=1.963, p=.068, *g*=.485] and no changes in DHEA were detected for losers [t(15)=1.183, p=.254, *g*=.344]. Furthermore, no significant effects of familiarity on DHEA levels were found [all β n.s; DHEA x Outcome x Familiarity: F(1, 15)=.105, p=.750, η_p^2=.006].

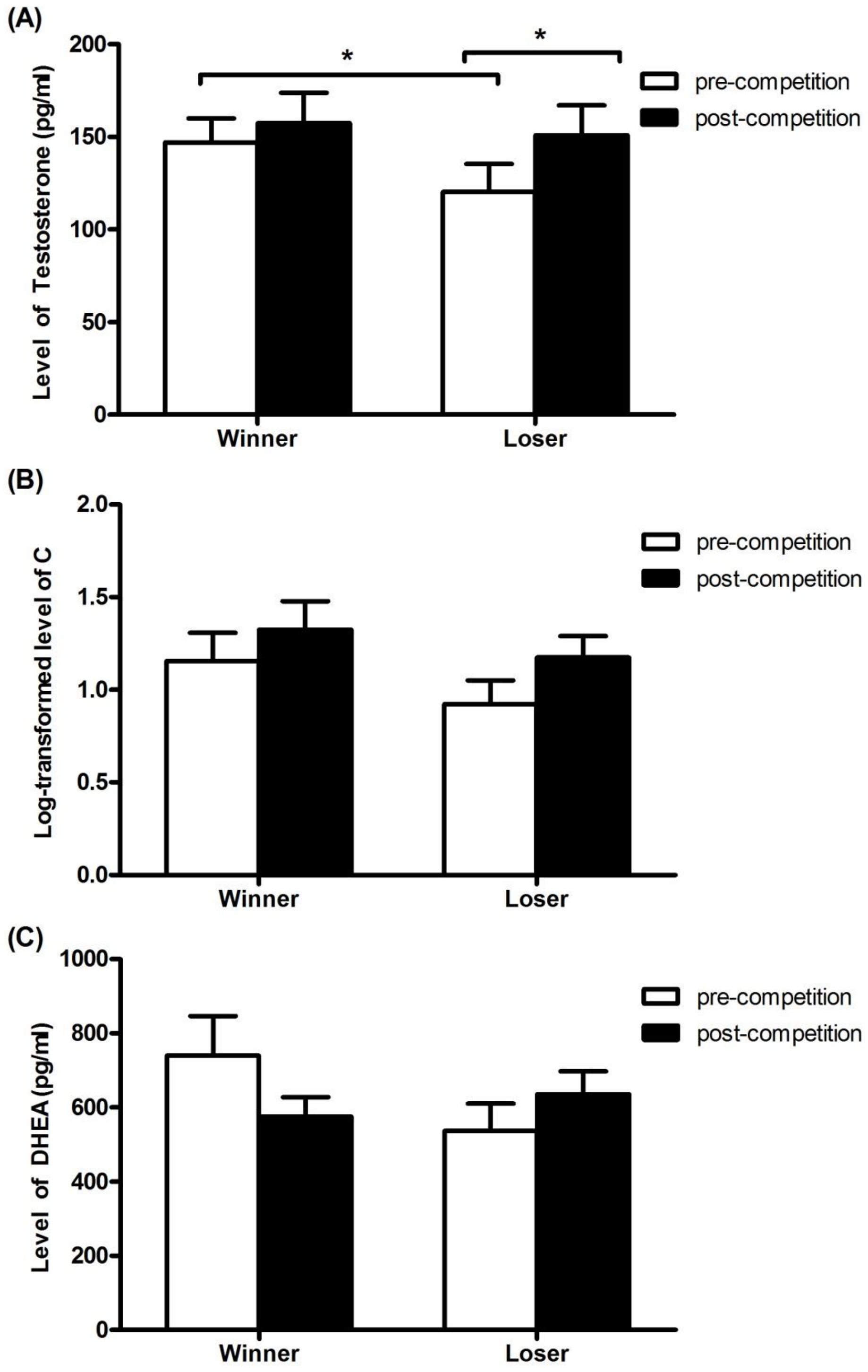

Figure 2. Hormone levels (Mean±SEM) measured at baseline (pre-competition) and 20 minutes after the competition (post-competition) for participants in the winner and loser condition with familiarity of the opponent as a covariate. A) Testosterone, B) Cortisol and C) DHEA. (*) indicates significant differences at p≤.05.

Association between hormones and psychological variables

No significant association between familiarity, appraisal and the post-competitive hormones levels were detected either for winners or for losers (Table 2).

Table 2

Pearson correlation coefficients between threat, familiarity and hormone levels 20 minutes after the competition for Winners (n=18) and Losers (n=18)

		Threat	Familiarity	T2	C2	DHEA2
Winner	Threat	1	-.308	.320	-.053	.150
	Familiarity	-.308	1	.067	.138	.192
Loser	Threat	1	.276	.113	-.069	.160
	Familiarity	.276	1	.003	-.218	-.196

Moderation analysis

The regression model predicting T variation as a function of Threat, Familiarity and Threat x Familiarity was not significant (R^2=.105, p=.658). Both predictors and the interaction term were also not significant (Threat: β=.367, p=.299; Familiarity: β=.206, p=.232; Threat x Familiarity: β=-.134, p=.372).

Discussion

In this experiment we aimed to investigate if the familiarity with the opponent and appraisal of challenge vs. threat moderated the T response to social competition in men. Participants assigned to the loser treatment significantly increased their T levels and no significant T change was observed in winners. This response to competition is specific to T, since for all other measured hormones no significant variation from pre- to post-competition levels was detected. These results for T cannot be fully explained by the biosocial model (Mazur, 1985) or the “challenge hypothesis” (Wingfield et al., 1990) since we have not found increased T in winners and decreased T in losers or a significant overall increase in T after the competition, respectively. The endocrine results for losers match previous findings with female samples using the same paradigm (G. A. Oliveira et al., 2013) and the T results in (Zilioli, Mehta, & Watson, 2014) with a female NTT competition that is only decided in the final trial (versus four trials in our experiment). Increases in T levels after losing a competition have been interpreted as an indicator of the individual's motivation to keep engaged in competition in order to regain the status lost in the previous interaction (Mehta & Josephs, 2006; G. A. Oliveira et al., 2013; Zilioli et al., 2014). The hypothesis that the T changes occurring after the resolution of a competition are relevant for subsequent interactions, rather than for the current one, is supported by research in human and non-human animals showing that the social decision-making mechanisms in the brain are sensitive to changes in circulating levels of T (G. A. Oliveira & Oliveira, 2014a). For example, the fear reducing properties of T (Hermans, Putman, Baas, Koppeschaar, & van Honk, 2006) may be of particular adaptive relevance to the individual in case of future agonistic interactions.

In our paradigm, the outcome of the competition was decided only in the last set of NTT trials and participants could monitor the score trial by trial. This may have influenced the losing participants' engagement in the competition and evaluation of their capacity to compete against the winners and thus dispute their status in future interactions. However, the possible effect of these variables in the T response is undetermined and cannot be tested post hoc in the current experiment. Some support to this hypothesis can be found in a recent article by Zilioli et al. (2014). These authors argued that the uncertainty of the outcome generated by the alternation of wins and losses, ending with a close resolution of the contest, replicates an unstable status hierarchy and therefore the classical predictions of the biosocial model may not apply. In their experiments, the increase of T in losers and decrease in winners has been interpreted as indicators of competition seeking and competition avoidance, respectively (Zilioli et al., 2014).

Since winners presented higher T than losers at the pre-competition measure, this experiment has limitations when it comes to findings related to the dynamics of T in male winners. For instance, we cannot exclude the possibility that the lack of a significant T increase in winners may be due to a ceiling effect. In fact before the competition subsequent winners had higher T levels than subsequent losers, but at the end of the competition T levels had increase in both groups and were not significantly different between them, yielding a significant increase from pre- to post-competition only in losers. Since winners and losers were experimentally assigned and randomized beforehand, the pre-competition difference between conditions cannot be attributed to a priori group differences or individual performance. Furthermore, experimenter bias was also controlled for, since the participants were free to choose their position in the competitive setting, thus self-selecting their experimental condition. Therefore, further research is required to clarify the inconclusive results for winners reported here.

Unlike a previous experiment with women in our lab (G. A. Oliveira et al., 2013), threat appraisal and opponent familiarity did not moderate the T increase found in men that lost the competition. Male losers also increased T and reported higher levels of threat than winners but neither variable was associated with opponent familiarity. Although previous research suggests a blunted or reduced T response in males when facing members of the in-group (Oxford et al., 2010; Trumble et al., 2012; Wagner et al., 2002), this effect may reflect group processes that are not present in individual competition and therefore these previous findings may not be directly moderated or mediated by the effects of familiarity with the opponent as it was operationalized here.

Together, these results suggest that the psychological moderators of these T changes may differ between sexes or may have different weights in the interaction between cognitive processes and the T response. In the context of our experiment, the outcome elicited similar challenge and threat appraisals to those previously reported in females (G. A. Oliveira et al., 2013), however sex differences may exist concerning the importance of familiarity. This is congruent with previous research in which women were found to be more sensitive to familiarity than men, suggesting that this variable may have greater adaptive relevance for females (Deaner, Shepherd, & Platt, 2007). Previous research with a male sample showed that the individual T levels were associated with the opponent’s self-efficacy, highlighting an evaluative process within the agonistic interaction in which the opponent’s characteristics relevant to the competition are assessed by the participants (van der Meij et al., 2010). Our results indicate that there may be sex differences in what is considered relevant in this evaluation. For instance the greater sensitivity to familiarity in women may explain the discrepancy of results using the same face to face competition. Furthermore, sex differences in psychological traits relevant to competition offer empirical support to this hypothesis. For example, a recent meta-analysis suggests that women are more sensitive to punishment and more averse to risk taking than men (Cross, Copping, & Campbell, 2011). Motivation toward competition is also different between the sexes. Men respond more strongly than women to intergroup conflict and therefore make more competitive choices in social dilemmas between groups than women (Van Vugt, De Cremer, & Janssen, 2007; Wildschut, Pinter, Vevea, Insko, & Schopler, 2003). Also, men are more motivated toward activities in which there are performance measures and opportunities to compete, when compared to women (Kilpatrick, Hebert, & Bartholomew, 2006). These sex differences however may be strongly influenced by social hierarchy and context, since although men compete more than women in patriarchal societies, this pattern is reversed in matriarchal societies (Gneezy, Leonard, & List, 2009) and differences in motivation toward competition are attenuated or absent in same sex competitions (Niederle & Vesterlund, 2011). Although risk aversion does not directly explain differences in willingness to compete (Niederle & Vesterlund, 2011), it may still be an important factor influencing the appraisal process. Since most of the aforementioned findings result from competitions that used economic games as a competitive task, different paradigms are required to clarify the generalization of these attitudinal sex differences in competition and for the sex differences in relevant components for appraisal suggested in this article.

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Chapter IV

Dear enemies elicit lower androgen responses to territorial challenges than unfamiliar intruders in a cichlid fish

Abstract

In many territorial species androgen hormones are known to increase in response to territorial intrusions as a way to adjust the expression of androgen-dependent behavior to social challenges. The dear enemy effect has also been described in territorial species and posits that resident individuals show a more aggressive response to intrusions by strangers than by other territorial neighbors. Therefore, we hypothesized that the dear enemy effect may also modulate the androgen response to a territorial intrusion. Here we tested this hypothesis in male cichlid fish (Mozambique tilapia, *Oreochromis mossambicus*) using a paradigm of four repeated territorial intrusions, either by the same neighbor or by four different unfamiliar intruders. Neighbor intruders elicited lower aggression and a weaker androgen response than strangers on the first intrusion of the experiment. With repeated intrusions, the agonistic behavior of the resident males against familiar intruders was similar to that displayed towards strangers. By the fourth intrusion the androgen response was significantly reduced and there was no longer a difference between the responses to the two types of intruders. These results suggest that the dear enemy effect modulates the androgen response to territorial intrusions and that repeated intrusions lead to a habituation of the androgen response.

Introduction

In territorial species, resident males have been shown to respond less aggressively towards an intrusion by a territorial neighbor than by a stranger male, a phenomenon known as the "dear enemy" effect (Temeles, 1994; Ydenberg, Giraldeau, & Falls, 1988). From an evolutionary perspective this phenomenon can be seen as an adaptation for territorial males to adjust their behavior according to the relative threat posed by the intruders.

The theoretical explanations for the dear enemy hypothesis rely on the familiarity existing between neighbours and on the relative threat posed by the different categories of intruders. The hypothesis based on the familiarity between neighbours proposes that territory owners are less aggressive towards neighbours either because familiarity decreases the risk of a role mistake (i.e. either contestant judging incorrectly its role as a likely winner/loser, Parker, 1984) due to previous interactions among them ("role mistake hypothesis", Ydenberg et al., 1988), or because they already have information on the resource holding power (RHP, (Parker, 1984) of their neighbours and therefore do not need further fights to get this information ("fighting to learn hypothesis", Getty, 1989). According to the latter explanation, the threat posed by stranger non-territorial floaters is higher than that posed by neighbours because the potential losses to strangers are higher (i.e. territory owners can lose both their territory and potential mates towards strangers but only potential males to neighbours that already have a territory) (Getty, 1989; Temeles, 1990, 1994). Moreover, assuming that territorial males hold information on the competitive ability of neighbouring males, obtained either actively from previous interactions or passively by eavesdropping on neighbours' interaction with third parties (R. F. Oliveira, Mcgregor, & Latruffe, 1998), the level of uncertainty in the interactions with territorial neighbours is lower and thus they pose a lower challenge than stranger males. Therefore, the reduced aggressive response towards a "dear enemy" permits an economic territory defence without compromising its efficiency (Leiser & Itzkowitz, 1999). These hypotheses are not mutually exclusive and have been extensively tested across a wide range of taxa [e.g. crabs (Booksmythe, Jennions, & Backwell, 2010), fish (Leiser, 2003), reptiles (Carazo, Font, & Desfilis, 2008), birds (Briefer, Rybak, & Aubin, 2008)].

In terms of proximate mechanisms the "dear enemy" phenomenon requires the ability of the resident male to discriminate between familiar and unfamiliar intruders, together with a habituation response to the presence of neighbours, which would explain the lower response that they elicit (Bronstein, 1994; Owen & Perrill, 1998). Hormones may also play a role on the dear enemy effect by modulating the cognitive mechanisms mentioned above or by acting directly on the motivation of residents to engage in fights. Androgens have been shown to respond to social challenges in a wide range of species (Hirschenhauser & Oliveira, 2006), and this response has been interpreted as a way to adjust the expression of androgen-dependent behaviours to social context (G. A. Oliveira & Oliveira, 2014; R. F. Oliveira, 2009). For example, it has been shown that transient changes in androgen levels triggered by agonistic interactions influences competitive behaviour in subsequent interactions [e.g. winner effect (R. F. Oliveira, Silva, & Canário, 2009; Oyegbile & Marler, 2005)], that bystanders not directly involved in the interaction also respond hormonally to observed social interactions (R. F. Oliveira, Lopes, Carneiro, & Canario, 2001), and that environmental cues contingent with an interaction can trigger an anticipatory hormonal response in a Pavlovian fashion (Antunes & Oliveira, 2009).

In this experiment, we test for the first time the hypothesis that the androgen responses to territorial intrusions may provide a simple mechanism underlying the dear enemy effect. Based on the evidence above, we predict a differential androgen response towards familiar vs. unfamiliar territorial intruders, so that territorial males should exhibit a lower androgen response when confronted with a familiar intruder, than when confronted with a stranger. Furthermore, we predict that due to habituation resident males should also gradually reduce their androgen response towards repeated territorial intrusions.

These predictions will be tested using an African cichlid, the Mozambique tilapia (*Oreochromis mossambicus*). In this species males establish territories in breeding aggregations to which they attract females to spawn with, and parental care is exclusively provided by the females (Baerends & Baerends van Roon, 1950; Bruton & Boltt, 1975). Territorial males adopt a typical black velvet colouration (Neil, 1964) and build display sites (i.e. bowers) in the substrate that act as extended phenotypes used by females in mate choice (Nelson, 1995). Non-territorial males move around in breeding aggregations as floaters and either try to take over territories or to sneak fertilizations when females spawn with territorial males (R. F. Oliveira & Almada, 1998; Turner, 1986). Previous work with other fish species [*Neolamprologus pulcher* (Frostman & Sherman, 2004); *Cyprinodon variegates* (Leiser, 2003); *Cichlasoma nigrofasciatum* (Leiser & Itzkowitz, 1999)] show that resident males are more aggressive towards unfamiliar males, however there is no information on how aggression towards neighbours and strangers varies over repeated territorial intrusions. This critical step to confirm the existence of a dear enemy effect will also be addressed in this experiment. Finally, we will also address a neglect potential confound in the test of the dear enemy effect, which is the modulation of the resident's behaviour by variation in the intruders' behaviour. Since neighbouring intruders are also more familiar with the resident male than stranger intruders, the former may act more boldly towards the resident and therefore induce higher levels of territorial defence, which do not reflect the mechanisms discussed above but rather a reflexive response to higher levels of aggression by the intruder.

Material and Methods

Animal housing

Experimental fish (n=15) were selected from a stock of individuals kept at the animal housing facilities of ISPA-IU. All fish were individually tagged with a magnetic transponder (Trovan ID 100: 2.2 x 11.5 mm; identification antenna: LID 500), which was implanted under anaesthesia (MS-222) in the peritoneal cavity. Aquaria were equipped with a bottom filter and continuous aeration. A layer of sand of ca.7 cm of height was deposited at the bottom of the aquaria, allowing males to dig spawning-pits that are essential for the full expression of their behavioural repertoire (Galhardo, Correia, & Oliveira, 2008).Water temperature was kept at 24 ± 2°C and the photoperiod regime was 12L: 12D. Fish were fed once per day with commercial fish flakes (Tropical Flake, Astra).

After the experiments all fish were returned to their original stock tank and none died or showed signs of chronic stress, during or after the experiment.

Experimental procedure

At the start of the experiment males were placed in individual tanks in which they could see one adjacent male and were allowed to become familiar with this neighbour over one week. After this period of time focal males received two 10 min. experimental intrusions on their territory per day, one from their neighbour and another from a stranger male. In order to study the "dear enemy effect" we monitored the agonistic behaviour of focal males during each intrusion test.

As a non-invasive alternative to blood sampling we measured androgens from fish urine. Urine was only sampled after the intrusion tests of the 1st and the 4th (and last) day of the experimental protocol in order to minimize handling stress during the experiment. Androgen concentration in urine has been interpreted as integrating recent circulating androgen levels (R. F. Oliveira, Almada, & Canario, 1996; Rocha & Reis-Henriques, 1996). In total, 15 replicates were carried out. Each replicate consisted of: 1) a focal male who established a territory and remained in the same aquarium during the whole experiment; 2) males that were used as “intruders” in the territory of the resident, but kept their own territories in their home-tanks; two types of intruders were used: (a) one neighbour who established a territory in the same aquarium as the resident, with a transparent partition separating both males, and (b) four strangers: individuals that were housed in tanks in visual isolation from the focal male but otherwise in similar conditions to the neighbour.

In order to standardize motivational states between the two types of intruders, stranger males were kept in individual aquaria (50 x 40 x 30 cm) with visual access to each other by transparent partitions during a period of eight days prior to the start of the behavioural trials (R. F. Oliveira et al., 1996). Similarly, residents and neighbours were placed in pairs in the test aquarium (100 x 40 x 50 cm) which had two divisions separated by a transparent sheet. In these aquaria, the resident male had more space than the neighbour (70 x 40 x 30 cm vs. 30 x 40 x 50 cm), so that the putative territory of strangers and neighbours was the same size. Thus residents and neighbours could interact visually and chemically with each other, while not having direct physical contact.

Resident and neighbour males were allowed to habituate to the new aquaria also for a period of eight days. The experiments involved 10 min intrusions of either a neighbour or a stranger male at the territory of the resident. This duration was chosen because it allows the expression of the full repertoire of aggressive behaviour but it is too short for males to risk physical injury (RF Oliveira & AFH Ros, personal observations). Resident males received two intrusions per day, one in the morning and one in the afternoon with balanced order for intruder type. Before introducing an intruder in the focal fish tank, an opaque partition was placed against the transparent partition that separates the neighbour from the focal fish territories. At the end of the 10 min period, intruder males were caught and returned to their own aquarium. Focal males that were confronted with a neighbour in the first intrusion subsequently received a stranger intruder and vice versa. This set-up was repeated during the following three days but with alternating the order each day and with a balanced design [i.e.

approximately half the residents received first a neighbour (n=8) and the other half a stranger (n=7)]. Both neighbours and all strangers were only used as intruders once per day.

In order to prevent the focal males from losing a fight and since body size is one of the best predictors of victory (Brandt, 1999; Neat, Huntingford, & Beveridge, 1998) we controlled the intruder's size so that the resident would always be the largest male in each replicate. The body size of the 5 intruder males within each replicate was kept as similar as possible (Mean ± SEM for coefficients of variation across replicates = 3.0 ± 0.4%). Condition factor (K = body weight/(standard length)^3) did not differ significantly between residents, strangers and neighbours [overall condition factor across the 3 groups (mean ± SEM) = 2.93 ± 0.06; Repeated measures ANOVA: $F(2, 28) = 2.07$, $p = 0.15$]. In addition, no difference in body length was found between males assigned as neighbours or strangers [$t(14) = .11$, $p = .92$]. Together this data suggested that differences in behaviour or androgen levels elicited by the intrusion tests should not be due to variation in physical characteristics of the intruder males between groups.

Behavioural measures

All experimental intrusion trials were recorded on video and subsequently analyzed using a multi-event recorder software (Observer XT, Noldus Inc., Holland). The video analysis was performed by an observer that was blind to the experimental treatments. The following behavioural categories were quantified based on the behavioural action patterns previously described for this species (Neil, 1964; R. F. Oliveira & Almada, 1998): Approach—focal fish swims towards the intruder becoming closer than 1 body length; Displays—all occurrences of frontal displays (in a frontal position towards the opponent the fish erects the dorsal fin and opens the gill covers and the branchiostegal membrane) and lateral displays (in a parallel or antiparallel position towards the opponent the fish fully erects the dorsal and anal fins and fully spreads its caudal and pelvic fins; at its maximum intensity it can be combined with erecting the branchiostegal membrane, and with tail beating); Attack—all occurrences of chase, bite and carouseling (i.e. the two fish circle each other in an anti-parallel position often trying to bite each other); Fighting—all occurrences of mouthfighting (the opponents grip each others' jaws, and having seized each other firmly by the mouth, they push and pull with tail beats) and pendelling (the two fish in a head to head position rush at each other with the dorsal and anal fins closed against the body; just before contact with the opponent the fish brakes to keep from colliding with it; often intersparsed with mouthfighting).

Frequency (number of occurrences per 10 min) and latency (time in seconds from the moment the intruder was introduced in the tank of the focal male until the queried behavior was observed, with 10 min, i.e. trial duration, set as maximum latency) were registered for all the behavioural categories mentioned above. Duration (in seconds) was also measured for the behavioural categories that are states (i.e. displays and fighting).

Urine sampling and analysis of androgen levels

Urine was collected by applying a small pressure on the lower part of the fish flanks behind the genital papilla (R. F. Oliveira et al., 1996). Androgens were measured from fish urine collected within 5 minutes after the intrusion tests of the 1st and the 4th day of the experimental protocol, in order to minimize handling stress during the experiment Androgen concentration in the urine is interpreted as integrating recent circulating androgen levels (R. F. Oliveira et al., 1996; Rocha & Reis-Henriques, 1996).We focused only on 11-ketotestosterone (KT) since it is the main androgen in teleost fish associated with the expression of male aggressive behaviour and of secondary sexual characters (Borg, 1994; Gonçalves & Oliveira, 2011). Urine samples were stored at -20°C until further processing. Free, glucuronated and sulphated fractions were extracted from each sample of 50 μl (R. F. Oliveira et al., 1996; Scott & Sorensen, 1994). A radioimmunoassay (RIA) was used to measure the concentrations of KT in each of these fractions. The RIA characteristics, including the cross-reactivity of the anti-bodies used, have been reported before (Scott & Sumpter, 1989). The intra and inter-assay variability was 8.2% and 11.6% respectively. Total levels of KT were calculated as the sum of all three fractions in each urine sample.

Statistical analysis

All behavioural variables were logarithmically transformed [log10 (x+1)] to meet parametric test assumptions. In order to account for the influence of the intruder’s behaviour on the behaviour of the resident fish, an index [resident behaviour/(resident behaviour + intruder behaviour)] was calculated for all the paired resident male behavioural variables (i.e.

attacks and displays). An escalation index was also calculated based the resident’s agonistic behavior to territorial intrusions [attack frequency/(display frequency + attack frequency)].

To test the effects of the type of intruder on the resident behaviour, we have used a General Linear Model with type of intruder as a within-subjects factor (neighbour, stranger) and the behavioural variables as a repeated measures factor (4 levels: day 1, 2, 3, 4). A Linear Mixed Model with type of intruder (neighbour, stranger) and KT levels (day 1, day 4) as fixed factors, and the intercept as a random effect, was used to test the hormone response to the territorial intrusion, to avoid loss of data due to missing values. Planned comparisons were used within the statistical models to check for differences between strangers and neighbours in each day of the experiment (t-test for the General Linear Models, z-test for the Linear Mixed Model).

Ethics statement

Since the goal of this study was to study the effect of opponent familiarity in behavioural and hormonal responses to social challenges, and given the fact that the efficiency of the manipulation of familiarity cues in dummies or video-playbacks is questionable, and the response of this species to either of them is very limited (R.F. Oliveira, personal observation), we have used real intruder which elicited aggressive encounters. However, we have kept the sample size to a minimum and have limited the agonistic interactions to 10 min, following the “Guidelines for the treatment of animals in behavioural research and teaching” of the Association for the Study of Animal Behaviour (“Guidelines for the treatment of animals in behavioural research and teaching,” 2012). No mortality of animals or serious physical injuries resulted from this experiment and all males were returned to their previous stock tanks after the experiments. All experimental procedures involved in this study were in compliance with the regulations on animal experimentation in Portugal and were approved by a permit (0421/000/000/2013) from the Portuguese Veterinary Authorities (Direcção Geral de Alimentação e Veterinária, Portugal).

Results

Effects of intruder familiarity and habituation on aggressive behavior

Overall resident males displayed sooner [F(1, 14) = 6.468, p = .023; Fig 1A], expressed more displays and attacks [Displays: F(1, 14) = 10.053, p = .006; Attacks: F(1, 14) = 5.046, p = .041; Fig 1B] and exhibited longer displays [F(1, 14) = 11.239, p = .004; Fig 1C], towards stranger intruders than towards intruding neighbours. Both the latency to display and the latency to attack intruders decreased over the 4 days of the experiment [Displays: F(3, 42) = 4.495, p = .007; Attacks: F(3, 42) = 4.897, p = .005; Fig 1A], whereas display frequency increased with the course of the experiment [F(3, 42) = 3.298, p = .029; Fig 1B]. A marginal non-significant trend for the frequency of attacks to increase over the 4 days of the experiment was also detected [F(3, 42)=2.248, p = .096; Fig 1B]. Shorter latencies to fight neighbours compared to strangers were detected on days 1 and 2, despite the lack of a significant main effect for this variable (F(1, 14)=4.079, p = .062; Fig 1A). Resident males also engaged more frequently in fights and these lasted longer when the intruder was a neighbour than when it was a stranger (Frequency: F(1, 14)=4.640, p = .049; Duration: F(1, 14) = 4.869, p = .044; Fig 1B–1C). Planned comparisons to test differences in the behaviour of the intruder to each type of intruder on a daily basis, confirmed the main effects described above for some of the days, particularly days 1, 2 and 4, while no significant differences between the neighbour and stranger intrusions were detected on day 3 for any of the measures used in this experiment (Table 1).

Table 1

Statistical values for the differences in the resident males' aggressive behaviours towards neighbour and stranger intruders over the course of the experiment

		Neighbour vs. Stranger							
		Day 1		Day 2		Day 3		Day 4	
	Measure	t	d	t	d	t	d	t	d
Displays	Latency	2.691*	.694	.797	.205	.455	.117	2.304*	.594
	Frequency	1.971#	.508	2.221*	.573	.883	.227	2.218*	.572
	Duration	1.858#	.479	2.523*	.651	.915	.236	2.578*	.665
Attacks	Latency	1.813#	.468	.865	.223	1.022	.263	2.019#	.521
	Frequency	1.107	.285	1.958#	.505	.089	.022	2.413*	.623
Fights	Latency	2.111*	.545	2.403*	.620	.168	.043	2.048#	.528
	Frequency	1.980#	.511	2.253*	.581	.108	.027	1.487	.383
	Duration	2.028#	.523	2.384*	.615	.113	.029	1.414	.365
Escalation	Index	2.324*	.600	.465	.120	.036	.009	.459	.118

t: values for contrasts (degrees of freedom=14) between neighbour and stranger intrusions for each day of the experiment; d: effect size estimate (Cohen's d); *significant for p≤.05; ** significant for p≤.01; # non-significant trend p≤.10

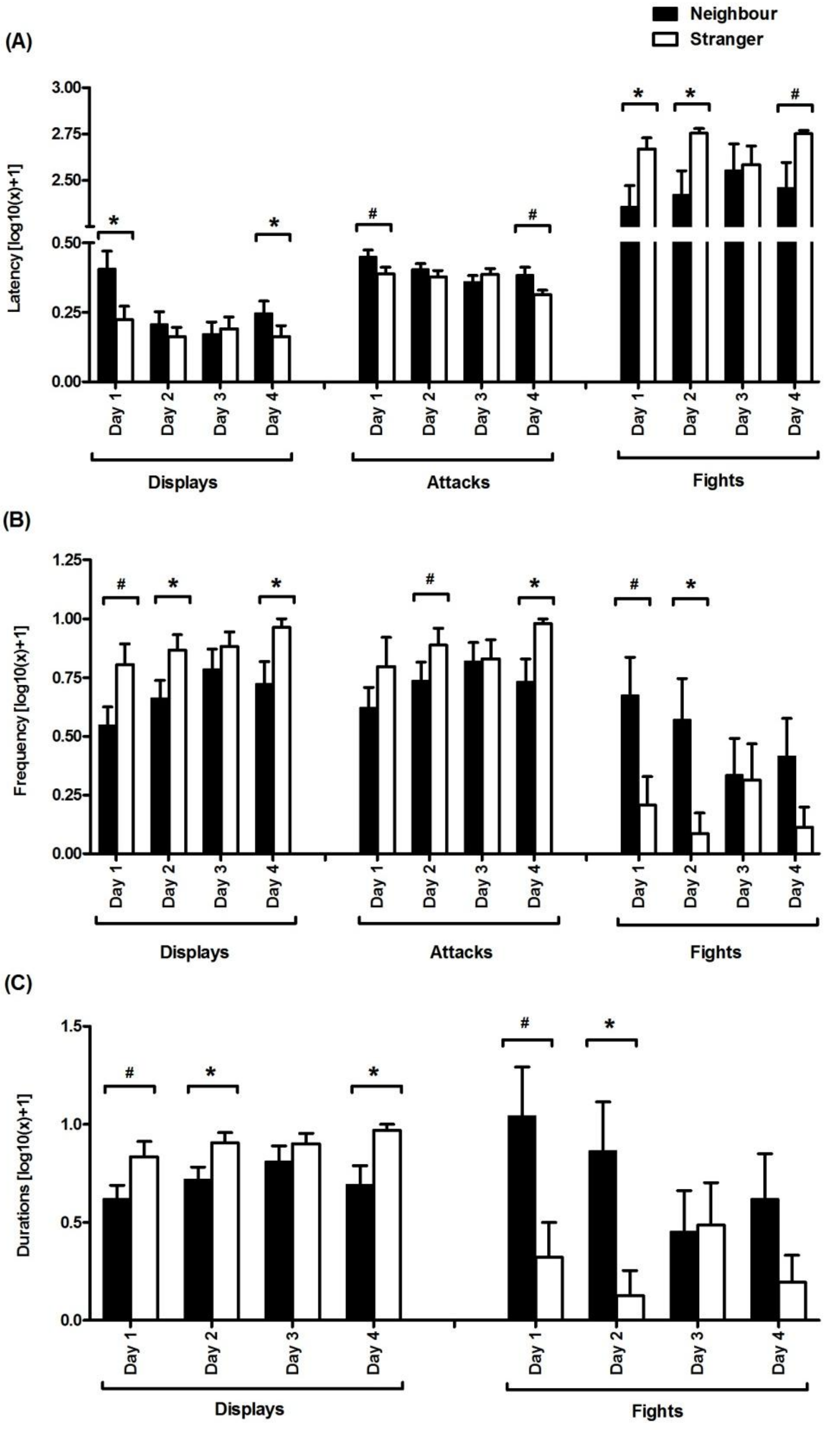

Figure 1. Aggressive behaviour displayed by resident males towards strangers and neighbour intruders during the 4 days of the experiment. A) Latency to displays, attacks and fights; B) Frequency for displays, attacks and fights; C) Duration of displays and fights; All plotted values for displays and attacks have been corrected for the influence of opponent's behaviour in the interaction. *significant for p≤.05; # non-significant trend p≤.10.

Although no main effect or interaction was detected for the escalation of fights, the resident males were more aggressive against stranger intruders than neighbours on day 1 (Intruder type: F(1, 14) = 1.642, p = .220; Time: F(3, 42) = ,81046, p = ,49525; Time x Intruder type: F(3, 42)= 2.0978, p = .114; Table 1, Fig 2).

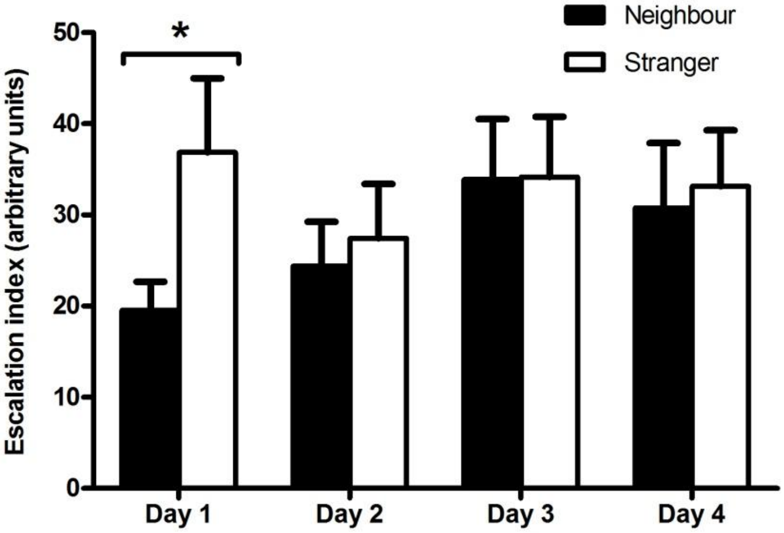

Figure 2. Resident males' escalation index for intrusions by neighbours and strangers. *significant for p≤.05.

Effects of intruder familiarity and habituation on KT levels

Overall levels of KT lowered from day 1 to day 4 (F(1, 29) = 15.219, p < .001). The resident male KT response to a territorial intrusion on day 1 was higher when the intruder was a stranger than when it was a neighbour (z = 1.928, p = .053, d = .674). This difference was no longer detected on day 4 (z = 1.034, p = .300, d = .494; Type of Intruder: F(1, 29) = 3.853, p=.059; KT x Type of Intruder: F(1, 29) = 0.508, p = .481; Fig 3).

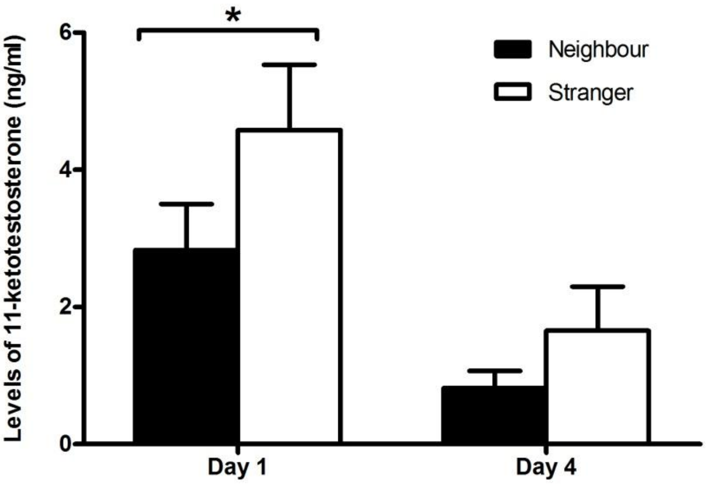

Figure 3. Resident males' 11-ketotestosterone response to intrusions by strangers and neighbours on the first and last days of the experiment. *significant for p≤.05

Discussion

In this experiment, we have tested the role of the androgen response to territorial intrusions as a mechanism underlying the dear enemy effect, using a paradigm of repeated territorial intrusions by neighbours and stranger males over the course of four days.

As predicted by the dear enemy hypothesis, resident males responded more aggressively towards territorial intrusions by strangers, as indicated by the differences in latency, frequency and duration for displays and attacks. Furthermore the escalation index confirms that the territorial intrusions by strangers elicited more aggression on the first day of the experiment. Unexpectedly, the fights against neighbours were longer and more frequent than against strangers. Since this experiment was carried out with real intruders and not with a standardized stimulus (e.g. dummies, video playbacks), it is possible that these paradoxical results for fights may be a consequence of differences in the behaviour of the two types of intruders (e.g. neighbour males being more familiar with the residents territory than strangers for whom it is novel). Moreover, all other behavioural measures were focused on the resident male and statistically corrected for the behaviour of the intruder, while no correction was possible for the fight measures, as it results from the behaviour of both males.

Contrary to the dear enemy predictions, we have not found evidence of a habituation effect on the residents' behavioural response to repeated territorial intrusions by neighbours.

Instead, the residents' aggression on intrusions by neighbours approached those displayed towards strangers on day 3 of the experiment for all the measured parameters, suggesting that the repeated intrusions may have led to a shift in the strategy adopted by the resident males. We hypothesize, based on the threat assessment and the familiarity hypotheses for the dear enemy effect (Temeles, 1994), that the repeated intrusions by neighbours caused a re-evaluation by the resident male of the threat posed by them to the resident's male territory. This implies that although familiarity is an intervening component in the threat evaluation process, it is not a sufficient estimator (Briefer et al., 2008). The increased value of threat of the neighbours would hence explain the increase in aggressive behaviour by the resident male. This hypothesis is congruent with findings in other species, showing a dissipation of the dear enemy effect over the course of repeated territorial intrusions (Monclús, Saavedra, & de Miguel, 2014) or as a consequence of changes in the context in which the familiar intruder is presented to the resident male [e.g. after a recent intrusion (Akçay et al., 2009); territorial eviction (Booksmythe et al., 2010); presence of a female (Leiser, 2003); or seasonality (Briefer et al., 2008)], suggesting that the dear enemy effect is not a fixed response, but a case of behavioural flexibility that can be modulated by the social environment.

In parallel to the dear enemy effect detected in the behavioural response, the resident males KT response to strangers was also higher than against familiar intruders on day 1. Furthermore, the KT response was lower at the end of the experiment, suggesting an habituation of the androgen response to repeated territorial intrusions. When compared to the behavioural findings, the results for KT match the findings for day 1, but are decoupled from those of day 4, in which most behaviours rebound in direction of a new dear enemy effect, after a period of similar aggression towards strangers and neighbours. These contrasting results confirm the previous finding that changes in social context (e.g. social instability, Almeida, Gonçalves-de-Freitas, Lopes, & Oliveira, 2014), may promote changes in the patterns of association between androgens and aggressive behaviour, which may become uncoupled.

Finally, it should be stressed that, in contrast to this experiment, in the natural environment resident males would also have access to information on the fighting ability of neighbours by eavesdropping on their agonistic interactions with third parties. This social phenomenon has been described for other fish species and effectively changes the fighting behaviour of bystanders (R. F. Oliveira et al., 1998), and thus may play a key role in the dear enemy phenomena. However, in the present study despite social eavesdropping not being accessible to resident males, a dear enemy effect was observed. This suggests that eavesdropped information on the relative fighting ability of the intruders, is not necessary for the dear enemy effect, and that threat assessment may rely on other social cues, such as familiarity and habituation/sensitization to intruders.

In summary, our results show for the first time that the dear enemy effect also modulates the androgen response to a social challenge, so that neighbours elicit a lower androgen response than strangers. Furthermore, this experiment along with other recent reports (Booksmythe et al., 2010; Monclús et al., 2014) suggests that the dear enemy effect is a flexible behavioural response modulated by social context and not a fixed response to familiar and unfamiliar intruders. These assumptions should be taken into account on future research in order to develop experimental designs that empirically test the predictions of the dear enemy effect in order to achieve a better understanding of this phenomenon.

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Chapter V

Unexpected winners decrease testosterone levels after a competition

Abstract

In recent years, evidence has accumulated showing that the testosterone (T) response to competition in humans does not always follow the direction predicted in the literature and that cognition may modulate this androgen response. This experiment aims to test the hypothesis that expectations about competition outcome act as a moderator of the T response to competition. Seventy-four female students enrolled in this experiment. First, participants completed a task in which expectations about the outcome of an upcoming competition were experimentally manipulated. Then, participants engaged in a competition that would confirm or violate their expectations. Levels of T and cortisol (C) were measured in several phases of the experiment. Results show that when expectations were violated, T decreased in winners and increased in losers, contrary to what was predicted by the literature. These results support the hypothesis that cognitive variables modulate the T response to competition and that the theoretical model's predictions may be reversed when hierarchies are perceived as unstable.

Keywords: testosterone, competition, expectations, appraisal, status instability

Introduction

Over the last decades several studies across different taxa have shown that agonistic interactions elicit an androgen response and that these changes in androgen levels may be integrated in social decision-making mechanisms in the brain underlying adaptive behavioral responses to changes in the social environment (G. A. Oliveira & Oliveira, 2014a; R. F. Oliveira, 2009).

Initially proposed by Mazur (1985) to explain the dynamics of testosterone (T) and dominance in primates, the biosocial model of status (BMS) has been extended to male humans (Mazur & Booth, 1998) and is one of the main theories for the social modulation of androgens. The BMS proposes a mutual reinforcing relationship between status and T, predicting outcome dependent changes in T levels in response to an agonistic encounter and a behavioral adjustment to the new position in the social hierarchy. Thus, according to the BMS, after a competition winners should increase their T levels promoting status-seeking behaviors and a decrease of T should be observed in losers along with status-avoidance behaviors in order to reduce the possibility of further losses (for a recent review see Hamilton, Carré, Mehta, Olmstead, & Whitaker, 2015; Mazur & Booth, 1998). In humans, the BMS has been studied in the context of sports and laboratory competitions, used as a proxy for agonistic encounters. Although some research is congruent with the predictions of the BMS for the direction of the T response to competition in humans, both sexes present a diversity of responses that cannot be fully explained by the BMS and this has been interpreted in recent reviews as resulting from a mediation/moderation of the androgen response by cognitive variables like appraisal (G. A. Oliveira & Oliveira, 2014b; Salvador & Costa, 2009).

Appraisal has been defined as a dynamic and recursive process between the individual and the environment, that takes into account the individual’s motivational and physiological state and the objective characteristics of the stimuli, in order to determine the significance of an event (Moors, Ellsworth, Scherer, & Frijda, 2013). Although some variation exists between different models of appraisal, most agree on a multi-component approach that includes stimulus evaluation checks for novelty, intrinsic pleasantness, predictability, goal significance, agency, coping potential and compatibility with personal and social standards (Ellsworth & Scherer, 2003). Appraisal can also be interpreted as a cognitive mechanism underlying behavioral plasticity (Faustino, Oliveira, & Oliveira, 2015). Since social environments are characterized by a high degree of complexity, as they involve interactions with other behavioral agents which generate a high level of unpredictability (Taborsky & Oliveira, 2012), for a behavioral response to be adaptive the individual must evaluate the probability of occurrence and consequences of an event, creating an expectation about the possible outcome of a stimulus (Scherer, 2001).

Previous studies have not found a relationship between T and the expectation of winning in participants playing a war videogame (Johnson et al., 2006) and in soccer fans watching the World Cup final (van der Meij et al., 2012). Recently, in a set of two experiments with female samples, Zilioli, Mehta, & Watson (2014) reported a T increase in losers compared to winners in a competition in which the outcome was manipulated in order to simulate a close defeat, a result that is congruent with previous findings in both sexes using a similar experimental paradigm (G. A. Oliveira et al., 2013, 2014). In a second experiment, the emotional state of surprise towards the competition outcome moderated the T increases of losers, so that the larger increases in T levels were found in participants that reported being more surprised with their defeat (Zilioli et al., 2014). , Since the T response present in these studies are not in the direction predicted by the BMS, it has been proposed that these results may have been driven by the status instability modeled in competitions in which victory or defeat are decided by a narrow (Zilioli et al., 2014). In support of this hypothesis, it has been shown that post-competitive increases in T levels predicts status seeking behavior after a decisive victory but not after a close victory in males (Mehta, Snyder, Knight, & Lassetter, 2014) and the increase of T levels in losers after a competition has been interpreted as an indicator of motivation to keep disputing status in future interactions (Mehta & Josephs, 2006; G. A. Oliveira et al., 2013; Zilioli et al., 2014).

However, the emotional state of surprise measured by Zilioli et al. (2014) in the second experiment can be interpreted as the endpoint of an evaluation process that is characterized by the violation of expectations (Scherer, Zentner, & Stern, 2004), and thus opens the possibility of a modulatory effect of appraisal components such as predictability and congruence with expectations on the T response to the outcome of a contest. Based on this we hypothesize that, in addition to the objective insufficient status asymmetry between winners and losers in cases of a close contest, the individuals' evaluation of the hierarchical structure and their own potential to induce hierarchical changes may be critical to reverse the direction of T response predicted by the BMS.

In support of the status instability hypothesis and of a potential role of appraisal in this process, previous research has shown that a low status group presents a threat physiological response compared to a high status group, but the possibility of a change in group status reversed the pattern and led to a threat physiological response in members of the high status group (Scheepers & Ellemers, 2005). These results were corroborated in an experiment manipulating group stability. When status was stable threat occurred in members of low status groups, but in the unstable status scenario, threat responses were found in the members of the high status groups (Scheepers, 2009). Moreover, high status individuals are more controlling and report more performance anxiety in unstable hierarchies (Georgesen & Harris, 2006).

The primary goal of this experiment is to test the hypothesis of the expectations of the outcome of a competition as a cognitive moderator of the T response to competition. For this purpose, instead of measuring the participants’ expectations or surprise when the outcome was revealed (van der Meij et al., 2012; Zilioli et al., 2014), in this study the participants’ expectations were experimentally manipulated and measured before the competitive task, based on previous work (Scheepers, Branscombe, Spears, & Doosje, 2002). Cortisol (C) levels were also measured to monitor HPA axis activity due to social stress (Schoofs & Wolf, 2011). We predicted that participants in the confirmation of expectations treatment would show a T response to the competition in the direction proposed by the BMS. The violation of expectations treatment, however, would allow us to confront the classic predictions of the BMS (Mazur & Booth, 1998) with the reversed winner-loser effect on T, proposed by the status instability hypothesis (Zilioli et al., 2014). Thus, based on the predictions of the BMS, winners should present an increase of T levels higher to the one presented by the winners in the confirmation of expectations treatment and T in losers should present a decrease in T to lower levels than those presented by the losers in the confirmation of expectations condition.

Materials and Methods

Participants and experimental design

Seventy-four undergraduate psychology female students (Mean: 20.972 ±.623 years) voluntarily enrolled in this experiment. Participants were informed that the experiment required physiological measures and were instructed to abstain from smoking, eating, drinking, consuming pH altering substances (examples for this option were explicit), physical exercise or brushing their teeth for 1 hour before the experiment. This was confirmed in each experimental session. Participants were tested in groups (Mean: 7.756 ±.377 individuals per session) and were randomly assigned to the conditions across the cells of a 2 x 2 (Result: Winner, Loser; Expectation: Confirmation, Violation) between subjects experimental design. All experimental sessions were conducted by the same male and female experimenters. Sessions lasted for approximately 45 minutes and were scheduled from 1:30pm to 2:30pm to control for circadian variation of hormone levels. All participants gave written consent and were rewarded with one course credit for their participation. This experiment was performed with the approval of the Ethics Committee of ISPA-IU’s Research Centre.

Psychological variables and instruments

Group identification measure: The level of group identification was measured using an adaptation of the scale developed by Doosje, Spears, & Ellemers (2002). Participants were asked to rate their degree of agreement with five items using a scale of nine points (ex. “At this moment, I can identify myself with my group”; 1=Disagree Completely, 9=Agree Completely).

Probability of winning: One item with a nine points scale was presented before the competition in order for the participants to rate their group’s probability of winning the competition (“How do you evaluate the probability of your group winning the upcoming competition?”; 1=Very poor 0%, 9=Very strong 100%).

Outcome predictability and congruence with expectations: Outcome predictability and congruence with expectations were assessed using two items with a nine-point scale (“I feel that the result of this competition is”: 1=Not predictable, 9=Very predictable; “Considering your expectations this result is: 1=Not expected, 9=Completely expected).

Influence of group performance in the competition outcome: Participants were asked to rate the influence of in-group and out-group performance, and of random events on the competition outcome. These variables used three items with nine points each (ex. “What influence had the performance of the adversary group in the result of the competition?”: 1=No influence, 9=Very influential).

Mood measure: To measure mood, defined as a low-intensity, unstable, continuous and diffuse affective state, we used a set of three mood state items, embedded in a six item questionnaire. Each mood item has an opposing pair of adjectives (good-bad, negative-positive, sad-happy), that must be rated using a 9 points scale (Garcia-Marques, 2004).

Appraisal measure: The appraisal of the competition and of competition outcome as a threat or challenge was measured using two semantic differential items with a nine-point scale each (i.e. "I feel that the upcoming competition could be: "1=Not threatening, 9=Very threatening; 1=Not challenging, 9=Very challenging; (G. A. Oliveira et al., 2013; Tomaka, Blascovich, Kelsey, & Leitten, 1993). Threat and challenge appraisals reflect the individual's evaluation of the task demands and the available coping resources in goal relevant situations like a competition. Threat occurs when the task demands are evaluated as exceeding the available resources; conversely, an event is evaluated as a challenge when the individual resources surpass the task demands (Blascovich & Mendes, 2000).

Procedure and data collection

Participants were asked to seat in individual cubicles separated by vertical partitions to prevent interactions between participants and the visualization of other computer screens besides their own. In the beginning of the experiment, participants provided a first saliva sample and were asked to complete a first measure of mood (Garcia-Marques, 2004). Upon completion of the mood questionnaire, the experimenters explained that the purpose of the experiment was to verify the relationship between perceptive styles and the discrimination of symbols and therefore the experiment was composed by a test to determine their perception style (manipulation of expectations) and a symbol discrimination task (competitive task). This cover story was used to keep participants unaware of the competitive nature of the second task and thus avoid anticipatory responses to the upcoming competition.

A dot estimation task (Scheepers et al., 2002) was presented to the participants as a test that will evaluate and categorize their perception style. The dot estimation task was composed by a series of twelve clouds of black dots (ranging from 20 to 40 dots) that were displayed for 2.5 seconds each. Task instructions focused on the importance of relying on intuition to make good estimates, since it would not be possible to count all the dots. These instructions aimed to withdraw the participant's control on the outcome of the dot estimation task, allowing the experimenters to give a more credible false feedback about the participant's belonging to a particular group. At the end of the dot estimation task, participants were categorized as members of the "holistic" or "detailed" perception style group depending of their assigned experimental condition.

In order to manipulate the expectations of the participants, individuals that were categorized as "holistic" were informed that previous research demonstrated that members of the "holistic" group had a high capacity to discriminate symbols and were 75% more likely to win the upcoming competition against the "detailed" group. Likewise, members of the "detailed" group were informed that their symbol discrimination capacity was poor and only had a probability of 25% to win against the "holistic" group. The experimenters reinforced the false feedback and informed the participants that the "holistic" and "detailed" groups would now compete on a symbol discrimination task.

A set of questionnaires was given to the participants to assess group identification (Doosje et al. 2002), mood (Garcia-Marques 2004) and appraisal of the competition (G. A. Oliveira et al., 2013). As a manipulation check, participants were asked to identify their group membership and how they evaluate the probability of their group winning the upcoming competition. After the completion of the questionnaires, the participants were asked to provide a second saliva sample and the competitive task was presented to them. The competitive task was composed by three sets (symbols, letters, numbers) of 20 trials, in which the participants had to identify the number of repetitions (ranging from 1 to 5) of a target character (Θ for the symbols, A for the letters and 4 for the numbers) embedded in a string of 12 characters (ex. 141774149141) exhibited on screen for 500msecs (a similar task can be found in Scheepers, 2009). The experimenters' instructions stressed the competitive nature of the task and participants were asked to remain engaged in order to provide fast and accurate answers.

Upon completion of the competitive task and before the result was announced to the participants, a third saliva sample was collected (12.8 ±.442 minutes after the end of the manipulation). The outcome of the competition was then revealed as a false feedback dependent of the participant's experimental condition and the participants were instructed to complete a third set of questionnaires. The third set of questionnaires was composed by two questions to confirm that the participants identified their group membership and competition outcome, followed by a measure of mood (Garcia-Marques, 2004), appraisal of the competition outcome as a threat/challenge (G. A. Oliveira et al., 2013), the items for outcome predictability and congruence with the expectations and finally the ratings for the influence of the group's performance on the competition outcome. When participants ended this set of questionnaires they were given personality questionnaires unrelated to this experiment to occupy them until the last saliva sample, collected 15 minutes after the announcement of the competition outcome (Carré & McCormick, 2008; Schultheiss et al., 2005). The collection of this saliva sample finished the experimental session.

Hormone assays

Saliva samples were collected by passive drool into 5 ml polypropylene tubes and were stored at -20◦C immediately after the end of the experimental session. The samples were then thawed and then centrifuged at 2245 g for 10 minutes at 20 °C and the supernatant stored once again at -20◦C until the assay. The hormonal assays were conducted using luminescence immunoassay kits (IBL-Hamburg, Germany) for T and ELISA kits (IBL-Hamburg, Germany) for C. The intra-assay and inter-assay coefficients of variance were respectively 2.9% and 6.7% for T and 1.6% and 7.3% for C.

Preliminary analysis

T measures were log-transformed in order to correct for skewed distributions (raw data is presented in Table 1). F measures did not require transformation and were analyzed based on their absolute values (Table 2). One individual presented contaminated saliva samples and was excluded from this study. Variables were scanned for extreme values (±3 standard deviations) and one participant was excluded based on this criterion. As in previous research (Mehta & Josephs, 2006; G. A. Oliveira et al., 2013), the unstandardized residuals of linear regressions were used as measures of T and C response to the manipulation of expectations and to the competition. On the manipulation hormone response measures, the baseline sampling point was used as a predictor of the hormone levels right after the competition and before the outcome was divulged (T: $R^2=.580$, $p<.001$; C: $R^2=.864$, $p<.001$). For the measures of hormonal response to the competition, the linear regression was composed by the hormone levels just before the competition as a predictor of the hormone levels 15 minutes after the result was divulged to the participants (T: $R^2=.732$, $p<.001$; C: $R^2=.936$, $p<.001$). No baseline differences in T or C were detected between winners and losers in the confirmation (T: t_{32}=1.310, p=.199, g=.439 ; C: t_{29}=.262, p=.794, g=.093) and violation of expectations conditions (T: t_{35}=.860, p=.395, g= .278; C: t_{35}=1.245, p=.221, g=.402).

Table 1

Testosterone levels for winners and losers in the confirmation and violation of expectations conditions (pg/ml)

Condition	Outcome	T1		T2		T3		T4	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Confirmation	Winner	22.655	3.297	20.323	3.576	20.791	3.161	24.727	4.029
	Loser	16.573	2.077	17.394	1.924	16.634	2.183	21.941	3.124
Violation	Winner	22.875	2.896	26.302	3.066	29.395	5.408	26.825	5.407
	Loser	27.824	5.107	28.349	6.274	25.353	4.767	27.361	5.275

Table 2

Cortisol levels for winners and losers in the confirmation and violation of expectations conditions (μg/dl)

Condition	Outcome	C1		C2		C3		C4	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Confirmation	Winner	.504	.053	.502	.062	.482	.060	.475	.057
	Loser	.483	.056	.479	.056	.470	.049	.471	.049
Violation	Winner	.563	.060	.587	.060	.568	.057	.478	.052
	Loser	.769	.165	.782	.158	.729	.152	.703	.154

Phase of the menstrual cycle was monitored, but not statistically tested since most participants reported using oral contraceptives (Confirmation/Winner=66.6%; Confirmation/Loser= 88.8%; Violation/Winner=50%; Violation/Loser=58.8%). Furthermore, previous research did not find effects of the menstrual cycle on T and C variation (Dabbs & Rue, 1991; Liening, Stanton, Saini, & Schultheiss, 2010). Participants taking oral contraceptives had marginally non-significant lower T levels than non-users ($F_{1,\ 67}$=3.743, p=.057) and no effect was found for C levels ($F_{1,\ 64}$=.100, p=.751). Since no effect of oral contraceptives was detected on the T or C response (see statistical analysis) to the manipulation ($F_{1,\ 65}$=1.186, p=.279; $F_{1,\ 62}$=2.189, p=.144) or to the competition ($F_{1,\ 60}$=.007, p=.932; $F_{1,\ 58}$=.062, p=.804), this factor was excluded from further analysis. These findings are congruent with previous research showing basal differences in T levels but no effect of oral contraceptives on the hormone variation in response to competition (Edwards & O'Neal, 2009).

Statistical analysis

Validation of the manipulation: Independent t-tests were used to test for differences in "probability of winning" and "group identification" between the Holistic and Detailed group. Appraisal of the competition (Threat, Challenge) was inserted as a repeated measures factor in an ANOVA with Group (Holistic, Detailed) as an independent variable. Differences in T and C response to the manipulation were assessed using independent t-tests (Holistic vs. Detailed).

Effects of the competition outcome: "Outcome predictability" and "Outcome congruence with expectations" were used as dependent variables on a MANOVA with Outcome (Winner, Loser) and Expectation (Confirmation, Violation) as independent variables. Separate General Linear Models with Outcome (Winner, Loser) and Expectation (Confirmation, Violation) as independent variables were used to test for differences in the following repeated measures factors: Influence of group performance on competition outcome (In-group, Out-group, Random), Mood (Baseline, After the manipulation, After the competition outcome), Appraisal of the competition outcome (Threat, Challenge). T and C response to the competition were inserted as dependent variables in separate factorial ANOVA with Outcome (Winner, Loser) and Expectation (Confirmation, Violation) as independent factors.

Mean and standard error of mean are presented as descriptive statistics throughout the manuscript. Planned contrasts were used for *a priori* comparisons within the statistical model. Effect sizes for main effects and interactions are presented as partial eta squared (η^2) and Hedge's *g* (Cohen's *d* corrected for sample size bias) for the planned comparisons (Lakens, 2013). Degrees of freedom vary between models due to insufficient saliva volume on some samples to conduct the hormone assays. All statistical analyses were conducted using the software package STATISTICA, version 12 (StatSoft, Inc., Tulsa, USA).

Results

Validation of the manipulation

Probability of winning: Participants assigned to the holistic group rated the probability of winning the competition higher than the members of the detailed group (independent t-test: t_{71}=2.431, p=.017, g=.563; Holistic: 5.771±.201; Detailed: 5.000±.241).

Group identification: No differences in group identification were detected between the members of holistic and detailed groups (independent t-test: t_{71}=.987, p=.326, g=.223; Holistic: 5.960±.214; Detailed: 5.631±.229).

Appraisal of the competition: The participants evaluated the competition as more of a challenge than a threat (Main effect: $F_{1,\ 71}$=123.00, p<.001, η^2=.634; Threat: 2.616±.198; Challenge: 5.945±.193) and this effect was not influenced by group membership (Appraisal x Group: $F_{1,71}$=.760, p=.386, η^2=.010).

Hormonal response to the manipulation of expectations: No differences between the holistic and detailed group were detected for T or C response to the manipulation (independent t-test for T: t_{69}=1.443, p=.153, g=.339; Holistic: -.069±.061; Detailed: .063±.068; independent t-test for C: t_{66}=.383, p=.702, g=.092; Holistic: -.006±.021; Detailed: .006±.025)

Effects of the competition outcome on cognitive and hormonal variables

Outcome predictability and congruence with expectations: The participants assigned to the confirmation condition rated the outcome of the competition as more predictable (Confirmation: 5.805±.381; Violation: 4.135±.307) and congruent with their expectations

(Confirmation: 6.000±.300; Violation: 4.161±.316) than the participants assigned to the violation of expectations condition (Main effect: Wilks λ=.786, $F_{2, 68}$=9.249, p<.001, η^2=.213). No main effect or interaction was found for the Outcome factor (Main effect: Wilks λ=.975, $F_{2, 68}$=.855, p=.429, η^2=.024; Outcome x Expectation: Wilks λ=.976, $F_{2, 68}$=.819, p=.445, η^2=.023).

Influence of group performance on the outcome of the competition: Participants rated the outcome of the competition as resulting more from the performance of the in-group and out-group than from random events (Main effect: $F_{2, 138}$=19.975, p<.001, η^2=.224) and this effect was moderated by the Outcome of the competition (Influence x Outcome: $F_{2, 138}$=4.018, p=.020, η^2=.055). Winners rated the performance of the in-group as having more influence on the outcome than losers (contrast: t_{69}=3.361, p=.001, g=.775; Winner: 7.052±.258; Loser: 5.628±.343), but no difference was detected between winners and losers neither for the performance of the out-group (contrast: t_{69}=0.553, p=.581, g=.139; Winner: 5.315±.363; Loser: 5.628±.369) nor for the influence of random events (contrast: t_{69}=.429, p=.668, g=.101; Winner: 4.473±.317; Loser: 4.257±.383). No effect of Expectation was detected (Main effect: $F_{1, 69}$=.980, p=.325, η^2=.014; Influence x Outcome x Expectation: $F_{2, 138}$=.039, p=.961, η^2<.001).

Mood: No main effect for Outcome ($F_{1, 69}$=.980, p=.325, η^2=.014) was detected, however a significant interaction was found between Mood and Outcome ($F_{2, 138}$=10.019, p<.001, η^2=.126). After the competition outcome was announced, winners had a more positive mood than losers (contrast: t_{69}=3.330, p=.001, g=.782; Winner: 6.929±.205; Loser: 5.809±.264). No differences were found for Mood at the beginning of the experiment (contrast: t_{69}=.161, p=.872, g=.033; Winner: 6.122±.207; Loser: 6.171±.262), or after the manipulation of expectations (contrast: t_{69}=.957, p=.341, g=.226; Winner: 6.087±.174; Loser: 6.380±.249). No effect of Expectation was found (Main effect: $F_{1, 69}$<.001, p=.994, η^2<.001; Mood x Outcome x Expectation: $F_{2, 138}$=.246, p=.782, η^2=.003).

Appraisal of the competition outcome: The participants evaluated the outcome of the competition as more challenging than threatening (Main effect: $F_{1, 69}$=126.815, p<.001, η^2=.647; Figure 1). The ratings given by the participants to the threat/challenge items depended on their assigned conditions for Outcome and Expectation (Appraisal x Outcome x Expectation: $F_{1, 69}$=7.355, p=.008, η^2=.096). When expectations were confirmed, the losers evaluated the outcome as more of a challenge than the winners (contrast: t_{69}=2.652, p=.009, g=.900) and no differences were found for the evaluation as a threat (contrast: t_{69}=.520, p=.604, g=.172). However, when the expectations were violated, the evaluation as threat was marginally higher for losers than for winners (contrast: t_{69}=1.765, p=.081, g=.560) and no differences were found for the appraisal as a challenge (contrast: t_{69}=.292, p=.770, g=.091).

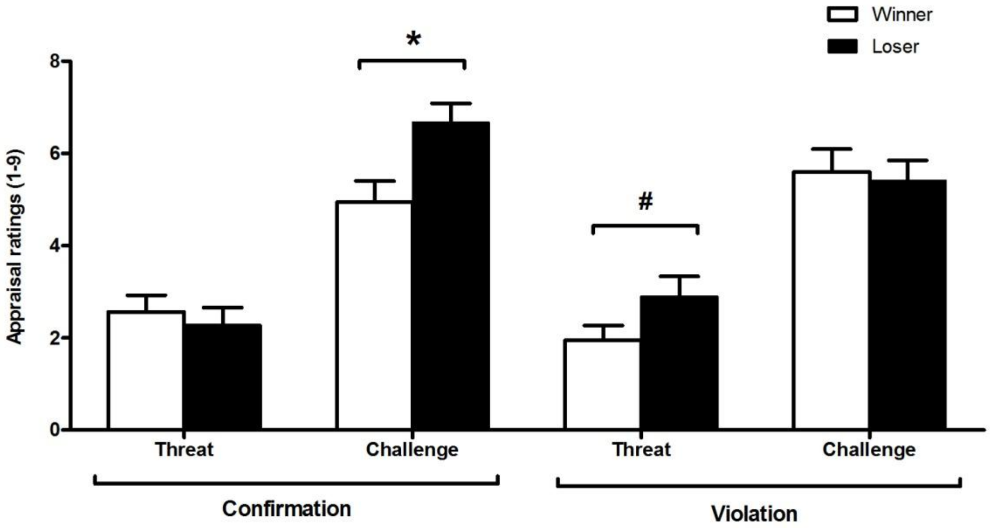

Figure 1. Ratings for the appraisal of the competition outcome as a threat and challenge (Mean±SEM). (*) significant difference for p≤.05; (#) non-significant trend p<.10.

Testosterone response to the competition: Significant differences were found between winners and losers when expectations were violated (contrast: t_{66}=2.212, p=.030, g=.746; Figure 2A), with winners decreasing and losers increasing their T levels in response to the competition outcome, but no differences were found between the T increases found in winners and losers assigned to the confirmation of expectations condition (contrast: t_{66}=.423, p=.673, g=.137). Differences were also found for the T response of winners in the confirmation and violation of expectations conditions (contrast: t_{66}=2.667, p=.009, g=.836), but not for the T response of losers (contrast: t_{66}=.021, p=.982, g=.007). No overall effect of Outcome on T was detected (Main effect: $F_{1,\ 66}$=1.548, p=.217, η^2=.022) and the effects for Expectation did not reach statistical significance (Main effect: $F_{1,\ 66}$=3.306, p=.073, η^2=.047; Outcome x Expectation: $F_{1,\ 66}$=3.422, p=.068, η^2=.049).

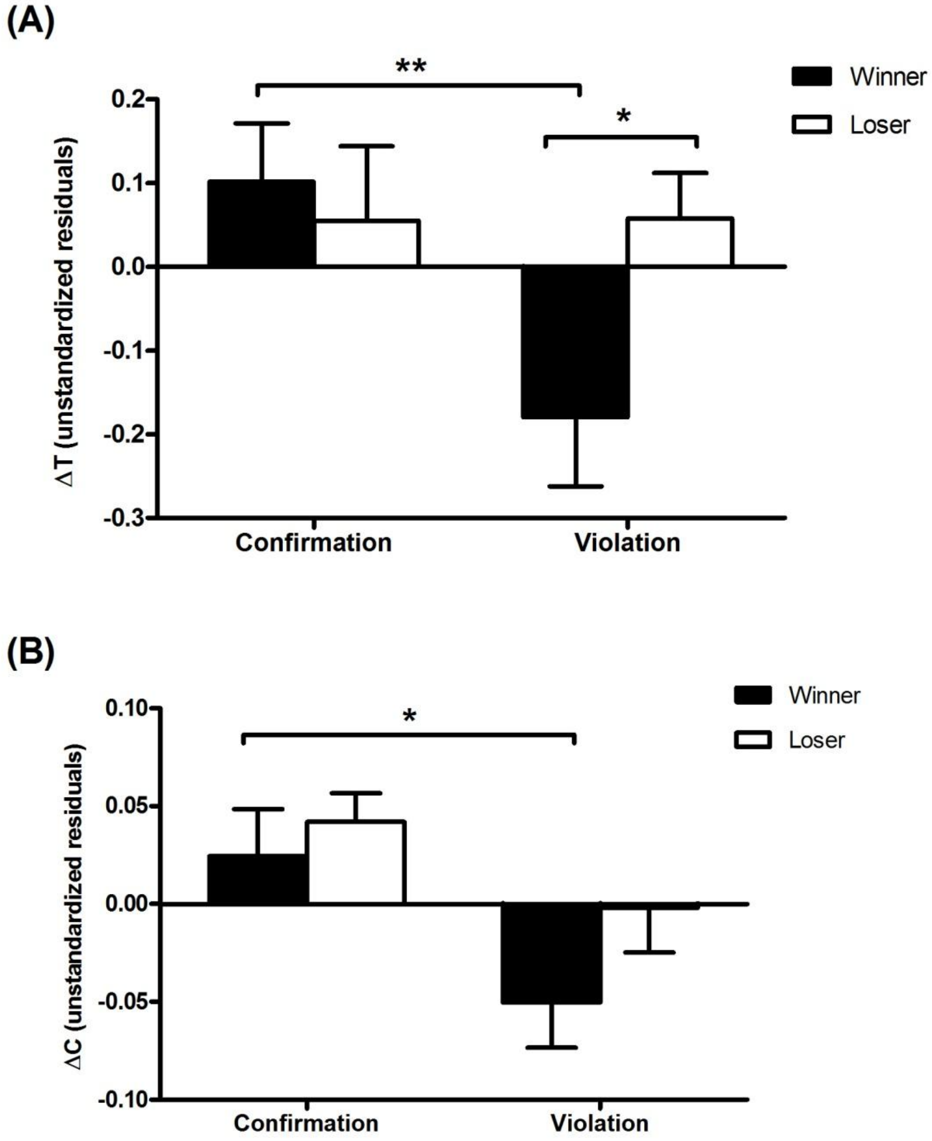

Figure 2. Hormone response to the competition (unstandardized residuals; Mean±SEM) in the confirmation and violation of expectations conditions. (A) Testosterone; (B) Cortisol. (*) significant difference for $p \leq .05$; (**) significant difference for $p \leq .01$.

Cortisol response to the competition: The C response to the competition was lower when the expectations about the competition outcome were violated than when they were confirmed (Main effect: $F_{1, 64}$=7.296, p=.008, η^2=.102). No differences were found when comparing the C response (Figure 2B) of winners and losers within the confirmation and violation condition (contrast: t_{64}=.545, p=.587, g=.223; contrast: t_{64}=1.630, p=.107, g=.475). Furthermore, no differences in C response were found when comparing losers between the confirmation and violation conditions (contrast: t_{64}=1.401, p=.166, g=.542). Winners in the violation of expectations condition showed a C response that was significantly lower than the one found for the confirmation of expectations (contrast: t_{64}=2.429, p=.017, g=.735). No effects for Outcome were detected (Main effect: $F_{1,64}$=2.263, p=.137, η^2=.034; Outcome x Expectation: $F_{1,64}$=.490, p=.486, η^2=.007).

Correlations between cognitive and hormonal variables

Predictability and congruence with expectations were not significantly associated with T response to the competition in each of the experimental conditions (.248≤p≤.996). The C response to the competition was only marginally associated with predictability (r=-.420, p=.093) and congruence with expectations (r=.429, p=.085) for the losers in the violation of expectation condition. Marginal positive correlations were found in winners in the confirmation of expectations treatment for mood after the competition and T response to the competition (r=.473, p=.055) and for the appraisal of the competition outcome as a threat and the C response to the competition (r=.460, p=.084). T and C responses to the competition were only associated for winners in the confirmation treatment (r=.748, p=.001; all other groups: .151≤p≤.845)

Discussion

The purpose of the current experiment was to test whether the T response to competition was moderated by the participant’s expectations of the outcome (winning or losing) and to contrast the direction of the observed T response to the competition with the predictions of the BMS (Mazur & Booth, 1998). Using a task similar to Scheepers et al. (2002), before the competition the participant’s expectations were manipulated with false feedback on their probability of winning the upcoming competition. The groups showed no differences in the degree of group identification and evaluated their probability of winning the competition in the direction of the false feedback given by our manipulation (holistic group with higher probability of winning). Furthermore, when the outcome was revealed, the participants in the confirmation of expectations condition considered the result as more predictable and congruent with their expectations than the participants in the violation of expectations condition. Overall, these results suggest that the manipulation was successful in creating two groups with significantly different expectations towards the competition outcome. Contrary to our predictions, the manipulation did not influence the appraisal of the competition as a threat/challenge since no effect of group membership was detected. We hypothesize, based on winners underestimating and losers overestimating their chances of winning the competition, that our false feedback was integrated and adjusted by the participant's internal state resulting in a lower asymmetry than the one we aimed to induce. We have not found a T or C response to this manipulation, even though it implied differences in group status (Scheepers et al., 2002; Scheepers & Ellemers, 2005).. It is possible that a hormonal response to the manipulation was masked by different endocrine dynamics elicited by the competition task making its' detection impossible with the current experimental design. Future studies should address this and aim to better decouple these two events in their experimental design.

Participants attributed the competition outcome as resulting more from group performance than from the influence of random factors, suggesting that the false feedback was credible. As expected, winners had a more positive mood after the competition than losers, but no consistent association was found between self-reported mood and the hormonal response to competition, a result that matches previous findings (González-Bono, Salvador, Ricarte, Serrano, & Arnedo, 2000; van Anders & Watson, 2007).

Based on the predictions of the BMS, we expected T to increase in winners and decrease in losers (Mazur & Booth, 1998). Moreover, we expected these androgen responses to be amplified in the violation of expectations treatment due to the unexpected gain or loss of status. On the confirmation of expectations treatment, no differences were found between the T response of winners and losers, with both conditions showing an increase in T levels. Although the T response in losers was not in the predicted direction these results may still be reconciled with the BMS, since the confirmation of expectations implies no changes in the social hierarchy and thus winners and losers retain high and low status respectively. Therefore, this increase in T can be interpreted as a more general response to the social challenge presented by the competition itself (Edwards & O'Neal, 2009; van der Meij, Buunk, Almela, & Salvador, 2010). In this sense, the increase of C levels also detected on both conditions corroborates this interpretation.

When the participants' expectations were violated, winners significantly decreased their T levels after the competition and losers increased T in a magnitude similar to that found in the confirmation of expectations treatment, supporting the status instability hypothesis

(Zilioli et al., 2014) and not the predictions of the BMS model (Mazur & Booth, 1998). The status instability hypothesis suggests that when an unstable hierarchy is modeled in a competition, the direction of the T response should be reversed, so that T decreases in winners and increases in losers, to promote status-avoidance and status seeking behaviors respectively (Zilioli et al., 2014). While in the Zilioli et al. (2014) experiment, the higher increases of T were found in losers that were more surprised with the competition outcome, no association for surprise was detected on winners. Although previous research (G. A. Oliveira et al., 2013, 2014) have also reported T increases in losers in close contests and interpreted this androgen response as a signal of motivation to keep disputing status supporting the Zilioli et al. (2014) status instability hypothesis, to the best of our knowledge this is the first time that experimental evidence is provided for a reversal of the T response in unexpected winners after a competition. In this experiment, the winners in the violation treatment were members of a low status group that were unexpectedly victorious against a high status group. Based on the hypothesis of a mutual relationship between T and status (Mazur & Booth, 1998; Mazur, 1985), the decrease of T levels in unexpected winners could be interpreted as a signal of status-avoidance in a social hierarchy that was perceived as unstable. Interestingly, an independent significant decrease of C levels was also detected on unexpected winners suggesting that, although these hormonal changes have the same direction, they might result from the down-regulation of different neuroendocrine axis since it has been suggested that in women the adrenal cortex and the ovarian stroma contribute equality to the production of T (Burger, 2002). Moreover, although it did not reach statistical significance, the participants that unexpectedly lost the competition against a lower ranked group, rated the outcome as more threatening than winners. This data is in the same direction as previous research showing threat responses in high status groups when there is a possibility for changes in the hierarchy or when status stability is manipulated (Scheepers & Ellemers, 2005; Scheepers, 2009). Taken together with the data for C already discussed, these results for the evaluation of the competition outcome suggests that for the winners in the violation of expectations treatment, the experience of winning against a higher ranked group was not as stressful and threatening as losing to a low status group.

In conclusion, in this experiment the manipulation of the participants' expectations of winning or losing a competition revealed that the predictions of the BMS (Mazur & Booth, 1998) are reversed when expectations are violated, supporting the status instability hypothesis (Zilioli et al., 2014) and a role for expectations as a moderator of the T response to competition. Further research is required to investigate if these findings can be extended to males and to provide experimental evidence for the behavioral relevance and implications of these reversed T responses in post-competitive contexts.

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Oliveira, G. A., Cavalheiro, A. S., Félix, A. S., & Oliveira, R. F. (Submitted). Unexpected winners decrease testosterone levels after a competition.

Chapter VI

Non-threatening negative affect decreases testosterone levels in females

Abstract

It has been suggested in the literature that mood could mediate the testosterone response to competition in humans. In this experiment, we have tested the direct effect of mood on T using emotional film clips. Since positive and negative affect are broad dimensions, we have decoupled amusement from tenderness and fear/anger from sadness, in order to better represent affective states with a distinct adaptive value. One hundred and sixteen undergraduate females participated in this experiment. We have found a significant testosterone decreases in the sadness based, non-threatening negative affect condition. Furthermore, post-stimuli decreases in testosterone levels were associated with attempts to suppress the emotional response to the film clips.

Keywords: mood, affect, testosterone, suppression, induction

Introduction

In recent years, the knowledge of the effects of testosterone (T) on human behavior has greatly increased, shifting the common view of T as a male hormone involved in aggression and reproduction, to an important modulator of social and affective behavior in both sexes (G. A. Oliveira & Oliveira, 2014b; van Anders, 2013).

In competition research, mood was one of the first psychological variables suggested as a mediator of the T response. According to this hypothesis, T increases after winning a competition when individuals' experience an increase in positive mood (Mazur & Lamb, 1980). This effect was also reported in other experiments (Booth, Shelley, Mazur, Tharp, & Kittok, 1989; Gladue, Boechler, & McCaul, 1989; McCaul, Gladue, & Joppa, 1992) and the hypothesis of mood as a mediator of the T response to winning has gained support in a recent review (Chichinadze, Lazarashvili, Chichinadze, & Gachechiladze, 2012). However, other studies have not found an effect between mood measures and androgen variation (e.g. González-Bono et al., 2000, 1999; Oliveira et al., 2009; van Anders and Watson, 2007).

The hypothesis of mood mediating T changes presupposes a direct effect of mood on T that can be experimentally tested using emotional stimuli even when the competition context is removed. Several experiments have shown that film clips can be used to elicit changes in the T levels of the participants, although this research has been performed with male samples. For example, T increases have been reported in men after watching a sexual stimuli (e.g. Pirke et al., 1974; Hellhammer et al., 1985) and after watching the video of a previous victory (Carré & Putnam, 2010), while T decreases have been reported in response to a stressful film (Hellhammer et al., 1985). In a more recent experiment with professional male athletes, T increased after watching training, erotic, humorous and aggressive films, but did not significantly varied after watching a sadness inducing film clip (Cook & Crewther, 2012).

Mood can be conceptualized as being comprised by the orthogonal dimensions of positive affect (PA) and negative affect (NA). This structure is supported by an extensive amount of research and implies that PA and NA can vary independently (e.g. Watson and Clark, 1997). However, for the present research, the dimensions of PA and NA could be too broad, by grouping sets of affective experiences that have a distinct adaptive value and originate different behavioral and physiological responses. Moreover, since it's hypothesized that the T changes elicited by the social environment have an adaptive function and that psychological variables may interfere in this process (G. A. Oliveira & Oliveira, 2014a), these different affective profiles within PA and NA could lead to different T responses, which may explain the inconsistencies in the associations between mood and T in the literature. Support for this hypothesis can be found in a recent experiment with a male sample showing different responses for anger (T increase) and sadness (non-significant T decrease), although both would be categorized as NA (Cook & Crewther, 2012). In order to surpass this issue, in this experiment, we have used validated stimuli that induce different emotional changes within the more general dimensions of PA and NA (Schaefer, Nils, Sanchez, & Philippot, 2010). For the dimension of PA, we have used stimuli to induce changes in amusement or tenderness, splitting positive affect into the Humorous and Affiliative conditions. For NA we have separated the emotions that signal threat (e.g. fear, anger) from the experience of sadness, originating the Threat and Sadness conditions. Based on previous research with men (Cook & Crewther, 2012), we expect that T reactivity to the emotional films will differ between the conditions of the same affective valence, reflecting the differences in the adaptive value of the induced affective experiences. We have controlled participants for alexithymia and emotional suppression, since these variables may interfere with how the emotional films are perceived by the participants (Mauss, Bunge, & Gross, 2007), and also for the use of oral contraceptives based on previous research showing that this variable could moderate the T response to affective experiences in women (Goldey & van Anders, 2011).

Material and Methods

Participants and experimental protocol

One hundred and sixteen undergraduate psychology female students (Age: 20.56 ±.419 years) participated in this experiment. Three participants were excluded due to contaminated saliva samples, bringing the final sample to one hundred and thirteen participants (Neutral: n=23; Humorous: n=22; Affiliation: n=22; Anger: n=23; Sadness: n=23). All experimental sessions were scheduled to occur between 12:30 and 14:30 to control for circadian variation of hormone levels. A male and a female experimenter were present in all experimental sessions. Participants were instructed to abstain from smoking, eating, drinking, consuming pH altering substances (items were explicit), brushing their teeth or doing physical exercise for 1 hour before the experiment. Compliance with these requirements was individually confirmed in each session. Previous visualization of the film clips used in this experiment was controlled *a posteriori* and this sample only includes the participants that reported not having seen the film specific to their assigned experimental condition. One course credit was awarded to all the individuals that voluntarily accepted to participate in this experiment. The experimental procedure was performed with the approval of ethics committee of ISPA's Research Centre and the written consent of all participants.

Procedure and data collection

Upon arrival at the laboratory, participants occupied individual private spaces, assuring that there was no visual contact between individuals throughout the experiment. Participants were then asked to provide a baseline saliva sample and to fill the first set of questionnaires composed by the modified Differential Emotions Scale (DES; Schaefer et al., 2010) and the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), in order to describe their current affective experience as in the original validation of the film clips (Schaefer et al., 2010). The PANAS is a widely used instrument composed by a PA and NA scale, each with 10 items. Participants use a 5 point scale to rate the extent to which they felt each emotional state. The DES is composed by 16 items and each item includes a set of emotional adjectives, representing different emotional dimensions (ex. Item 2: joyful, happy, amused; Item 5: fearful, scared, afraid), although this instrument was build based on emotion theory (e.g. Izard, 2009) , the adjectives can also be combined in a general PA and NA score (Schaefer et al., 2010). In the DES, participants are required to rate the emotion intensity using a 7 point scale (1- not at all; 7 – very intense).

After completing the first set of questionnaires, participants were informed that they should focus their attention to the screen and not look away during the exhibition of a short film. Participants would then watch the emotional film specific to their assigned experimental condition (films were counterbalanced between sessions). The emotional films for the Affiliation (scene from “Forrest Gump“ featuring an interaction between father and son), Threat (“American History X” scene featuring the murder of a man by a skinhead) and Sadness (“City of Angels” scene depicting the death of a woman) conditions used on this experiment are part of a larger emotional film database (Schaefer et al., 2010) and were selected based on the intensity they elicited the target emotions, without completely overlapping the emotional profile elicited by the other film clip with the same valence. For the Humorous condition, we used a film clip taken from “Mr. Bean” that successfully induced amusement and kept tenderness at a nominal level during a pre-test stage. Finally, on the Neutral condition, participants watched a film clip from a BBC documentary on Yellowstone National Park that effectively induced a pleasant, relaxing state on previous research (de Groot, Semin, & Smeets, 2014). When the emotional film ended, participants were given a second set of questionnaires including a second measure for the modified DES (Schaefer et al., 2010) and PANAS (Galinha & Pais-Ribeiro, 2005; Watson, Wiese, Vaidya, & Tellegen, 1999). Participants were also asked to evaluate the visualization of the emotional film as a threat and as a challenge (G. A. Oliveira et al., 2013; Tomaka, Blascovich, Kelsey, & Leitten, 1993), using a 9 point scale (ex. 1- not threatening; 9- very threatening). Evaluation as a threat or as a challenge reflects the individual's balance between perceived task demands and the available coping resources that can be modulated by affective states (Blascovich & Mendes, 2000). We have also used two items from the Geneva Appraisal Questionnaire (Scherer, 2001) to investigate how the participants attempted to suppress the emotions elicited by the film clips. These items ("To what extent did you try to reduce the intensity of your emotional experience and to shorten its duration?"; "To what extent did you try to control or mask the expression of your feelings to keep them from being observed by others?") were scored using a 5 point scale (1- Not at all; 5-Extremely). Finally, participants filled the revised version of the Toronto Alexithymia Scale (TAS-20; Verissimo, 2001; Bagby et al., 1994), which is composed by twenty items scored from 0 to 4 and grouped in three subscales, reflecting the theoretical construct of alexithymia (F1- difficulty in identifying and distinguishing between feelings and bodily sensations; F2 – difficulty in describing feelings; F3 – externally-oriented thinking). In order to keep participants occupied until the final saliva sampling point, filler personality questionnaires were given to the participants as in previous experiments (e.g. Oliveira et al., 2013, 2014). Fifteen minutes after the end of the emotional film clip, participants were asked for a second saliva sample, concluding the experimental session.

Hormone assays

The participant's saliva was collected in 5ml polypropylene vials and stored at -20ºC right after the end of the experimental sessions. Samples were thawed and centrifuged at 2245 *g* for 10 minutes and the supernatant stored at -20º C until the hormone assay. Commercial luminescence immunoassay kits were used to determine the concentrations of free T (IBL-Hamburg, Germany; kit reference: RE62031). The intra-assay and inter-assay coefficients of variance were 2.5% and 8.2%.

Statistical Analysis

Indexes of change (Δ) for the DES and PANAS were calculated by subtracting the baseline score from the post-stimuli score (complete data for these instruments is available as supplementary material – Appendix B). Multivariate ANOVA (MANOVA) were used to check for baseline differences in the emotional profile of the participants (baseline score for the 16 emotions measured by the DES) between conditions and to assess the overall effectiveness of the emotional films (measures of change for the 16 emotions of the DES). Follow-up pair-wise comparisons with Bonferroni correction were used to check for differences in the specific emotions targeted by the film clips (fear, tenderness, anger, amusement, sadness) between the experimental conditions. For the other psychological measures, the experimental conditions were compared with the Neutral condition using the Dunnett test, after a separate MANOVA for Appraisal and changes in PA/NA (as measured by the DES and PANAS), and after the ANOVA for the TAS-20 score and for Emotion suppression (sum of both items).

Testosterone levels were log-transformed due to skewness (see Table 1 for hormone concentrations) and a one-way ANOVA was used to test for baseline differences between conditions. A measure of T response to the emotion induction procedure (ΔT) was created by using the unstandardized residuals of the regression of T levels post-stimuli on the baseline levels as in previous research (G. A. Oliveira et al., 2013; Zilioli, Mehta, & Watson, 2014). A one-way ANOVA was used to check for differences in ΔT between conditions. Specific comparisons for ΔT between the conditions of the same valence (Humorous vs. Affiliation; Threat vs. Sadness) were performed using contrasts within the ANOVA model. Post-hoc comparisons were performed using the Dunnett test with the Neutral condition as a reference group.

Table 1

Mean and standard error of the mean for the testosterone levels before and after the emotional film.

Condition	Baseline (pg/ml)	Post-stimuli (pg/ml)
Neutral	24.796±4.759	31.268±6.600
Humorous	29.087±3.862	30.338±6.043
Affiliation	29.845±3.302	32.698±3.782
Anger	23.224±1.930	26.376±2.208
Sadness	27.038±4.467	22.693±3.067

Results

Changes in the emotions targeted by the emotional films

No overall differences between conditions were detected on the initial measure of the DES [Wilks λ =.502, F(64, 346.78)=1.040, p=.401, η_p^2=.161]. However, after being exposed to the emotional films, significant changes were detected in the emotional profile of the participants [Wilks λ =.087, F(64, 366.35)=4.945, p<.001, η_p^2=.463]. The pair-wise Bonferroni corrected analysis on the five emotions targeted by the film clips, showed differences between the emotional changes elicited by the films and the neutral condition. Furthermore, movies of the same valence were also differentiated by changes in specific emotions (Figure 1). The Threat condition showed increases in anger and fear significantly different than the Sadness condition, while the changes in tenderness were significantly different between the Humorous and Affiliation conditions (Figure 1).

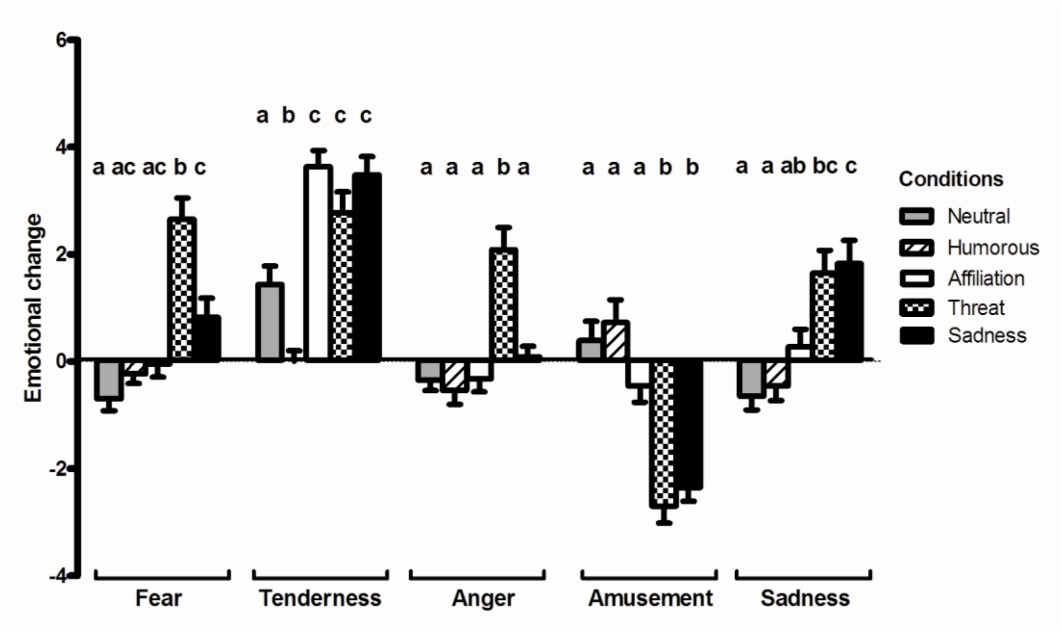

Figure 1. Changes in the emotions targeted by the emotional film clips. Different letters denotes a significant difference between experimental conditions on that specific emotion.

Changes in positive and negative affect

The changes in PA and NA (Figure 2), as measured by the DES and PANAS, significantly varied across the experimental conditions [DES: Wilks λ =.355, F(8, 214)=18.143, p<.001, η_p^2=.404; PANAS: Wilks λ =.352, F(8, 212)=18.154, p<.001, η_p^2=.406]. Post-hoc analysis showed the same pattern of response on the DES and PANAS scales, with increases in PA and decreases in NA on the Humorous and Affiliation conditions that were not significantly different from the Neutral group (Table 2), while the participants in the Threat and Sadness conditions showed significant decreases in PA and increases in NA when compared with the Neutral condition (Table 2).

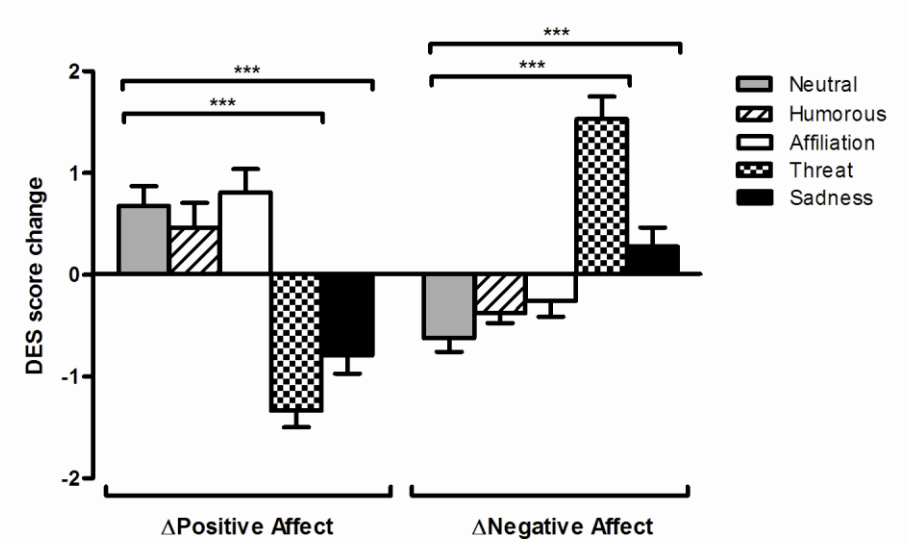

Figure 2. Changes in positive and negative affect as measured by the Differential Emotions Scale (DES). (***) significant for p≤.001

Table 2

Post-hoc comparisons for the participants' scores on psychological measures between the neutral group and the other emotional conditions

	Neutral	Humorous		Affiliation		Threat		Sadness	
	Mean±SEM	Mean±SEM	p	Mean±SEM	p	Mean±SEM	p	Mean±SEM	p
ΔPA_{DES}	.678±.194	.463±.245	.873	.809±.229	.975	-1.332±.165	***	-.791±.181	***
ΔNA_{DES}	-.625±.131	-.375±.099	.670	-.255±.157	.335	1.532±.220	***	.277±.188	***
ΔPA_{PANAS}	4.391±1.501	2.809±1.871	.831	4.090±1.299	.999	-8.869±1.037	***	-3.695±.950	***
ΔNA_{PANAS}	-1.608±.406	-.666±.386	.836	-.909±.487	.944	10.913±1.582	***	2.434±.746	***
Threat	.273±.075	.640±.221	.767	.722±.234	.625	1.656±.388	**	1.200±.362	.069
Challenge	4.608±.650	4.400±.617	.997	4.781±.539	.998	4.939±.515	.984	5.113±.551	.926
Alexithymia	25.608±2.643	27.409±2.276	.944	27.454±2.149	.940	28.130±2.034	.836	28.304±1.950	.802
Suppression	3.478±.439	3.908±.413	.888	3.681±.350	.991	4.826±.395	.077	4.956±.480	*

SEM: Standard error of the mean; ΔPA_{DES}, $\Delta NA_{DES:}$ changes in positive and negative affect measured by the DES; ΔPA_{PANAS}, $\Delta NA_{PANAS:}$ changes in positive and negative affect measured by the PANAS; p: p-value of the Dunnett test comparing the experimental condition with the Neutral group; *significant for p<.05; *** significant for p<.001

Threat/Challenge appraisal

Threat evaluation was only significantly higher on the Threat condition, and marginally non-significant for the Sadness group, when compared to Neutral [Table 2; Main effect: Wilks λ =.875, F(8, 214)=1.833, p=.072, η_p^2=.064]. No other differences between the other conditions and the Neutral group were detected. No differences were detected for the appraisal of the emotional films as a challenge (Table 2)

Alexithymia (TAS-20) and emotional suppression

No significant differences were found for the TAS-20 total score between the conditions [Main effect: F(4, 108)=.234, p=.918, η_p^2=.008; Table 2]. The multivariate analysis also suggests that there are no differences between the conditions for the individual factor scores of the alexithymia scale [Wilks λ =.921, F(12, 280.74)=.736, p=.714, η_p^2=.030].

Participants in the sadness condition attempted to suppress more their emotions than the participants in the Neutral condition (Table 2; F(4, 107)=2.648, p=.037, η_p^2=.090). No other differences were found between the participants in the other emotional conditions and the Neutral group for emotional suppression.

Testosterone response to the emotional films

No baseline T levels differences were found between the experimental conditions [Main effect: F(4, 108)=.800, p=.527, η_p^2=.028]. The analysis for the T response to the emotional films showed that only the participants in the Sadness condition significantly decreased their T levels when compared to the Neutral group [Dunnett test: Sadness: p=.024; Happiness: p=.099; Affiliation: p=.909; Threat: p=.977; Main effect: F(4, 108)=2.845, p=.027, η_p^2=.095; Figure 3]. Furthermore, the T response in the Sadness group was significantly lower than the T response to Anger [t(108)=2.316, p=.022], but no differences were found between the Humorous and Affiliation conditions [t(108)=1.508, p=.134]. These results were not moderated by the use of oral contraceptives [Main effect: F(1, 103)=.611, p=.436, η_p^2=.005; Interaction: F(4, 103)=.886, p=.474, η_p^2=.033]

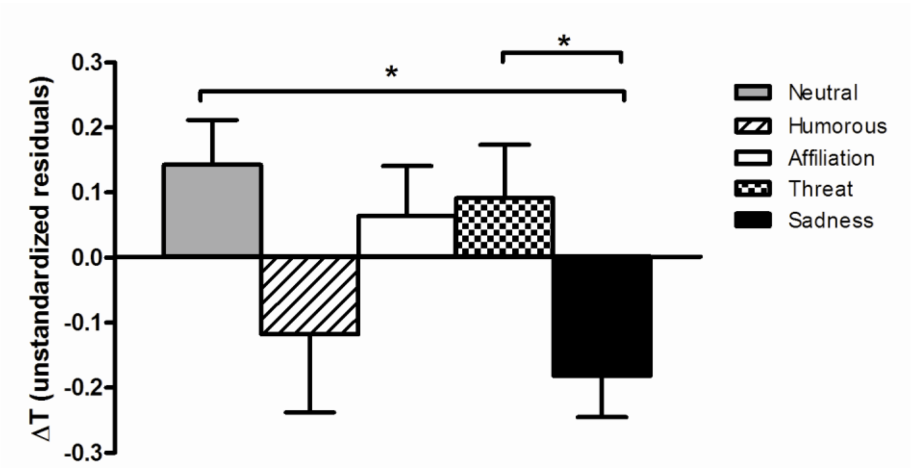

Figure 3. Testosterone response to the emotional film clips (unstandardized residuals; Mean±SEM). (*) significant difference for p≤.05.

Associations between cognitive variables and the testosterone response

Significant associations were detected between the ΔPA (r=.730, p<.001) and ΔNA (r=.819, p<.001) scores of the DES and PANAS. The participants that evaluated the emotional films as more threatening also show higher scores on the TAS-20 (r=.283, p=.002). Moreover, a positive correlation was also found between the attempt to suppress the emotional expression and the TAS-20 (r=.201, p=.033). A negative correlation was found between T response and the attempt to suppress the emotional expression (r=-.196, p=-.038). Participants that scored the visualization of the emotional film as more of a challenge also decreased their T levels after the film (r=-.191, p=.042), but no association was detected for threat (r=-.122, p=.196). No association was found between T and the TAS-20 (r=.032, p=.732)

Discussion

Overall, the results suggest that most of the emotional film clips successfully induced different discrete emotional profiles and presented changes in the condition specific target emotions compared to other conditions.

For the negative condition films, we have detected a significant decrease in PA and increases in NA compared to Neutral. The participants in the Sadness group presented a post-stimuli decrease in T levels significantly different from Threat and Neutral conditions and this effect was not moderated by the use of oral contraceptives. A T decrease for sadness has also been reported for men but was not significant (Cook & Crewther, 2012), however, the direction is congruent with the hypothesis of negative mood eliciting decreases in T levels (Mazur & Lamb, 1980), but other explanations are still possible since the changes in the discrete emotion of sadness in this condition, although higher (as in the validation procedure, Schaefer et al., 2010), were not significantly different from the Threat condition. Therefore, it is unclear whether the T decrease was elicited by a sadness based negative affect or from negative affect characterized by the absence of threat, as measured by the anger and fear scores and the appraisal of the emotional film.

Interestingly, emotional suppression was only significantly higher in the Sadness condition compared to Neutral and suppression was associated with post-stimuli decreases in T levels in our sample. To the best of our knowledge this association has not been previously reported in the literature. Suppression has been described as a response-focused form of emotion regulation that involves the voluntary inhibition of an ongoing response to the emotional stimulus. In contrast with reappraisal, suppression may be successful in reducing the behavioral response, but does not decrease the emotional experience (Gross, 2002). Previous research, has found an association between the activation of the orbitofrontal (OFC) and the medial prefrontal cortex (MPFC), brain regions involved emotion regulation (Banks, Eddy, Angstadt, Nathan, & Phan, 2007), and the perceived difficulty in suppressing sadness (Beauregard, Paquette, & Lévesque, 2006). It has been suggested that the OFC is an important relay in the amygdala and MPFC cortex interactions (Banks et al., 2007) and the administration of exogenous T decreases the functional connectivity between the OFC and the amygdala (Mehta & Beer, 2010). Together, the data from these experiments opens the possibility of a T effect on emotion suppression that could be tested in future experiments.

The Threat condition presented the highest scores for NA and for fear and anger compared to the other conditions, matching the results previously reported for this film (Schaefer et al., 2010). Unexpectedly, this condition only elicited a non-significant increase in T levels, despite being evaluated as the most threatening film of the experiment. This contrasts with previous research showing T increases in females participants exposed to angry faces of either sex (Zilioli, Caldbick, & Watson, 2014) and the association between threat evaluation and increases in T after a competition (G. A. Oliveira et al., 2013). Conceptually, anger and fear are signals of social threat, but they could generate different adaptive responses, reflecting the activation of motivational states of approach and avoidance, respectively (Pichon, de Gelder, & Grèzes, 2009). Therefore, it is possible that the mixed emotional state induced by the Threat condition may have led to an ambiguous evaluation of the threat and a blunting of the T response to the stimulus. This hypothesis could be tested in a future experiment by dissociating the induction of fear and anger.

On the positive conditions, we have found that both Humorous and Affiliation elicited changes in PA and NA of a similar magnitude, but these levels of PA were achieved with the contributions of different discrete emotions, since the Affiliation condition presented higher scores for tenderness than the Humorous condition. Furthermore, these changes for tenderness also allowed a differentiation between Affiliation and all other conditions including the Neutral group. In contrast, Humorous presented discrete emotional changes similar to the Neutral condition and could not be differentiated from this condition on any of the measures used in this experiment. Moreover, the T response to the films used in the positive conditions suggest a T decrease in the Humorous condition and an increase in the Affiliation condition, but neither of these changes was significantly different to the Neutral group. Previous research with a male sample has shown T increases after a humorous film clip (Cook & Crewther, 2012), but T decreased in women after an induction procedure involving the imagination of a positive event (Goldey & van Anders, 2011). Our findings for the positive conditions are inconclusive and it is unclear at this point whether there are sex differences in the T response to positive affect and how it may vary depending on the activation of discrete emotions.

As mentioned before, we have used effectiveness in inducing the emotional states targeted by our research as the primary criterion for stimuli selection. As a consequence, and to avoid the problems emerging from conditions composed by multiple emotional stimuli, the duration of the film clips varied. This is a potential limitation to our findings, especially since a T response was only detected for Sadness which was the longest film. Previous research with a male sample, using emotional films of approximately 4 minutes, detected transient T changes (Cook & Crewther, 2012) that we have not found in our female sample, despite the successful induction of the target emotional states, as in the case of the Affiliation and Threat conditions. Future research could test if the extension of these stimuli to 4 minutes is sufficient to trigger an androgen response in women as observed in men.

In conclusion, we found that the transient T response to a negative mood induction could be different depending on the array of discrete emotions elicited by the film clips, so that the participants that experienced sadness, without changes in the emotions signaling threat, significantly decreased their T levels. Moreover, we have found that an association between the use of emotional suppression and decreases in T that could be an interesting topic for future research.

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Chapter VII

Social modulation of androgens and threat detection in humans

Abstract

The adaptation of an organism to the social environment depends to a great extent on efficient threat detection. Previous research has suggested that threat detection in humans can be modulated by dominance and androgens like testosterone (T). In this experiment, we tested how the outcome of competition influences threat detection and what is the contribution of post-competition T levels in this process. For this purpose, forty female students engaged in a group competition followed by an emotion identification task. Results show that winners were faster and better discriminators of anger than losers. Furthermore, anger discrimination was enhanced when participants had high post-competition T levels and a high sense of power. These findings suggest that cognitive and physiological markers of dominance facilitate the detection of threat, which may give dominant individuals an advantage in the social group.

Keywords: threat, emotions, status, testosterone, competition, signal detection theory

Introduction

The social environment is characterized by its high variability and complexity, as it involves interactions between behavioral agents which may be modulated by social context and by the internal states of the interacting agents, generating a high degree of unpredictability (Taborsky & Oliveira, 2012). The successful adjustment to this fluctuating environment, requires context-dependent action selection, supported by the cognitive abilities of the organism (Faustino, Oliveira, & Oliveira, 2015). Thus, in order to match the challenges of the social environment, organisms should be particularly sensitive to threatening stimuli and automatically direct attention towards them (Ohman & Mineka, 2001). In this regard, an efficient threat detection system can be seen as an evolutionary advantage supporting behavioral flexibility and the appropriate selection of fight-or-flight responses depending on context and internal state.

In humans, angry and fearful facial emotional expressions are social relevant stimuli that can be used to signal potential threats in the social environment, prompting the observer to prepare an adaptive response (Parkinson, 2005). Although both anger and fear are characterized by high arousal and negative valence, they respectively reflect the activation of approach and avoidance motivational systems (Carver & Harmon-Jones, 2009). Congruent with the hypothesis above, there is evidence suggesting that humans are sensitive to facial expressions of threat and detect them efficiently (Fox, Russo, Bowles, & Dutton, 2001; Öhman, Lundqvist, & Esteves, 2001). On the other hand, since social behaviors may depend on status, dominant and subordinate individuals may display behavioral differences in response to the same stimuli, in order to reduce or avoid the fitness costs of agonistic interactions (R. F. Oliveira, 2009). Indeed, research suggests that social status is an important social cue and influences threat detection. For example, angry facial expressions of high-status individuals are detected early and perceived as more persistent in time than those of low-status individuals (Ratcliff, Franklin, Nelson, & Vescio, 2012). Furthermore, individuals with a stronger social dominance orientation are more accurate when detecting anger on high-status faces than on low-status individuals (Ratcliff, Bernstein, Cundiff, & Vescio, 2012).

Androgens, such as testosterone (T), are known to respond to social challenges and theories for the social modulation of androgens (e.g. biosocial model of status: Mazur, 1985; challenge hypothesis: Wingfield, Hegner, Dufty, Jr., & Ball, 1990) have hypothesized that socially-driven changes in peripheral (e.g. gonadal) androgen levels underlie adaptive behavioral responses to changes in the social environment by acting on the neural circuitry of social decision-making (G. A. Oliveira & Oliveira, 2014a). Results from competition experiments in humans (reviewed in Carré and Olmstead, 2014; Oliveira and Oliveira, 2014b), used as a parallel of the agonistic interactions in non-human animals, support this hypothesis and show that post-competitive T effects on social cognition depend on the competition outcome (e.g. T mediates the effect of winning the competition on aggressive behavior, Carré et al., 2013; T is associated with willingness to compete again in losers, Carré and McCormick, 2008; Mehta and Josephs, 2006). Previous research has revealed that not only threat detection can be modulated by T levels but also that the exposure to angry faces also elicits a T response in participants of both sexes (Zilioli, Caldbick, & Watson, 2014).

However, there is no clear evidence on what happens to emotion perception as a consequence of that T increase. Experiments using interference paradigms (stroop like tasks) have shown an association between higher endogenous T levels and selective attention towards angry faces (Van Honk et al., 1999; van Honk et al., 2000; Wirth & Schultheiss, 2007), while the administration of exogenous T reduced the attentional bias towards masked fearful faces compared to placebo (Van Honk, Peper, & Schutter, 2005) and reduced several physiological parameters of the fear response (Hermans, Putman, Baas, Koppeschaar, & van Honk, 2006; Roelofs, Bakvis, Hermans, van Pelt, & van Honk, 2007), hence suggesting a fear reducing effect of T. Recent experiments corroborated this interpretation by showing that the administration of T reduces avoidance and increases approach to threatening stimuli, with concomitant increases in amygdala activity (Enter, Spinhoven, & Roelofs, 2014; Radke et al., 2015).

One study that directly tested the effects of T on explicit detection of threat (van Honk & Schutter, 2007) by using short films morphing a neutral face into other emotional expressions, has shown that participants detected anger at later stages after T administration when compared to placebo. Although these authors interpreted these results as a T induced reduction of sensitivity to anger (van Honk & Schutter, 2007), a more conservative explanation is that after T administration the participants required a less ambiguous emotional expression of anger in order to respond. Using signal detection theory (Macmillan & Creelman, 2004) it is possible to distinguish between these two processes. While sensitivity reflects the perceived separation between the distributions of signal and noise, the criterion describes the participant’s strategy of response and implies a decision making process in which one type of response is favored over the other (response bias).

Together the experiments described above show an effect of anger and threat in increasing individuals’ T levels and the impact that endogenous and exogenous T levels exert on the detection of emotional signals of threat. However, none of these experiments has yet shown how T and social status jointly influence threat detection in humans. In this paper, we addressed this hypothesis by testing the assumption that the experience of winning or losing a social contest should modulate threat detection in the social environment. Furthermore, based on the theories for the social modulation of androgens (Mazur, 1985; Wingfield et al., 1990) and the previous research on T and threat detection reviewed above, we expected that the individual’s T levels after the competition should have an adaptive function and should therefore influence the detection of emotional signals of threat. Signal detection theory (Macmillan & Creelman, 2004) also allowed us to test if the effects of T are specific to the perceptual or decision-making processes involved in the explicit detection of emotions that convey threat. Cortisol levels (F) were also measured since this hormone is known to interact with T in status-seeking behaviors (Mehta & Josephs, 2010) and there is evidence on the effect of F on social threat (Roelofs et al., 2007).

Materials and Methods

Participants and experimental protocol

Forty female undergraduate psychology students (Age Mean ±SEM: 21.102 ±1.011 years) participated in this experiment. Sessions lasted approximately one hour and were scheduled from 14:30 to 17:30 to control for circadian variation of hormone levels. Participants engaged in a group competition using a symbol matching task in order to determine their status (winner vs. loser), followed by an explicit emotion recognition forced-choice procedure in a divided visual field paradigm. Competition outcome was randomly assigned. One participant was excluded from this experiment due to contaminated saliva samples bringing the total of participants to thirty-nine (Winners: n=20; Losers: n=19). Although at the beginning of the experiment, the participants were informed that only the members of the winning team would receive a 10 Euros gift card, all participants received payment after debriefing. This experiment was performed in accordance with the declaration of Helsinki and with the approval of the Ethics Committee of ISPA’s Research Center. Written consent was given by all participants.

Stimuli and apparatus

Stimuli presentation and response collection procedures were implemented in E-Prime version 1.1 (Psychology Software Tools, Inc., Pittsburg, PA, USA; Schneider et al., 2002) and run individually on Hewlett-Packard computers equipped with 19 inch CRT monitors running at 100 Hz (10 ms resolution).

Competition task - Matrices were composed by an average of 60 different symbols displayed in 4 distinct colors, created using Adobe® PhotoShop®. The symbols were non-commonly used, rotated in all degrees and obtained from 30 commercial font types.

Emotional identification task - Twelve color photographs from MacBrain Face Stimulus Set (Tottenham et al., 2009) (four actors, two females and two males, each with either a happy, fearful, angry, or neutral expression) were re-sampled to have 7 cm wide and 10 cm high in size (sustaining a visual angle of ~8.0^{o} and ~11.3^{o}, 50 cm from screen), grey-scaled (16-bit), and matched to luminance and contrast. These facial expressions were presented in the left visual field or right visual field, against a white background (to avoid reflection). Stimuli were centered vertically with the innermost edge at 5cm (5.7^{o}) left or right of the fixation cross. The backward mask was created from Gaussian noise using Adobe® PhotoShop®.

Data collection and psychological variables

Participants were asked to form two teams based on acquaintance and the teams were asked to sit on the opposite sides of the laboratory, in cubicles without visual contact with the other participants. The participants provided a baseline saliva sample and filled a mood assessment questionnaire (Garcia-Marques, 2004) for the first time. The mood questionnaire is composed by three mood state items and each item has an opposing pair of adjectives (good-bad, negative-positive, sad-happy), that must be rated using a 9 points scale. Upon completion of the mood questionnaire, the group competition task was explained to the participants.

In the competition task, participants were asked to focus on a target symbol displayed on the screen for 1 second and then identify it within a matrix of distracting symbols as fast as possible over the course of 10 trials (target symbol was different on each trial). The experimenters stressed that the speed and accuracy of each team member was critical to team performance and only the members of the winning team would receive a 10 Euros gift card.

After each trial, the outcome (winner and loser team) was verbally given as false feedback and also written on a score board visible to all participants. Winning and losing trials were manipulated in order for the outcome to be a draw on the sixth trial, after which the group in the winner condition would win the 4 remaining trials. Immediately after the end of the competition, participants filled a second measure of the mood questionnaire and were asked to evaluate the competition outcome as a threat and as a challenge using 2 items, each of them associated with a 10cm line anchored by “Non threatening/challenging” and “Very threatening/challenging” (G. A. Oliveira et al., 2013; Tomaka, Blascovich, Kelsey, & Leitten, 1993). The participants were also asked to fill the sense of power scale (Anderson & Galinsky, 2006), as a subjective measure of how participants evaluate their own power. This instrument is composed by 8 items each with a 7 points rating scale and has been shown to correlate with the status of the individual in the social hierarchy (Anderson & Galinsky, 2006). Personality questionnaires were then distributed in order to keep the participants busy until it was time to provide a second saliva sample, which was collected 15 minutes after the end of the competition.

After the post-competition saliva sampling, the emotion identification task (Figure 1) was presented to the participants (adapted from Tamietto et al., 2006). This task consisted of 4 counterbalanced emotional blocks (neutral, happy, fear, anger), each composed by 56 randomized trials (totaling 224 trials), 20 trials with the target emotion and 36 trials with lures (12 trials of each of the other non-target emotional categories). Before each block, a screen identified the emotion that participants should aim to identify. In order for the participants to focus their attention on the emotional stimuli, each trial started with a central fixation cross that had to be clicked from 3 to 5 times with the mouse cursor until the stimulus appeared for 150ms. Each mouse click shifted the fixation cross up or down 2mm in order to maintain the participant’s focus on the center of the screen. The stimulus presentation was followed by a Gaussian noise backward mask (300ms) to reduce afterimage effects.

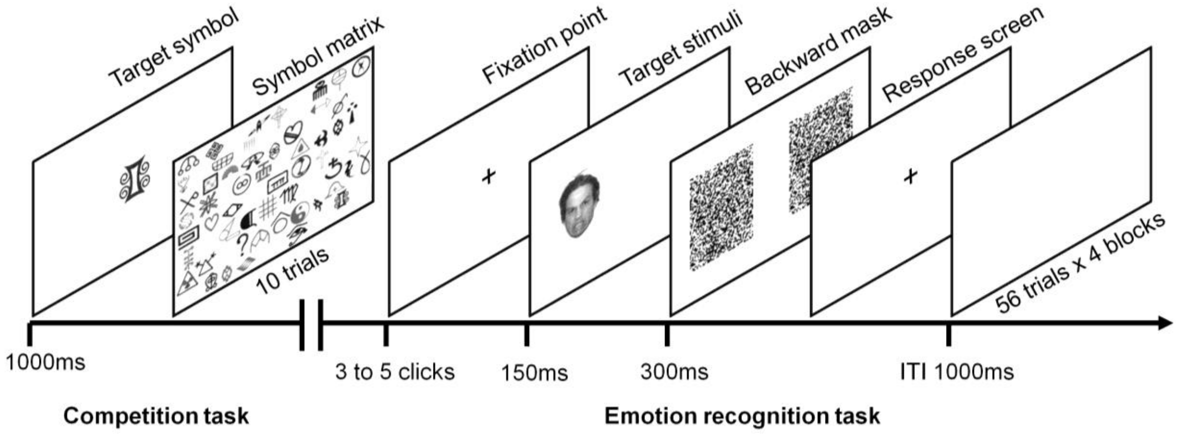

Figure 1. Overview of the experimental procedure

A forced-choice procedure was used for the emotion identification task. Match trials (target emotion was displayed) were answered by pressing the response key (space bar) and the mouse button was used for no-match trials (lure emotion). To increase unpredictability, inter-individual variability and avoid possible ceiling effects, half of the stimuli were presented on the left visual field and the other half on the right. Two practice blocks were performed before testing blocks, comprising 5 match (targets: sad or disgust expressions) and 5 no-match trials (lures) each.

Emotion identification task measures

Classic signal detection theory (Macmillan & Creelman, 2004; Stanislaw & Todorov, 1999) was used to calculate responses discriminability and biases in identifications. The two measures take into account the proportion of hits (responding that the target emotion is present in match trials) and the proportion of false alarms (responding that the target emotion is present in no-match trials). The discrimination parameter [ ] is a measure of sensitivity and reflects the individual's ability to discriminate the target emotion from the non-target (lures) emotional expressions. A higher *d'* reflects a higher ability to distinguish a target emotion from other emotions. The criterion parameter [ ] is a measure of response bias and reflects the individual's tendency of response: $c>0$ reflects the use of a conservative criterion that bias individuals to neglect the presence of the emotion; $c<0$ reflects to the use of a more liberal criterion that bias individuals to falsely identify the presence of the emotional facial expressions. To calculate these indexes the proportions of hits and false alarms were adjusted to avoid divisions by zero (Stanislaw & Todorov, 1999). Response latency of correct responses (hits and correct rejections) to each target emotion was also measured.

Hormone assays

Saliva samples were collected in 5ml polypropylene vials immediately after the end of the experimental sessions. Samples were thawed, centrifuged at 2245 *g* for 10 minutes and the supernatant stored at -20 C° until further processing. To determine the concentrations of free T and F, we used commercial IBL (Hamburg, Germany) luminescence immunoassay kits (T: RE62031; F: RE62111). The intra-assay and inter-assay coefficients of variance were 1.2% and 10.5% for T, and 1% and 9.3% for F.

Statistical analysis

To test the competition induced changes in the participant's mood (difference between pre-competition and post-competition score) and differences in the sense of power scale ratings, between winners and losers independent variables t-tests were used. A repeated measures ANOVA with outcome (winner, loser) as a between subjects factor was used to check for differences in the appraisal of the competition outcome (threat, challenge). The hormone response to the competition was assessed using a separate General Linear Model (GLM) for T (baseline, post-competition) and for F (baseline, post-competition) with outcome (winner, loser) and oral contraceptives (non-user, user) as between-subjects factors. The hormonal values in these GLM required log-transformation due to skewness (see Table 1 for hormone concentrations). To check for differences in the emotion recognition task parameters according to outcome and post-competitive hormone levels, we have used GLMs with Outcome (Winner, Loser) as a between-subjects factor and each task parameter (*d'*, *c*, response latency) was inserted as a repeated measures factor with 4 levels (Neutral, Happy, Fear, Anger). Planned comparisons (t-test) were used to compare levels within each GLM.

Table 1

Testosterone and cortisol concentrations for winners and losers before and after the competition

		Baseline (Mean±SEM)	Post-competition (Mean±SEM)
Winners	Testosterone (pg/ml)	13.880±1.575	13.627±1.916
	Cortisol (μg/dl)	.308±.047	.267±.038
Losers	Testosterone (pg/ml)	18.186±2.869	14.873±1.884
	Cortisol (μg/dl)	.272±.042	.215±.031

A path analysis was performed in order to assess the contribution of the competition outcome, appraisal of the competition outcome and hormonal levels on the parameters of emotion recognition that were found to be sensitive to competition and therefore differentially expressed by winners and losers. Parameters were estimated using the maximum-likelihood method and bootstrapped to 1000 samples with a 95% bias-corrected interval of confidence. By removing the non-significant paths of the initial model in the path analysis and in successive iterations until an adequate fit was reached, we defined the best model to describe the relations between the variables at study. Chi-squared test (χ^2) allowed us to test the null hypothesis that the predictions of the model adequately fit the observations. The Comparative Fit Index (CFI) and the Tucker Lewis Index (TLI) allowed us to compare the proposed model with an independence model (i.e. there is no relation between the variables), and the Parsimony-adjusted Comparative Fit Index (PCFI) was used in order to favor simpler models and penalize more complex solutions. Along with the previous measures, the Akaike Information Criterion (AIC) was used to compare the goodness-of-it of the initial and final models. All statistical analyses were conducted using the software package IBM SPSS Statistics v. 21 with AMOS Graphics v.21.

Results

Competition

Cognitive variables: After the competition winners and losers changed their mood in opposite directions: winners increased and losers decreased the positivity of their mood [Winners: .633 ±.172; Losers: -.473 ±.244; t(37)=3.725, p<.001, d=1.193]. The competition outcome did not affect perceived threat or challenge [Main effect: F(1, 37)=1.037, p=.315, η_p^2=.027, Outcome x Appraisal: F(1, 37)=.250, p=.619, η_p^2=.006]. Overall, the participants perceived the competition more as a challenge than as a threat [Main effect: F(1, 37)=71.912, p<.001, η_p^2=.660; Threat - Winner: 2.625 ±.605; Loser: 2.263 ±.443; Challenge - Winner: 6.635 ±.416; Loser: 5.826 ±.565**]**. Winner and losers did not report differences in their sense of power [Winners: 4.927±.180; Losers: 5.059±.132; t(37)=.580, p=.565, d=.185].

Hormone response to competition:

A decrease of T levels was detected for losers [t(35)=2.918, p=.006, d=.669] while winners maintained their levels of T after the competition [t(35)=.145, p=.884, d=.032; Outcome x T: F(1,35)=3.864, p=.057, η_p^2=.099; Figure 2A]. No differences were found between winners and losers at the baseline [t(35)=1.525, p=.136, d=.488] or 15 minutes after the competition [t(35)=.505, p=.616, d=.161]. Although the participants using OC had lower T levels and non-users [Main effect: F(1, 35)=3.947, p=.054, η_p^2=.101], this variable did not interact with the T response observed in winners and losers [OC x Outcome x T: F(1, 35)=.021, p=.884, η_p^2<.001].

A decrease in F levels was observed in both winners and losers from baseline to 15 minutes after the competition [Winners: t(35)=1.978, p=.055, d=.442; Losers: t(35)=3.388, p=.001, d=.777; Outcome x F: F(1, 35)=.388, p=.537, η_p^2=.010; Figure 2B]. No differences were detected between winners and losers at the baseline [t(35)=1.244, p=.221, d=.398] or 15 minutes after the competition [t(35)=1.594, p=.119, d=.510]. No effect of OC was detected on F levels [Main effect: F(1, 35)=1.192, p=.282, η_p^2=.032; OC x Outcome x F: F(1, 35)=.374, p=.544, η_p^2=.010].

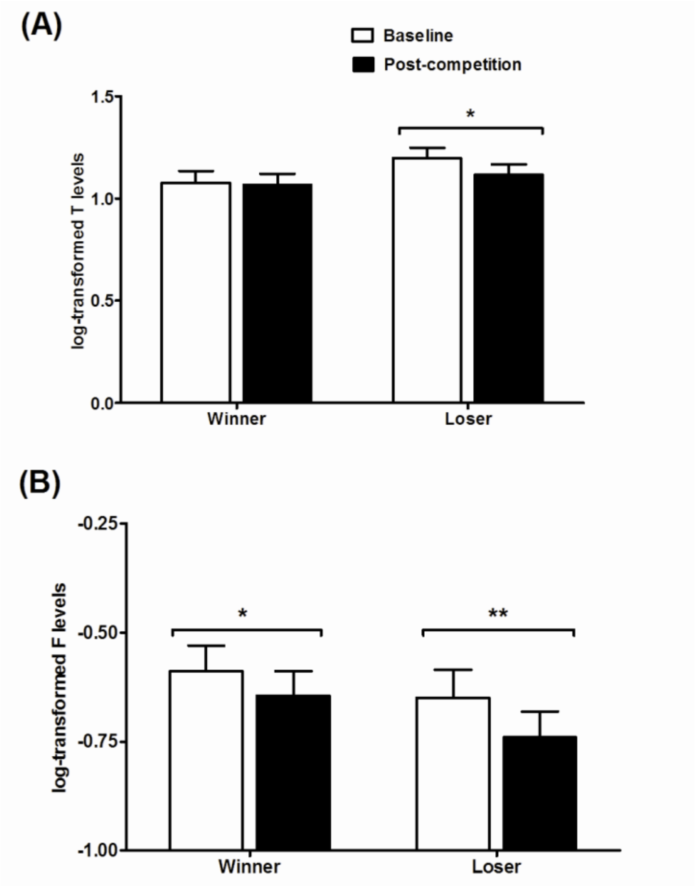

Figure 2. Log-transformed hormone levels (Mean±SEM) measured at baseline and 15 minutes after the end of the competition for winners and losers. (A) Testosterone; (B) Cortisol. (*) significant difference for p≤.05; (**) significant difference for p≤.01.

Emotion identification task

Discrimination

Discrimination varied depending on the target emotion [Main effect: F(3, 111)=76.312, p<.001, η_p^2=.673] with fear being the hardest emotion to discriminate. Winners discriminated anger significantly better than losers [t(37)=2.081, p=.044, d=.666], but this difference did not reach statistical significance for fear [t(37)=1.734, p=.091, d=.555]. No other differences between winners and losers were found on this parameter [Happy: t(37)=.205, p=.838, d=.065; Neutral: t(37)=.301, p=.764, d=.096; Main effect for Outcome: F(1, 37)=1.552, p=.220, η_p^2=.040; *d'* x Outcome: F(3, 111)=1.559, p=.203, η_p^2=.040; Figure 3A].

Criterion

Overall, participants were more conservative when the target emotion was fear and less conservative when identifying neutral faces [Main effect for *c*: F(3, 111)=15.465, p<.001, η_p^2=.294; Figure 3B]. No effect of the competition outcome was detected for any of the emotional blocks [Neutral: t(37)=1.026, p=.311, d=.328; Happy: t(37)=1.155, p=.255, d=.370; Fear: t(37)=.426, p=.672, d=.136; Anger: t(37)=.527, p=.600, d=.168; Main effect for Outcome: F(1, 37)=1.238, p=.272, η_p^2=.032; *c* x Outcome: F(3, 111)=.209, p=.889, η_p^2=.005].

Response latency

Participants' average response latency was different for each emotional block of trials [Main effect: F(3, 111)=3.641, p=.014, η_p^2=.089]. The response latency for the emotional faces of anger was significantly lower for winners when compared with losers [t(37)=2.885, p=.006, d=.924] and the reverse effect was detected for neutral faces, for which winners had a higher latency than losers [t(37)=3.007, p=.004, d=.963]. No differences in response latency were found between winners and losers for happy or fearful faces [Happy: t(37)=.133, p=.894, d=.042; Fear: t(37)=.796, p=.430, d=.255; Main effect for Outcome: F(1,37)=.680, p=.414, η_p^2=.018; Response latency x Outcome: F(3, 111)=14.243, p<.001, η_p^2=.277; Figure 3C].

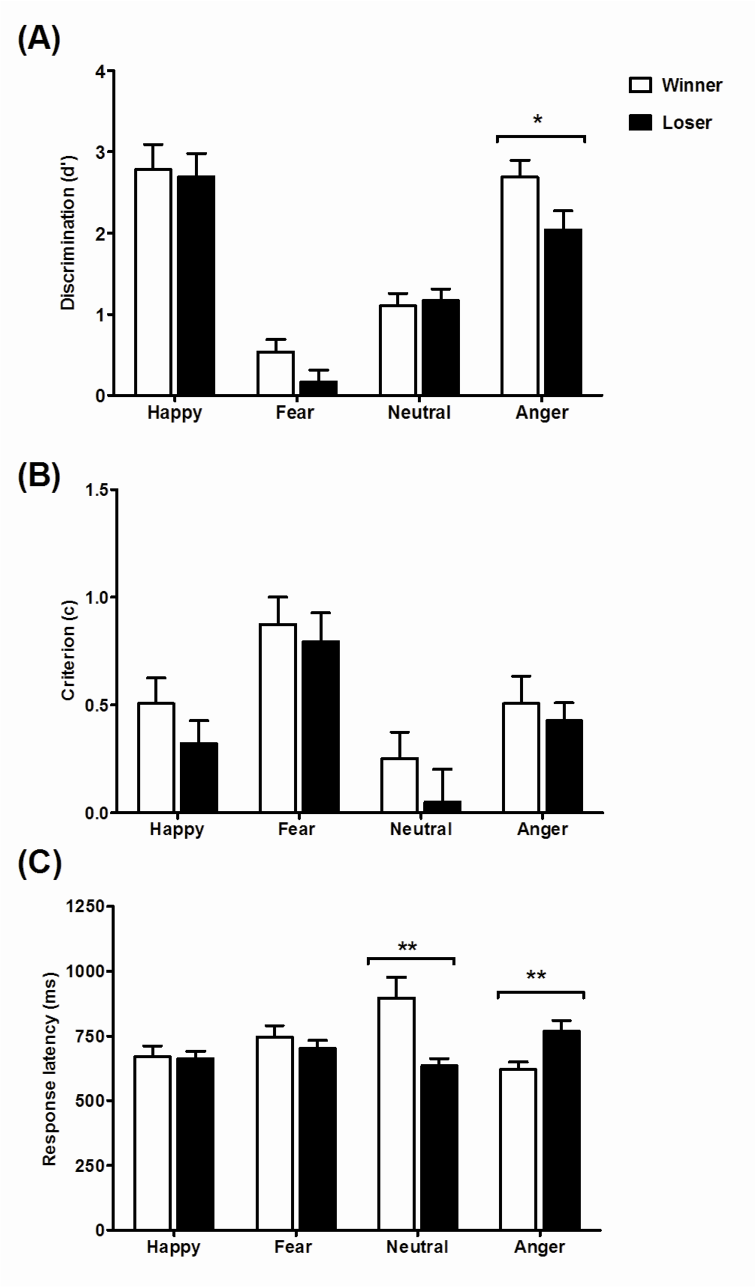

Figure 3. Emotion identification task parameters (Mean±SEM) for participants in the winner and loser condition. (A) Discrimination; (B) Criterion; (C) Response latency. (*) significant difference for $p \leq .05$; (**) significant difference for $p \leq .01$.

Path analysis

A path analysis was performed to test the effect of the competition outcome, the appraisal of the competition outcome and the post-competitive hormonal values for T and F on the parameters of the emotion identification task that were sensitive to the experience of winning or losing the competition. Based on the emotion identification task results, we have elected anger discrimination, response latency to neutral and angry faces as the variables that were effectively modulated by the competition outcome.

Results showed that the initial model (Fig. 4A) generated predictions adequate to what was observed (χ^2=9.214, p=.512; CFI=1; TLI=1), but was severely penalized by its complexity (PCFI=.278). All path coefficients for the initial model are available as supplementary information (Appendix C). After removing the non-significant paths, we have reached a final model (Fig. 4B) that showed an improvement in all the indices used for goodness-of-fit (χ^2=17.884. p=.530; CFI=1; TLI=1; PCFI=.679) and comparatively fitted the data better than the initial model (Final model AIC=51.884; Initial model AIC=79.214).

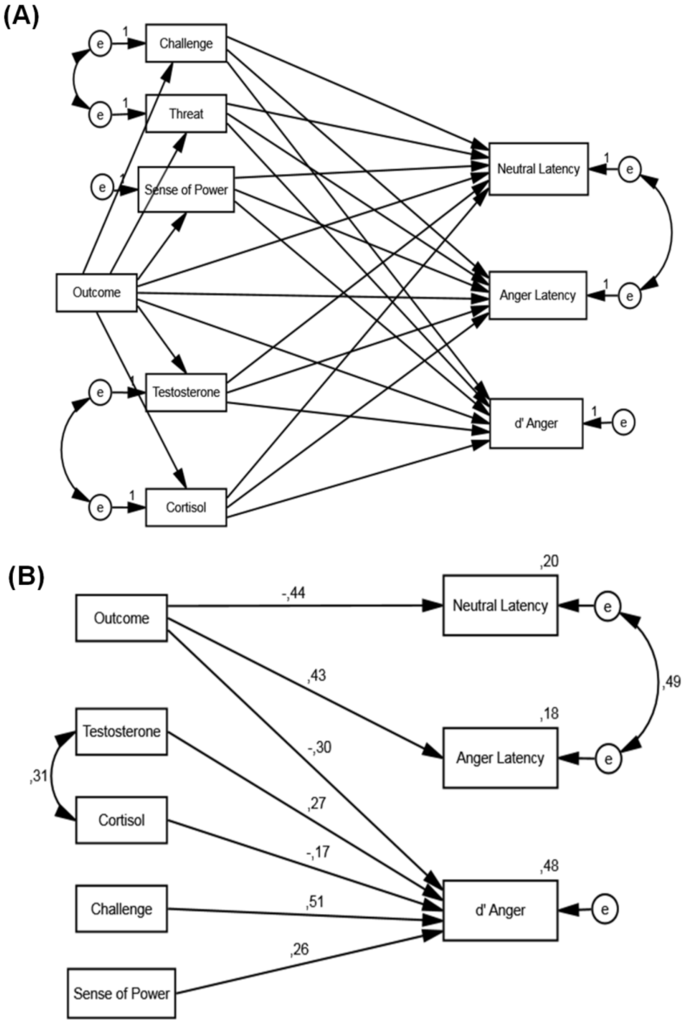

Figure 4. Initial and final model for the path analysis on the emotion recognition task parameters sensitive to the competition outcome. (A) Initial model; (B) Final model.

In the final model (Fig. 4B), we have found that the discrimination of anger was negatively affected by losing the competition (β=-.295; p=.011; Bootstrap: p=.028), but enhanced when the participants had higher post-competitive T levels (β=.268; p=.029; Bootstrap: p=.044), reported a higher sense of power (β=.259; p=.026; Bootstrap: p=.028), and evaluated the competition outcome as a challenge (β=.505; p<.001; Bootstrap: p=.002). The effect of the post-competitive F levels on anger discrimination was non-significant (β= -.171; p=.163; Bootstrap: p=.485). However, without controlling for F levels, the effect of T on anger discrimination becomes marginal and non-significant (p=.076; Bootstrap: p=.108). Response latency to angry and neutral faces was only predicted by the competition outcome (Anger: β=.429; p=.003; Bootstrap: p=.01; Neutral: β=-.443; p=.003; Bootstrap: p=.004).

Discussion

In this experiment, we found evidence for an effect of winning or losing a laboratory competition game on the identification of emotional threat related facial expressions, but not on post-competitive T-levels. Because of this, the hypothesized mediation of post-competitive T levels on the parameters of emotion identification sensitive to changes in social status was not tested. However, direct effects of T levels on threat detection were observed.

Overall, the results from the emotion identification task conducted after the group competition were congruent with our predictions. Winners were more efficient than losers in identifying anger, in a pattern that is consistent with the activation of approach in winners (low latency, high discrimination) and avoidance in losers (high latency, low discrimination). The increased discrimination and readiness in the identification of social signals of threat after winning a dominance contest can be seen as an adaptive advantage, since it allows individuals an effective and early detection of an upcoming social challenge, enabling the establishment or maintenance of their dominance in a social group (Wirth & Schultheiss, 2007). Alternatively, an angry face may also signify a positive outcome of a dominance contest to the perceiver (Wirth & Schultheiss, 2007).

The path analysis showed that independently the different indices of social dominance had a facilitating effect on anger discrimination, since along with winning the competition, sense of power, post-competition T levels and the evaluation of the competition outcome as a challenge, were significant indicators of a higher identification of angry faces. Interestingly all these variables were also linked to the activation of the approach motivational system

(Anderson & Jennifer, 2002; Blascovich, Vanman, Mendes, & Dickerson, 2011; Lacreuse et al., 2010).

The competition surprisingly did not produce differences in the participant's sense of power. However it should be noted that although sense of power is a concept closely related to dominance, it has been defined as the internal representation of the relative power an individual has towards others (Anderson & Galinsky, 2006). The competition outcome implied a conscious attribution of high and low status to winners and losers, respectively, but since sense of power is affected by the individual position in multiple social hierarchies (Anderson, John, & Keltner, 2012) participant's may have perceived their power as extending beyond the current hierarchical position elicited by the competition outcome and, by doing so, losers with a high sense of power avoided the effects of a social defeat on their ability to detect angry faces. Previous research has shown that dominant individuals detect anger with greater accuracy when anger is displayed by other high ranked individuals (Ratcliff, Bernstein, et al., 2012). Since no dominance manipulation accompanied the stimuli used in this experiment, it is not possible to check if the effect of sense of power on anger discrimination reported here is related to the perceived dominance of the displayer. This is of course an empirical question to respond in future studies.

In our experiment, high T individuals were better discriminators of anger. Although previous research has shown that high T individuals have better anger detection (Ersche et al., 2015), the cognitive process had not been identified. The effect for T was only significant when the participant's F levels were controlled in the path analysis supporting an interplay between these hormones, as proposed by the dual-hormone hypothesis (Mehta & Josephs, 2010) and reflecting the physiological communication between these two neuroendocrine axes (Viau, 2002). Together with the fear reducing and approach promoting effects of T, high T participants are also expected to engage more readily in status-seeking behaviors (Enter et al., 2014; Hermans et al., 2006). The interpretation of T levels as a marker of social dominance is supported by the reciprocal relationship between T and status proposed by both theories for the social modulation of androgens (biosocial model of status: Mazur, 1985; challenge hypothesis: Wingfield et al., 1990). Although some discussion exists surrounding the pattern of T response to the competition outcome (Carré & Olmstead, 2014; G. A. Oliveira & Oliveira, 2014b), the link between T, status and dominance is well established in the literature (Hamilton, Carré, Mehta, Olmstead, & Whitaker, 2015). Although the ability to efficiently detect threat in the social environment is not a status-seeking behavior *per se,* it can be interpreted as a precursor mechanism that allows the appropriate selection of status-seeking or status-avoidance behaviors.

The results presented here for T did not match the findings reported by Van Honk and Schutter (2007), in which anger detection was delayed in participants with exogenously increased levels of T, when compared to those in a placebo condition. Beside the differences in the stimuli and lack of a common measure that would facilitate the direct comparison of the findings in both experiments, in our experiment the emotion recognition task was performed after a social contest that elicited changes in the social status of the participants. As previous research has shown (Carré et al., 2013; Carré, Putnam, & McCormick, 2009; Mehta & Josephs, 2006) the effects of T on social cognition are very sensitive to the participant's status and this coupling of T and social context may result in different interactions that are not present in the experimental design used by Van Honk and Schutter (2007). Moreover, it is also possible that the T administration in the experiment by Van Honk and Schutter (2007) impaired anger discrimination by inducing supraphysiological T levels, thus moving the participants to the right tail of the dose-response curve (Adkins-Regan, 2005). Further research is necessary to address these different results and, in this regard, signal detection theory is a useful tool to compare findings and pinpoint the mechanisms involved in threat detection.

Although the detection of anger is usually associated with the activation of the motivational system of approach, empirical evidence suggests that this may not occur when an appropriate adaptive response is not available to the individual (Carver & Harmon-Jones, 2009; Harmon-Jones, Sigelman, Bohlig, & Harmon-Jones, 2003; Vescio, Gervais, Snyder, & Hoover, 2005). The selection of an adaptive response to a stressor or to a more specific social challenge depends greatly on the evaluation of the event and the coping capacity of the organism (Blascovich & Mendes, 2000; Blascovich et al., 2011). According to the biopsychosocial model of challenge and threat (Blascovich & Mendes, 2000), an event may be evaluated as a threat when task demands are greater than the individual resources or as a challenge appraisal when the coping resources are evaluated as greater than the task demands. Our results suggest that the participants with the higher ratings for the evaluation of the competition outcome as a challenge were capable of mobilizing their cognitive resources and obtain a better performance in anger discrimination. This interpretation is supported by previous research showing that a challenge appraisal is linked to a better cognitive (Tomaka et al., 1993) and athletic performance (Blascovich, Seery, Mugridge, Norris, & Weisbuch, 2004).

Unexpectedly, we have also found that the response latency to neutral faces was higher for winners than for losers. Neutral faces carry a certain degree of ambiguity that can be misinterpreted as a threat by individuals with high attentional vigilance as in the case of social anxiety patients (Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006; Yoon & Zinbarg, 2008). Although winners had a higher response latency than losers on neutral faces, no difference was detected on the discrimination parameter, suggesting that the winners' recognition of neutral faces was not impaired, and that winners simply required more time to correctly identify the emotion being expressed. The increased response latency for neutral faces in winners can thus be interpreted as increased attentional vigilance to an ambiguous signal in order to avoid the fitness reducing costs of a missing a threat display in the social group that could jeopardize their position in the social hierarchy.

There are several limitations present in this experiment. First, although we have found a clear difference between winners and losers for anger discrimination, a marginal non-significant difference for fear discrimination (in the same direction as anger) was also detected. Therefore, we cannot exclude the possibility of a more general effect of status and social dominance on the discrimination of emotions that convey threat (anger, fear), which could be detected in a similar experiment with a larger sample and higher statistical power. Second, we have not found the expected difference between the T levels of winners and losers after the competition as predicted by the biosocial model of status (Mazur & Booth, 1998; Mazur, 1985). Similar results have been reported in the literature and it has been suggested that the T response to competition may be modulated by cognitive variables (G. A. Oliveira & Oliveira, 2014b). Future research that achieves a significant peripheral change in androgen levels depending of the competition outcome may expand the results presented here for threat recognition.

In conclusion, in this experiment we have found that post competition T levels specifically modulated the discrimination parameter involved in anger detection and that threat detection can be modulated by competition induced changes in social status. The efficient recognition of anger displays found in the winner condition could be an adaptive advantage in the detection of potential agonistic interactions in the social group and thus help to secure or advance to a higher position in the social hierarchy. Furthermore, since a better performance in anger discrimination can also be predicted from trait and physiological markers of dominance, these factors may protect the individual from the impairing effects of a social defeat.

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Chapter VIII

General discussion

General Discussion

The experiments presented in this thesis were designed based on the observation of discrepancies between the empirical results and the direction of the T response to competition in humans predicted by the theories for the social modulation of androgens (BMS: Mazur, 1985; "Challenge hypothesis": Wingfield, Hegner, Dufty, Jr., & Ball, 1990). We have suggested that the T response to the competition outcome can be better understood in an interaction between cognition, physiology and the environment (G. A. Oliveira & Oliveira, 2014a, 2014b). In this integrative perspective, we have specifically selected appraisal as a key moderator of the T response to competition and most of the empirical work presented here was devoted to the experimental testing of this hypothesis. Although both theories now consider the possibility of a cognitive modulation of the endocrine response (Edwards, 2006; Kempenaers, Peters, & Foerster, 2008), the BMS by establishing clear predictions for the directions of the outcome dependent T responses to competition, offers a richer theoretical framework to investigate the cognitive modulation of T response to competition and for the most part will be used as a reference.

Cognitive modulation of the testosterone response to competition

Based on the “dear enemy” effect, a phenomenon described in non-humans animals according to which the territorial intrusions performed by stranger males elicits higher levels of aggression than territorial intrusions done by a familiar male (Temeles, 1994), we have first tested the effect of the familiarity component of appraisal on the T response to competition. The experiment described on Chapter II provides the first empirical evidence of a cognitive moderation of the T response to competition in humans. In this experiment, women showed greater increases in post-competition T levels when they were defeated by an unfamiliar opponent and evaluated the outcome of the competition as more threatening. The follow-up experiment with a male sample (Chapter III) did not detect the moderation effect of familiarity and threat on the T response to competition, despite male losers increasing T levels and evaluating the competition outcome as more threatening than winners, matching the results reported on Chapter II for women. Together these experiments suggest that there could be sex differences for the cognitive moderators of the androgen response to competition. In this case, the familiarity component within the appraisal process, through which the participants evaluated their opponent, could be more important for women than for men. The hypothesis of a sex difference for the effect of familiarity on the androgen response is supported by previous research suggesting that women are more sensitive to the effects of familiarity than men (Deaner, Shepherd, & Platt, 2007). However, reduced or blunted T responses to competition have been reported in experiments performed in an in-group context, suggesting that the familiarity parameter could still be relevant for men (Oxford, Ponzi, & Geary, 2010; Trumble et al., 2012; Wagner, Flinn, & England, 2002). If these assumptions are correct, it is possible that the continuous measure of opponent familiarity used in the male experiment was too subtle and did not allow an effective differentiation of the opponents based on familiarity. This hypothesis could be experimentally tested using a simple dichotomous manipulation of familiarity (e.g. familiar, stranger) in a face-to-face competition setting.

The findings on Chapter II and III prompted us to revisit the "dear enemy" effect on Chapter IV and continue the research on the effects of familiarity and threat assessment on the aggressive and androgen response. Using a cichlid fish, in this experiment we showed for the first time that the "dear enemy" effect also modulates the androgen response to repeated territorial intrusions. On the first day of territorial intrusions, stranger males elicited a higher androgen response than familiar males. However, on the fourth day of territorial intrusions, the androgen levels were dramatically lower and the difference between stranger and familiar males was no longer detected, suggesting a habituation effect of the androgen response to repeated territorial intrusions. Moreover, since most of the research on the "dear enemy" effect used a single intrusion (Frostman & Sherman, 2004; Leiser & Itzkowitz, 1999; Leiser, 2003), the variation of the resident male's aggressive response over multiple intrusions by strangers and neighbors has not been properly described in the literature. In our experiment, the "dear enemy" effect detected on the first day dissipated with repeated intrusions, as the aggressive response to intrusions by neighbors became similar to those by stranger males. This effect suggests that familiarity influences the resident's male evaluation of the relative threat posed by neighbors and stranger males and this effect is stronger on the first day of intrusions, however the repeated intrusions seems to have led to a re-appraisal of the threat posed by the neighbors and the aggressive response has been adjusted accordingly.

From a comparative perspective, bridging the findings from Chapter II, III and IV, it would be interesting to investigate the effect of multiple challenges by familiar and unfamiliar opponents on the androgen response to competition in humans. The competition experiment with women (Chapter II), showed an association between high familiarity and lower threat appraisal and the slope for the T response to competition when facing a familiar opponent was not significant. However, the behavioral results on Chapter IV indicate that the threat evaluation of a familiar opponent can be updated based on the successive territorial intrusions. Hypothetically, these results suggest that repeated challenges by familiar opponents could trigger an androgen response based on their increased threat value. On the other hand, it is also possible that the increases in T levels that we have reported for the face-to-face competition against an unfamiliar opponent could become non-significant after repeated interactions, matching the habituation effect detected for cichlids.

On chapter V, we have tested the effect of the expectations component, continuing the research on appraisal as a moderator of the T response to competition in humans. Moreover, by manipulating the participant's expectations on the outcome of the competition, this experiment also tested the association between surprise and T increases previously described in the literature (Zilioli, Mehta, & Watson, 2014), assuming that surprise is an emotion triggered by an appraisal process characterized by a violation of expectations (Scherer, Zentner, & Stern, 2004). Our results are consistent with the hypothesis of expectations as a moderator of the T response to competition in women. The participants assigned to the violation of expectations treatment (e.g. victory or defeat occurring against their expectations), showed differences in their T response to winning or losing, so that winners significantly decreased T levels after the competition, while for losers only a non-significant increase was found. These results are compatible with previous research linking surprise and T (despite the differences in the significance of the effects), showing significant increases in T for losers and non-significant decreases in T for winners that were more surprised with their defeat or victory, respectively (Zilioli et al., 2014).

The Chapter V experiment on expectations complements the work presented on Chapter II for the familiarity component and supports the hypothesis of appraisal as a moderator of the T response to competition. Together they also underline the need to include specific measures on how participants evaluate the outcome of the competition and the social context in which the competition is taking place. Componential models of appraisal (Ellsworth & Scherer, 2003) can be a useful tool for this research program, since the partitioning of the appraisal process in specific components facilitates their operationalization in an experimental context. As it can be seen on Chapter V, the self-report measures for the expectations component validated the manipulation, but they did not correlate with the T changes elicited by the competition and neither did the measures for the evaluation as a threat/challenge. In our perspective, although the treat/challenge self-report measures could be complemented by their cardiovascular indices (Blascovich, Vanman, Mendes, & Dickerson, 2011) this would still be insufficient. The cardiovascular indices for threat/challenge are also proxy measures of the endpoint of an evaluation process and therefore do not allow the disentanglement of the intervening components. In this regard, the explicit manipulation of appraisal components seems to be the more robust experimental approach.

On the other hand, although the manipulation of isolated appraisal components may be a suitable method to determine their relevance, this route provides only a limited perspective on the effects of appraisal, since there will be no information on the key characteristics of appraisal, namely, the interaction of multiple components in a dynamic and recursive process to determine the significance of an event (Scherer, 2009). Therefore, in order to better match the construct, it is indispensable that future research starts combining the measurement and the manipulation of multiple appraisal components to investigate their relative importance and the effect of their interaction on the androgen response to social challenges. Curiously, the same issue can be found in the research of appraisal in non-human animals; there is evidence suggesting the existence of appraisal components in non-human animals, but little information exists on the contribution and interaction of these different components for the evaluation of a stimuli (Faustino, Oliveira, & Oliveira, 2015). In this regard, the measurement of aggressive and endocrine responses to agonistic interactions in a laboratory setting could be a useful tool for appraisal research in non-humans, especially since there is accumulating evidence that phenomena like the “dear enemy” are not fixed responses and can be modulated by the social context, as seen on Chapter IV and on other experiments (Booksmythe, Jennions, & Backwell, 2010; Monclús, Saavedra, & de Miguel, 2014).

Besides appraisal, on Chapter VI we have tested the hypothesis of mood as mediator of the T response to competition, according to which T increases in winners due to increases in positive mood and T decreases in losers in response to the negative mood induced by the defeat (Booth, Shelley, Mazur, Tharp, & Kittok, 1989; Mazur & Lamb, 1980). In this experiment, we have tested the direct effect of affective changes induced by emotional film clips on T levels, decoupling the changes in affective state from competitive context. We have found that participants decreased their T levels after being exposed to a film clip that successfully induced negative affect characterized mainly by increases in sadness, while on all other conditions no T response was detected. The T change in the Sadness condition was significant and in the predicted direction and since sadness is associated with avoidance

(Carver & Harmon-Jones, 2009), this could be a mechanism inhibiting losers from disputing status in future interactions, as predicted by the BMS (Mazur, 1985). To what extent these results can be translated to a competition context depends greatly on the motivational system that is being activated by the outcome (approach vs. avoidance; Carver & Harmon-Jones, 2009). For instance, in our competition experiments with humans (Chapter II, III, V) losers consistently evaluated the outcome of the competition as more threatening than winners. It is expected that a defeat evaluated as a threat triggers the proper adaptive emotional responses to the event, specifically fear (avoidance activation) and anger (approach activation) since these are emotions that signal threat (Pichon, de Gelder, & Grèzes, 2009). In Chapter VI, our condition for Threat induced both fear and anger and did not produce a significant T response, thus in this experiment we don’t have empirical data on what happens to T when anger is induced. However, other experiments have found positive associations between anger and T levels (Peterson & Harmon-Jones, 2012). This suggests that, hypothetically, increases in anger following a defeat could also induce increases in T levels as observed in the experiments described in Chapter II and III, and this effect would be congruent with the interpretation of T increases in losers as a signal of motivation to keep disputing status (e.g. Mehta & Josephs, 2006).

In conclusion, although this experiment complements a field lacking research on how women’s T responds to affective stimuli, the findings in this experiment are not sufficiently strong to address the original hypothesis. More research is required to test if the T response is elicited by changes in mood or from a covariate of mood that is relevant for the current competition or for future interactions, for example appraisal of the outcome or changes in the participant’s sense of power following a victory or defeat.

Predictions of the theoretical models for the social modulation of androgens

After showing how cognition can moderate the androgen response to competition, we may now contrast our findings with the predicted direction of the T response to competition and the assumption that the T responses to agonistic interactions have an adaptive function, influencing future interactions. The direction of the T response in these experiments with the direction of the T response to competition predicted by the theoretical models (Mazur, 1985; Wingfield et al., 1990). The experiments described in Chapter II, III and V do not show the pattern of response predicted by the BMS (increases in winners, decreases in losers) or by the

“Challenge hypothesis” (general increase, no outcome dependent prediction). As we have already discussed above, appraisal contributes to the understanding of these unpredicted results, however an alternative explanation resides in the unstable hierarchies that are modeled in these laboratory competitions.

The status instability hypothesis (Mehta, Snyder, Knight, & Lassetter, 2014; Zilioli et al., 2014) proposes that when the competition outcome is decided by a narrow margin, the classic predictions of the BMS may not apply and in fact they could be reversed, so that losers increase and winners decrease T levels after the competition encouraging status-seeking and status-avoidance behaviors, respectively. The competition task in Chapter II and III is very similar to the one used by Zilioli et al. (2014) in their first experiment. Due to the manipulation of the trials, the participants assigned to the winner and loser condition experienced victories and defeats over the course of the competition and the interaction was only resolved in the final set of four trials. The T increase found for the losing women in Chapter II was replicated in the Zilioli et al. (2014) first experiment and later also detected for men in the Chapter III experiment. These T increases in losers have been interpreted in the literature as an indicator of the participant’s willingness to keep disputing status (e.g. Mehta & Josephs, 2006; Schultheiss et al., 2005), which in the case at hand is also congruent with the predictions of the status instability hypothesis (Zilioli et al., 2014). In Chapter V, a complete reversal of direction of T response can be observed for winners and losers in the violation of expectations treatment. This experiment also simulates an unstable hierarchy, since the winners assigned to the violation of expectations condition had a low status before the competition and climbed up the social hierarchy against their expectations by winning against a high status group. The T decrease detected for these unexpected winners is in the direction predicted by the status instability hypothesis and can be interpreted as an inhibition to engage in future competitions, due to the perceived fragility of the current hierarchical position (Zilioli et al., 2014). In sum, the competitions tasks used in the Chapter II, III and V experiments match an unstable hierarchy and the effects for T support the predictions of the status instability hypothesis (Zilioli et al., 2014) and not those of the BMS (Mazur, 1985).

It is known from experiments in social psychology that there is an interaction between the objective structure and dynamics of the social hierarchy and the individual’s evaluation of these events. For example, individuals in high status groups show a cardiovascular response consistent with a threat evaluation when facing a social challenge that could change their status or when the social hierarchy is perceived as unstable (Scheepers & Ellemers, 2005;

Scheepers, 2009). Therefore, the status instability hypothesis and the cognitive modulation of the androgens proposed in this thesis should not be seen as mutually exclusive explanations. In fact, they simply focus on different aspects relevant to the competition and by doing so complement each other. Together they may provide a more complete understanding on the interactions between the social hierarchy, intra-individual processes and androgens when individuals are facing a social challenge.

The theories for the social modulation of androgens also propose a reciprocal relation between androgens and behavior. In this interaction, the androgen response elicited by agonistic interactions are thought to have an adaptive function and feedback to the brain adjusting cognitive and motivational processes to the changes in the social environment and carrying over to future interactions (R. F. Oliveira, 2009). Based on previous research, it is possible to infer the adaptive function of the T changes detected in response to the social and affective stimuli used in the experiments we have already discussed (e.g. willingness to compete for the T increases in losers; Mehta & Josephs, 2006); T decreases as avoidance; (Zilioli et al., 2014). However, these experiments were designed solely to test the effects of cognition on the T response and did not provide any empirical evidence on the hypothetical effects of T on cognition.

The experiment in Chapter VII addresses this reciprocal relation between androgens and behavior and tests the effect of the post-competition T levels on threat detection. We have found that the winning participants were faster and discriminated angry faces better than losers. The effects of T on threat detection and specifically on anger have been thoroughly reported in the literature, but the cognitive process underlying these effects have never been properly identified. Using signal detection theory we have found that post-competition T increased the sensitivity to angry faces. The effects of post-competition T on threat detection described above match the predictions of the BMS for the reciprocal relationship between status and T (Mazur & Booth, 1998). The enhanced sensitivity to anger displays found in high T individuals after winning a competition could confer them an advantage in the detection of challenges to their dominance status and support other status-seeking behaviors (Wirth & Schultheiss, 2007).

Although the findings in Chapter VII are merely correlational and no causal relationship can be established between the high levels of peripheral T and the enhancement of threat detection functions in the CNS, this relationship is congruent with our view, exposed in the introduction, of the social-decision making processes in the brain as a target for modulation by peripheral/gonadal hormones. Our assumption of a brain-body-environment goal-oriented coupling (e.g. Beer, 2009) seems to be backed by the data in this experiment, since the statistical analysis showed that a better performance for the discrimination of anger could also be predicted from high trait dominance and ability to cope with the competition outcome. These variables, along with T, have been linked with the activation of the approach motivational system (Anderson & Jennifer, 2002; Blascovich et al., 2011; Lacreuse et al., 2010), suggesting an integrative and coordinated adaptive response to a signal of threat.

Within this framework, the hypothetical functions for the post-competitive or post-stimuli T reported in the previous experiments (also mostly supported by correlational data) requires that these gonadal T changes feedback to the brain, influencing the goal-relevant cognitive processes. The inclusion of the assumption of a reciprocal mutually reinforcing relationship between T and status (e.g. Mazur & Booth, 1998) allows the circumscription of brain regions that could potentially be targeted by peripheral T in a social competition context. It should be expected a T effect on the brain regions involved in reward and in the motivational systems of approach (supporting the interpretation of losers increasing T on Chapter II and III) and avoidance (supporting the interpretation of winners decreasing T on Chapter V; sadness inducing a T decrease on Chapter VI). These effects have been described mostly in neuroimaging studies in which the T levels of the participants were increased by the administration of exogenous T (e.g. Hermans et al., 2010; Lombardo et al., 2012; Simon et al., 2010). It is possible to interpret these experiments as a manipulation of the peripheral T signaling decoupled from the context of an agonistic interaction, enabling a test of the direct effect of increased T in the brain. The ecological validity of this "simulation" is questionable due to the supraphysiological levels of circulating T that are being induced in the individual (e.g. Tuiten et al., 2000). The brain areas involved in the process of appraisal are also potential targets for T. If the reciprocal model is correct, post-competition T should also feedback into these regions, altering the way a dominance contest is evaluated by the individuals. Moreover, winners and losers should also present different patterns of activation and T could play a role in this effect. So far there is no empirical evidence for such an effect in humans. Alternatively, since reward and motivation interface with the appraisal process itself (Scherer, 2001), it is certainly possible that T modulates the appraisal process indirectly by acting on these circuits, instead of the more cortical areas identified in the literature (Ohira et al., 2008; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008) that may not be involved in the more automatic appraisal processes addressed in this thesis.

Concluding remarks

The theories for the social modulation of androgens were initially proposed based on data from non-human animals and later extended to human males (Archer, 2006; Mazur & Booth, 1998). In recent years, more competition experiments have been performed with women and it is becoming clear that the problems initially identified for women's T response to competition are also present in competitive events with males (see Chapter II and III for unpredicted results in men and women).

In this thesis, we have found evidence in support of our hypothesis of a cognitive moderation of the T response to social competition in women. Specifically, we found that competition-induced changes in women's T levels could be moderated by the evaluation of the outcome as a threat and by the appraisal components of familiarity and expectations. The only experiment performed with men did not replicate the findings for familiarity and threat described for females, hinting a possible sex difference in the relative importance of appraisal components of the competition. Although, men and women do not show differences in motivation to compete against members of the same sex (Niederle & Vesterlund, 2011), sex differences have been reported on competition relevant traits, for example, women show a greater aversion to risk-taking and higher sensitivity to punishment than men (Cross, Copping, & Campbell, 2011). These factors may explain why the same competitive task could induce identical T responses in men and women (e.g. Jiménez, Aguilar, & Alvero-Cruz, 2012) or lead to sex differences in T (e.g. Mazur, Susman, & Edelbrock, 1997), and change the emphasis from questioning the relevance of T for women competition, to the identification of the common and specific cognitive (this thesis) and contextual parameters (e.g. status instability hypothesis; Zilioli et al., 2014) that influence the T response to competition in men and women.

In our experiments with women, the use of oral contraceptives lowered the T levels of the participants. Previous research have shown that oral contraceptives decrease the levels of circulating androgens in its free form by inhibiting ovarian production and increasing steroid hormone binding globulin synthesis (Graham, Bancroft, Doll, Greco, & Tanner, 2007;

Wiegratz et al., 2003). Importantly, despite the lower concentrations of T, we have not found any evidence suggesting an effect of oral contraceptives on the T response to the competitive tasks or to the affective stimuli. These results add to the accumulating evidence suggesting that oral contraceptives do not impair the reactivity of T in response to social challenges (e.g. Edwards & O’Neal, 2009; Zilioli et al., 2014). However, there is still insufficient research on the effect of oral contraceptives on T response to affective stimuli. Our results in Chapter VI contrast with the moderation effect detected for oral contraceptives on the T response to imagined social situations of different affective value (Goldey & van Anders, 2011). In sum, in agreement with a previous commentary (Josephs, 2009), we conclude that the exclusion of women using oral contraceptives from competition experiments based on potential differences in the T response does not seem necessary. Despite this, it is still advisable that this variable is properly controlled in experiments since it may potentially lead to free T baseline differences between treatments. Moreover, the use of measures sensible to the intra-individual changes in T levels is recommended, since this method avoids the overshadowing of variations in the absolute values of T for oral contraceptives users.

In conclusion, the experiments in this thesis support the hypothesis that T in women is also sensible to changes in social status and plays an effective role in the adaptation to changes in the social environment. Furthermore, we have found evidence of a cognitive moderation of the outcome-dependent changes in T which may contribute to understanding of sex differences reported in the literature for this topic. More importantly, this thesis along with other contemporary research suggests that the unpredicted T responses to competition have an adaptive function and therefore should not be interpreted as anomalies. In turn, this underlines the need for the theoretical models for the social modulation of androgens to explicitly integrate cognitive and contextual parameters in their predictions.

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Appendix A

Summary of humans studies reporting effects of competition on androgen levels (sorted by year of publication)

Authors	**Paradigm**	**Sex (n)**	**Measure**	**Competition effect**	**Winner vs Loser**	**pre-pos Winner**	**pre-pos Loser**
(Mazur & Lamb, 1980)	Tennis	M(8)	Plasma	↓(close match)	n/a	↑(decisive match)	↓(decisive match)
	Lottery	M(14)	Plasma	n.s.	n/a	n.s.	n.s.
(Elias, 1981)	Wrestling	M(15)	Plasma	↑	W>L (T%change)	n/a	n/a
(Booth, Shelley, Mazur, Tharp, & Kittok, 1989)	Tennis	M(6)	Saliva	n/a	n.s.	n/a	n/a
(Gladue, Boechler, & McCaul, 1989)	Reaction time task	M(39)	Saliva	n/a	W>L	n/a	n/a
(Mazur, Booth, & Dabbs, 1992)	Chess regional	M(8)	Saliva	n/a	W>L	n/a	n/a
	Chess tournament	M(8)	Saliva	n/a	W>L	n/a	n/a
(McCaul, Gladue, & Joppa, 1992)	Exp 1: Coin toss	M(28)	Saliva	n/a	W>L (p=.079)	n/a	n/a
	Exp 2: Coin toss	M(101)	Saliva	n/a	W>L	n/a	n/a
(Mazur, Susman, & Edelbrock, 1997)	Videogame	M(28)	Saliva	n.s.	n.s.	n/a	n/a
		F(32)	Saliva	↓	n.s.	n/a	n/a
(Bernhardt, Dabbs, Fielden, & Lutter, 1998)	Watching basketball	M(8)	Saliva	n/a	n/a	↑	↓
	Watching soccer	M(21)	Saliva	n/a	n/a	↑	↓
(González-Bono, Salvador, Serrano, & Ricarte, 1999)	Basketball	M(16)	Saliva	n/a	n.s.	n.s.	n.s.
(Schultheiss, Campbell, & McClelland, 1999)	NTT	M(42)	Saliva	n/a	n.s.	n/a	n/a
(Suay et al., 1999)	Judo	M(28)	Plasma	↑	n.s.	n/a	n/a

(González-Bono, Salvador, Ricarte, Serrano, & Arnedo, 2000)	Basketball (winners)	M(16)	Saliva	n/a	n/a	Team1:↑ (p=.058) Team 2: n.s.	n/a
(Serrano, Salvador, Sanchís, & Suay, 2000)	Judo	M(12)	Saliva	n.s.	n.s.	n.s.	n.s.
(Filaire, Maso, Sagnol, Ferrand, & Lac, 2001)	Judo	M(18)	Saliva	n/a	W<L	n.s.	n.s.
(Bateup, Booth, Shirtcliff, & Granger, 2002)	Rugby	F(17)	Saliva	↑	n.s.	n/a	n/a
(Schultheiss & Rohde, 2002)	NTT	M(66)	Saliva	n/a	n.s.	n/a	n/a
(Wagner, Flinn, & England, 2002)	Domino	M(8)	Saliva	n/a	n.s.	n.s.	n.s.
(Kivlighan, Granger, & Booth, 2005)	Ergometer	M(23)	Saliva	↑	n/a	n/a	n/a
		F(23)	Saliva	n.s.	n/a	n/a	n/a
(Schultheiss et al., 2005)	SRT task	M(95)	Saliva	n/a	n/a	n/a	n/a
		F(75)	Saliva	n/a	n/a	n/a	n/a
(Edwards, Wetzel, & Wyner, 2006)	Soccer	M(22)	Saliva	n/a	n/a	↑ (p=.08)	n/a
		F(18)	Saliva	n/a	n/a	↑	↑
(Josephs, Sellers, Newman, & Mehta, 2006)	NTT	M(92)	Saliva	n/a	n/a	n/a	n/a
(Mehta & Josephs, 2006)	NTT	M(64)	Saliva	n/a	n/a	n/a	n/a
(Parmigiani & Bartolomucci, 2006)	Judo	M(22)	Plasma	↑ (total T)	W<L (free and total T)	n/a	n/a
(Stanton & Schultheiss, 2007)	SRT	F(49)	Saliva	n/a	n/a	n/a	n/a
(van Anders & Watson, 2007)	Vocabulary task (ability determined)	M(37)	Saliva	n/a	n.s.	n.s.	↓ (p=.055)
		F(38)	Saliva	n/a	n.s.	n.s.	n.s.
	Vocabulary task (chance	M(31)	Saliva	n.s.	n.s.	n.s.	n.s.
		F(43)	Saliva	n.s.	n.s.	n.s.	n.s.

	determined)						
(Carré & McCormick, 2008)	PSAP	M(38)	Saliva	↑	n/a	n/a	n/a
(Mehta, Jones, & Josephs, 2008)	Dog competition	M(93) F(91)	Saliva	n/a	n/a	n/a	n/a
	Intelligence test	F(70)	Saliva	n/a	n/a	n/a	n/a
(Carré, 2009)	Hockey (winners)	M(10)	Saliva	n/a	n/a	↑	n/a
(Carré, Putnam, & McCormick, 2009)	NTT+PSAP	M(39)	Saliva	↓	n/a	n/a	n/a
		F(60)	Saliva	↓	n/a	n/a	n/a
(Edwards & O’Neal, 2009)	Sports	F(80)	Saliva	↑	n/a	n/a	n/a
(Hamilton, van Anders, Cox, & Watson, 2009)	Wrestling	F(21)	Saliva	n/a	n.s.	↑	↑
(Mehta, Wuehrmann, & Josephs, 2009)	Intelligence test	M(30) F(30)	Saliva	n/a	n/a	n/a	n/a
(T. F. Oliveira, Gouveia, & Oliveira, 2009)	Soccer	F(33)	Saliva	n/a	W>L	↑	↓
(Pound, Penton-Voak, & Surridge, 2009)	Lab task	M(57)	Saliva	n/a	W>L	↑	n.s.
(Stanton, Beehner, Saini, Kuhn, & Labar, 2009)	Elections	M(57)	Saliva	n/a	W>L	n.s.	↓
		F(106)	Saliva	n/a	n.s.	n/a	n/a
(Carré, Gilchrist, Morrissey, & McCormick, 2010)	PSAP	M(139)	Saliva	n.s.	n/a	n/a	n/a
(Edwards & Kurlander, 2010)	Volleyball (winners)	F(15)	Saliva	n/a	n/a	↑	n/a
	Tennis (losers)	F(13)	Saliva	n/a	n/a	n/a	↑
(Oxford, Ponzi, & Geary, 2010)	Videogame (teams)	M(42)	Saliva	n/a	n/a	↑ (between teams	↑ (between teams

						competition)	competition)
(Steiner, Barchard, Meana, Hadi, & Gray, 2010)	Poker	M(32)	Saliva	n/a	n.s.	↑	↑
(van der Meij, Buunk, Almela, & Salvador, 2010)	Intelligence test	M(84)	Saliva	n/a	n.s.	↑	↑
(Slatcher, Mehta, & Josephs, 2011)	Lab task	M(76)	Saliva	n/a	n/a	n/a	n/a
(Costa & Salvador, 2012)	Squares and letters	F(40)	Saliva	n/a	W>L	n/a	n/a
(Jiménez, Aguilar, & Alvero-Cruz, 2012)	Badminton	M(27)	Saliva	n/a	W>L	↑	↓
		F(23)	Saliva	n/a	W>L	↑	↓
(Trumble et al., 2012)	Soccer	M(88)	Saliva	↑	n.s.	n/a	n/a
(van der Meij et al., 2012)	Watching soccer (winners)	M(25)	Saliva	n/a	n/a	n.s.	n/a
		F(25)	Saliva	n/a	n/a	n.s.	n/a
(Zilioli & Watson, 2012)	Tetris	M(70)	Saliva	n/a	W>L	n.s.	↓
(Carré, Campbell, & Lozoya, 2013)	Videogame	237 M(48%)	Saliva	n/a	W>L	n/a	n/a
		F(52%)	Saliva	n/a	n.s.	n/a	n/a
(Denson, Mehta, & Ho Tan, 2013)	RT task	F(53)	Saliva	n/a	W>L	n/a	n/a
(G. A. Oliveira et al., 2013)	NTT	F(34)	Saliva	n/a	W<L (p=.097)	n.s.	↑

Abbreviations: W= winner; L = loser; ↓ = significant decrease; ↑ = significant increase; n.s. = non-significant differences; M= male; F = female; n/a = not tested in original paper; NTT= number tracking test; SRT= serial response task; PSAP= point subtraction aggression paradigm. Reprinted from "Androgen responsiveness to competition in humans: the role of cognitive variables." by G.A. Oliveira and R.F. Oliveira 2014, *Neuroscience and neuroeconomics, 3*, p.22. Copyright 2014 by the authors. Reprinted with permission.

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Appendix B

Scores for the Differential Emotion Scale measures

Table B1

Scores (Mean ±SEM) for the initial measure of the Differential Emotion Scale

	Neutral		Humorous		Affiliation		Threat		Sadness	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Attention	5.347	.240	4.590	.299	4.727	.238	4.608	.241	4.565	.250
Fear	1.695	.230	1.409	.142	1.409	.169	1.478	.225	1.608	.206
Anxiety	2.913	.371	2.409	.306	2.500	.299	2.304	.254	2.434	.313
Tenderness	1.260	.143	1.318	.137	1.136	.099	1.086	.060	1.434	.164
Anger	1.391	.206	1.727	.295	1.363	.242	1.478	.207	1.347	.148
Shame	1.869	.237	1.454	.170	1.863	.303	2.217	.332	2.130	.283
Elation	2.826	.364	2.666	.326	2.000	.271	2.545	.269	2.565	.293
Joy	4.333	.438	4.090	.328	3.409	.291	4.043	.304	4.000	.259
Sadness	2.000	.301	1.818	.204	1.954	.258	1.608	.257	1.913	.258
Satisfaction	4.217	.355	3.904	.364	3.363	.298	4.173	.232	4.086	.250
Surprise	3.173	.285	3.045	.363	2.454	.352	2.304	.329	2.652	.336
Love	4.869	.316	4.363	.319	4.090	.262	4.000	.338	4.695	.284
Guilt	1.478	.250	1.136	.074	1.409	.193	1.173	.080	1.434	.164
Disgust	2.045	.363	1.318	.166	1.318	.274	1.391	.233	1.521	.234
Scorn	1.304	.193	1.227	.146	1.181	.181	1.086	.086	1.347	.184
Calm	5.130	.394	4.818	.352	4.909	.293	4.565	.331	4.956	.291

Table B2

Scores (Mean ±SEM) for the post-stimuli measure of the Differential Emotion Scale

	Neutral		Humorous		Affiliation		Threat		Sadness	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Attention	5.608	.279	5.318	.385	6.045	.232	5.782	.188	5.739	.228
Fear	1.045	.045	1.181	.106	1.363	.192	4.130	.423	2.434	.293

Anxiety	1.913	.332	1.681	.231	2.227	.308	4.652	.390	3.130	.362
Tenderness	2.695	.346	1.318	.166	4.772	.301	3.869	.362	4.913	.332
Anger	1.043	.043	1.181	.141	1.045	.045	3.565	.439	1.434	.151
Shame	1.000	.000	1.045	.045	1.045	.045	2.130	.357	1.217	.108
Elation	3.782	.387	4.227	.388	2.454	.277	1.347	.148	1.652	.161
Joy	4.347	.324	4.818	.434	2.954	.325	1.347	.161	1.652	.161
Sadness	1.260	.129	1.363	.203	2.227	.315	3.260	.422	3.739	.345
Satisfaction	4.909	.321	4.500	.398	3.545	.340	1.304	.171	1.869	.211
Surprise	4.521	.416	3.380	.399	3.227	.321	3.956	.329	2.565	.313
Love	5.000	.338	3.500	.409	4.318	.368	1.391	.150	2.739	.289
Guilt	1.130	.071	1.045	.045	1.045	.045	1.521	.187	1.434	.197
Disgust	1.173	.135	1.000	.000	1.000	.000	3.260	.436	1.260	.129
Scorn	1.000	.000	1.000	.000	1.000	.000	2.478	.411	1.304	.132
Calm	5.826	.336	3.818	.375	4.727	.373	2.217	.251	3.695	.254

Appendix C

Coefficients and significance for each path of the initial model

Path			β	p-value	Bootstrap p-value
Outcome	→	Testosterone	.076	.639	.658
Outcome	→	Cortisol	-.168	.292	.284
Outcome	→	Challenge	-.181	.256	.299
Outcome	→	Threat	-.076	.638	.605
Outcome	→	Sense of Power	.095	.556	.646
Outcome	→	Neutral Latency	-.430	.004	.003
Outcome	→	Anger Latency	.489	<.001	.001
Outcome	→	Anger d’	-.295	.016	.030
Testosterone	→	Neutral Latency	.042	.779	.770
Testosterone	→	Anger Latency	.087	.552	.585
Testosterone	→	Anger d’	.269	.030	.046
Cortisol	→	Neutral Latency	.161	.291	.218
Cortisol	→	Anger Latency	.136	.360	.344
Cortisol	→	Anger d’	-.171	.172	.555
Challenge	→	Neutral Latency	-.047	.753	.606
Challenge	→	Anger Latency	.201	.165	.236
Challenge	→	Anger d’	.507	<.001	.002
Threat	→	Neutral Latency	.038	.794	.802
Threat	→	Anger Latency	-.081	.573	.584
Threat	→	Anger d’	-.006	.961	.894
Sense of Power	→	Neutral Latency	.068	.632	.781
Sense of Power	→	Anger Latency	-.129	.355	.317
Sense of Power	→	Anger d’	.260	.027	.036