University of Valencia

Department of Psychobiology

NEUROSCIENCE Ph.D.

Organizational and Activational Effects of Testosterone in Two Populations with High Androgenization Susceptibility

Efectos organizadores y activadores de la testosterona en dos poblaciones susceptibles de alta androgenización

Dissertation

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Abbreviations

ADHD = Attention Deficit Hyperactivity Disorder

ASD = Autism Spectrum Disorders

BAP = Broader Autism Phenotype

C = Cortisol

CG = Control Group

CNS = Central Nervous system

DSM-V = Diagnostic and Statistical Manual of Mental Disorders-V

HPA axis = Hypothalamic-pituitary-adrenal axis

HPG axis = Hypothalamic-pituitary-gonadal axis

T = Testosterone

T/C ratio = Testosterone/Cortisol ratio

Abreviaturas

C = Cortisol

DSM-V = Diagnostic and Statistical Manual of Mental Disorders-V

Eje HHA = Eje Hipotálamo-hipofiso-adrenal

Eje HHG = Eje Hipotálamo-hipofiso-gonadal

FAA = Fenotipo Autista Amplio

Ratio T/C = Ratio Testosterona/Cortisol

SNC = Sistema Nervioso Central

T = Testosterona

TDAH = Trastornos por Déficits de Atención con Hiperactividad

TEA = Trastornos Espectro Autista

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Chapter 1

INTRODUCTION

General introduction

Sex steroids such as testosterone (T) have relatively permanent organizational effects on the structure and functions of the central nervous system (CNS) (Filová, Ostatníková, Celec, & Hodosy, 2013; Lust, Geuze, Van de Beek, Cohen-Kettenis, Bouma, & Groothuis, 2011). Early brain exposure to T could prime individuals to be sensitive to the activational effects of T in adulthood (van-Honk, Schutter, Bos, Kruijt, Lentjes, & Baron-Cohen, 2011). Moreover, fluctuations in socio-cognitive abilities may be associated with the transient activational effects of T (Celec, Ostatnikova, Putz, & Kudela, 2002; Ostatníková, Laznibatová, & Dohnányiová, 1996; Ostatníková et al., 2007; van-Honk et al., 2011; van Rooij et al., 2012; Wirth & Schultheiss, 2007). Thus, behavior and cognitive processes may be a result of interaction between organizative and activational hormonal effects. Nonetheless, there is insufficient research to address these gaps in the literature.

Early T exposure has been associated with specific cognitive profiles (such as high levels of systemizing and low empathy) with implications for physiological variables and health (Nakayama, Takahashi, Wakabayashi, Oono, & Radford, 2007) including: proneness to aggressive behavior (Bailey & Hurd, 2005; Hampson, Ellis, & Tenk, 2008); lack of empathy (Von-Horn, Bäckman, Davidsson, & Hansen, 2010); and sensation seeking (Hampson et al., 2008). Due to the difficulty of analysing the masculinization of the CNS it is useful to employ peripheral indicators such as the 2D:4D ratio. This ratio is the quotient between the index (2D) and ring finger (4D) lengths and displays an inverse relationship with fetal T exposure. Hence, high prenatal T exposure corresponds to smaller or masculinized 2D:4D ratio (Bull, Davidson, & Nordmann, 2010). However, the relationship between circulating T and the 2D:4D ratio in adults is weak or absent (Hönekopp, Bartholdt, Beier, & Liebert, 2007). It is suggested that high intrauterine T levels may play a role in the development of autism spectrum disorders (ASD), specifically those with higher-functioning autism (Scheeren & Stauder, 2008; De Bruin, Verhiej, Weigman, & Ferdinand, 2006), as well as attention deficit hyperactivity disorders (ADHD)-related symptoms (Ames & White, 2010; Sucksmith, Roth, & Hoekstra, 2011). A positive association was found between the 2D:4D ratios of children with ASD and the 2D:4D ratio of their relatives. For this reason, it has been hypothesized that those progenitors (fathers and mothers) with low or masculinized 2D:4D ratios may provide further biological features

(especially high prenatal T) which increase the likelihood of their offspring developing ASD (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001).

Populations with these disorders – which are highly heritable – are suffering from overlapped behavioral and cognitive disorders. A study of families with ASD parents showed that they may have masculinized cognitive traits, although they do not present ASD (Kose, Bora, Erermiş, Ozbaran, Bildik, & Aydın, 2013). Nevertheless, little is known about the activational effects of T on this population. Moreover, ASD persons present common behavioral problems such as high levels of non-adaptive aggressive behavior, emotional reactivity to stressful situations, and anxiety (Kaartinen, Puura, Helminen, Salmelin, Pelkonen, & Juujärvi, 2012; Pouw, Rieffe, Oosterveld, Huskens, & Stockmann, 2013; Saenz & Alexander, 2013; Schwichtenberg et al., 2013). Nevertheless, the role of T in the relationship between ASD and high levels of aggression is not as well established as in IPV perpetrators.

Many studies with animals and humans associate T with aggression and violence. In this sense, the T decreases the threshold for irritability, hostility, and violence. The classical hypothesis establishes a causal relationship between T and aggression. However, T is currently considered a modulating factor that interacts with other factors (genetic, hormonal, neurochemical, environmental, etc.) to increase the likelihood of aggressive behavior (Moya-Albiol, 2010). Men who perpetrate intimate partner violence (IPV) are a specific population in which the activating T effects may be prone to aggressive behavior. Nevertheless, there is no empirical evidence that analyzes whether organizational T effects could facilitate the adoption of aggressive behavior to specific stimuli.

The main aim of this Ph.D work is to analyze the organizational and activational T effects in two specific populations employing common variables in both studies (2D:4D ratio, circulating T and C levels), one associated with prenatal hyper-masculinization (particularly parents of people with ASD), and the other related with greater activation of aggressive behavior, as is the case of male perpetrators of IPV.

Parents of people with autism spectrum disorders

Hyper-masculinized behavior may be associated with excessive prenatal exposure to testosterone (T) (Knickmeyer & Baron-Cohen, 2006). Moreover,

early high T exposure could increase the likelihood of developing ASD, characterized by 'extreme male brain' (Baron-Cohen, 2010a). Individuals with ASD present poor empathy skills and poor accuracy when inferring the thoughts and feelings of others (Baron-Cohen, 2010b). However, they present a highly systemizing mind (Baron-Cohen, 2010a), characterised by restricted interests and problems in switching attention. Furthermore, subtle cognitive masculinization traits can be detected in ASD parents. Hence, they present mild forms of autistic-like characteristics (without clinical diagnosis) that have been defined as a broader autistic phenotype (BAP) (Scheeren & Stauder, 2008). BAP in ASD parents is characterized by higher autistic and systemizing traits and lower empathy than the normative population. Moreover, these traits are combined with neuropsychological deficits – particulary in executive domains (Sucksmith et al., 2011) and subsyndromic attention and hyperactivity impairments. Recent research demonstrated the last result because ASD and ADHD exhibit a high rate of comorbidity (Sucksmith et al., 2011), and a plausible biological risk factor for both disorders may be elevated (T) levels (James, 2008).

Considering the evidence that could refer to an idiosyncratic endophenotype of parents of offspring with ASD, the purpose of these papers was to examine whether they have features that differentiate them from the general population. For this purpose, different markers of masculinization, with a particular emphasis on the 2D:4D ratio, have been employed. An additional aim was to analyze whether the 2D:4D ratio could be used as a predictor of other psychobiological features of masculinization in this particular population (especially with high current T levels), and of the severity of ASD and ADHD comorbid symptoms in their offspring. It could be expected that ASD parents would present a smaller or more masculinized 2D:4D ratio than the normative population values due to early T exposure (Manning et al., 2001). In fact, this high masculization could prime individuals to be sensitive to the activational effects of T (Moffat & Hampson, 1996; van Honk et al., 2011). Hence, the analysis of BAP in ASD parents may help provide a better explanation of the role of T in the etiology of ASD.

Male perpetrators of intimate partner violence

The T in men, as an indicator of the activity of the hypothalamic-pituitary-gonadal (HPG), is not only related to the development of neuropsychiatric disorders, but also plays a key role in the modulation of violent behavior. In addition, greater prenatal T exposure (defined by a masculinized 2D:4D ratio)

has been associated with a greater likelihood of physically expressing anger (Bailey & Hurd, 2005). Elevated basal T levels were described in various types of violent men – such as rapists, offenders with alcohol dependence, and criminals with antisocial character (Moya-Albiol, 2010).

Cortisol (C) is a product of the hypothalamic-pituitary-adrenal (HPA) axis and could act antagonistically with testosterone to reduce proneness to aggressive behavior by modulating T levels, due to an inverse relationship between the HPG and HPA axes (Terburg, Morgan, & van Honk, 2009). Low levels of C are associated with the most violent subjects (Popma et al., 2007). Thus, a hypoactive HPA axis may increase proneness to aggressive behavior. The quotient between the T and C has been employed as an indicator of social aggression (Terbug et al., 2009). The reciprocal relationship between both axes modulates anger expression in interaction with neuropsychological deficits in executive domains (Teichner, Golden, Van Hasselt, & Peterson, 2001), in empathy (Babcock, Green, & Webb, 2008), and in several personality traits such as anger and hostility (Norlander & Eckhardt, 2005). These deficits may lead to social inadequacy and even cause the adoption of inappropriate behavior that predisposes violent action (Tirapu-Ustárroz, Pérez-Sayes, Erekatxo-Bilbao, & Pelegrín-Valero, 2007).

T could be considered a predictor of masculinized characteristics – including autistic traits and high levels of aggression (Saenz & Alexander, 2013). Hence, we analyzed the effects of early T exposure in two specific populations by studying the 2D:4D ratio, basal T levels, T changes in response to an acute laboratory stressor, and the relationship of these variables with the rest of the masculinization variables. This analysis is made from from a holistic perspective (including psychological, neuropsychological techniques, and hormonal parameters) in order to analyze the modulatory relationship between T and cognition and behavior in subjects with differing masculization levels.

The main aims and hypotheses of this Ph.D work are summarized below:

1. *Characterize ASD parents using masculinization indicators (2D:4D ratio and T baseline), hormonal (C baseline), and psychological variables (anger, empathy, cooperation, cooperation, and autistic traits).* Furthermore, we analyzed the relationship between these markers with the ASD of the offspring. It is hypothesized that parents of people with ASD would be more masculinized than the normal population. Accordingly, they would be

framed in the BAP because the 2D:4D ratio is a good indicator of this condition (Manning et al., 2001). Furthermore, in line with the results obtained in previous studies at a cognitive level (Baron-Cohen, 2010b), and given its high androgenisation, we hypothesise that this indicator does not differ when comparing parents of people with ASD. Finally, the 2D:4D ratio predicts the severity of the symptoms of offspring with ASD, so a lower ratio would be associated with more severe symptoms (Manning & Bundred, 2000).

2. *Analyze the role of masculinization in ASD parents on the comorbid symptoms of ADHD in their offspring with ASD.* We hypothesized that the T of ASD parents would mediate the relationships between a masculinized 2D:4D ratio of ASD parents and ADHD symptoms of the parents and their offspring, respectively. Hence, high current T levels will be related with masculinized 2D:4D ratios and the more inattentive symptoms in ASD parents. And finally, highly inattentive symptom scores in ASD parents will be related with a masculinized 2D:4D ratio and increased ADHD symptoms in their offspring. Moreover, the relationship between masculinization and ADHD symptoms will be stronger with inattentive symptoms than hyperactivity symptoms – as revealed by previous research (McFadden, Westhafer, Pasanen, Carlson, & Tucker, 2005).

3. *Study the T and C response to acute stress in male perpetrators of IPV in comparison with controls.* Moreover, the relationship between these hormonal parameters and other variables such as neuropsychological and psychological variables is studied. The main aim is to analyze the modulating role of T on aggressive behavior. We hypothesized that IPV perpetrators would show higher basal T levels (Soler, Vinayak, & Quadagno, 2000) and lower C levels (Moya-Albiol, 2010) than controls. IPV perpetrators would also present cognitive dysfunctions such as poor cognitive flexibility (Teichner et al., 2001) and poor recognition of emotions or thoughts (Holtzworth-Munroe & Smutzler, 1996), especially for neutral expressions (Babcock et al., 2008). Finally, we hypothesized that IPV perpetrators would report lower affective empathy (Covell, Huss, & Langhinrichsen-Rohling, 2007) and a masculinized or smaller 2D:4D ratio (Bailey & Hurd, 2005). The 2D:4D ratio could explain the high levels of sensitivity to T effects (van Honk et al., 2011) and may predict T changes, especially in IPV perpetrators. As supported in a previous study, heightened feelings of anger are

related to high T levels (Herrero, Gadea, Rodríguez-Alarcón, Espert, & Salvador, 2010). For this reason, we expect that feelings of anger will be related with high T levels in both groups – although this relationship could be stronger in IPV perpetrators than controls due to their violent pasts. Additionally, for the reversal relationship between T and C and its effects on the neuropsychological and empathic performance, we hypothesized that the neuropsychological performance (Wisconsin card sorting test [WCST]) would be positively related with T levels (Muller, Aleman, de Haan, & van der Schouw, 2005) and negatively related to C levels (Egeland et al., 2005). Moreover, emotional recognition (reading the mind in the eyes) will be negatively related to T levels (van Honk & Schutter, 2007), and positively related to C levels (Smeets, Dziobeck, & Wolf, 2009).

4. *Validate the use of the ratio between the T and the C, together with several variables as a marker of proneness to aggressive behavior in male perpetrators of IPV*. It has been hypothesized that IPV perpetrators, as dominant men who are highly prone to aggression, show higher T/C ratios (Terburg et al., 2009) and higher self-esteem (Bushman, Baumeister, Thomaes, Ryu, Begeer & West, 2009) than controls – which could lead to better mental health (Mann, Hosman, Schaalma, & de Vries 2004).

Chapter 2

Study 1: The 2D:4D ratio and its relationship with other androgenisation parameters in parents of individuals with autism spectrum disorders

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Introduction

The 2D:4D ratio or quotient between the index and ring finger lengths is considered a non-direct indicator of androgenisation (Schneider, Pickel, & Stalla, 2006). Accordingly, the greater the exposure and sensitivity to prenatal testosterone and corresponding reductions in oestrogens, the greater is the likelihood of developing a lower ratio (Manning, Bundred, Newton, & Flanagan, 2003). Therefore, men will show, in general, lower values for this parameter than women (Breedlove, 2010). Additionally, the stability of the ratio over time in humans from two years of age has been proven, and there are no major variations during puberty (Knickmeyer & Baron-Cohen, 2006).

In humans, the 2D:4D ratio has been associated with health, cognitive profiles, physiological variables, personality patterns, and with the activator effects of hormones in adulthood. However, the results that relate these variables have been conducted, in general, with different samples and in separate studies. This fact makes it difficult to extract integral conclusions when explaining which role this indicator plays as a predictor of possible interactions between androgenisation, cognition, and health.

In terms of cognitive patterns, men often have more interest in objects and the laws underlying reality than for people and so they are more systematic (Baron-Cohen, 2002). This cognitive style is named 'S type', whereas an ex-cessive systematising has been named 'S extreme', and is re-lated to people affected by autism spectrum disorder (ASD). According to empathy and systematising theory, this cognitive style represents one of the endpoints of a continuum where people who are excessively empathetic will be situated at a far end called 'extreme E' (Baron-Cohen, 2010a). These cognitive styles have been related with the 2D:4D ratio, which means low ratio-values are associated with high values in systematising and less in empathy (Von-Horn, Bäckman, Davidsson, & Hansen, 2010). Furthermore, women with lower 2D:4D ratios, or with more androgenisation, show a more systematic cognitive style (Valla et al., 2010), whereas women with no androgenisation show 'E type' cognitive style or greater empathy (Wakabayashi et al., 2006). There is empirical data showing a high susceptibility for developing depression and neuroticism depending on a certain cognitive style, which means men with 'E type' style and women with 'S type'. A physiological indicator of this effect could be higher cortisol levels than people with a standard cognitive style (Nakayama, Takahashi, Wakabayashi, Oono, & Radford, 2007).

The 2D:4D ratio has been related with psychological trait-variables such as aggressivity. In this respect, low ratio-values have been associated with physical aggression in men (Bailey & Hurd, 2005) and with reactive aggression in women (Benderlioglu & Nelson, 2004). There is a greater level of cooperation in young adults of both genders with lower 2D:4D ratios (Millet & Dewitte, 2006). This indicator has been related to either the organising effects or activating effects of testosterone and cortisol. At a prenatal level, a predominance of the levels of testosterone compared to oestrogens in the amniotic fluid has been associated with the development of a smaller 2D:4D ratio (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). The results on the relationship between testosterone and the 2D:4D ratio in adults are inconsistent, while in infertile men a negative correlation between both parameters has been described (Manning et al., 2004), whereas there is no relationship with the non-clinical population in both genders (Hönekopp, Bar-thold, Beier, & Liebert, 2007). However, a recent study has shown that women (non-clinical population) with lower values of the 2D:4D ratio was susceptible to the effects of the exogenous administration of testosterone and decreased their scores for empathy (van-Honk et al., 2011). Moreover, there is also evidence that the 2D:4D ratio is related to other hormones such as cortisol. The levels of this hormone predicted values of the 2D:4D ratio for the right-hand in non-clinical populations of both genders (Beaton, Rudling, Kissling, Taurines, & Thome, 2011) although the significance of this relationship has not yet been clarified.

These indicators of androgenisation have been used in general for the analysis of clinical populations such as people with ASD, but a possible use to characterise the idiosyncra-sies of their parents has been neglected. It has been hy-pothesised that these people have, to a greater extent than the general population, autistic traits reflected in lower val-ues of the 2D:4D ratio and high scores on the autism quo-tient (Wheelwright, Auyeung, Allison, & Baron-Cohen, 2010). This data allows us to frame this population in the broad autism phenotype (BAP) (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001) and they are supported by a genetic predisposition in the etiology of these disorders (Buxbaum, Baron-Cohen, & Devlin, 2010).

Considering the evidence that could refer to an idiosyncratic endophenotype of parents of offspring with ASD, the purpose of this paper is to examine if the parents of offspring with ASD have idiosyncratic features that differentiate them from the general population, using different markers of

androgenisation, and with a particular emphasis on the 2D:4D ratio. Another aim is to analyse whether this indicator could be used as a predictor of other psychobiological variables related with androgenisation and the severity of ASD. It is hypothesized that parents of people with ASD would be more androgenised than the normal population. Accordingly, they would be framed in the BAP as the 2D:4D ratio is a good indicator of the condition (Manning et al., 2001). Furthermore, in line with the results obtained in previous studies at a cognitive level (Baron-Cohen, 2010b) we hypothesise that this indicator does not differ when comparing parents of people with ASD, given its high androgenisation. Finally, the 2D:4D ratio predicts the severity of the symptoms of offspring with ASD, so a lower ratio would be associated with more severe symptoms (Manning & Bundred, 2000).

Method

Participants

The final sample consists of 85 people aged between 31 and 63 years (M = 44.27, SD = 6.58) and with a body mass index (BMI) of M = 26.80, SD = 4.70 kg / m2, who partici-pated voluntarily and signed an informed consent in accor-dance with ethical standards for human research. The ex-perimental group consisted of 43 subjects, all fathers (n = 16) or mothers (n = 27) of offspring with ASD. The control group consisted of 42 fathers (n = 19) and mothers (n = 23) with similar characteristics to the experimental subjects, and whose children do not suffer any ASD or other chronic dis-orders. The ages of the offspring with ASD ranged from 5 to 30 years (M = 14.25, SD = 5.73), with a gender distribu-tion of 34 men and 7 women.

Procedure

Participants were scheduled to attend the laboratories of the psychology faculty at the University of Valencia, having previously been asked not to eat food or drink (except wa-ter, brushing teeth, or chewing gum) during the two hour period before arrival at the laboratory. The experimental ses-sions lasted approximately an hour and a half and were car-ried out between 16:00 and 19:00 pm (when cortisol levels are most stable) (Dickmeis, 2009).

After arrival in the laboratory, subjects were taken to a room with a constant temperature (22±1°C) and the anthropometric variables including weight, height, 2D:4D ratio, and socio-demographic variables were recorded. Two samples of saliva were then collected for determining hormone cortisol

(C) and testosterone (T), the first of the sample was taken at the end of the collection of anthropometric data and the last collection was within 20 minutes. Participants then completed questionnaires that assessed the psychological dimension feature type and, in the case of par-ents of offspring with ASD, a questionnaire aimed at assessing the degree to which their children suffer from autism.

Variables and measurement instruments

2D:4D ratio

To calculate the 2D:4D ratio three separate measure-ments of the length of the index finger (2D) and the ring finger (4D) of both hands were made. Measurements were made on the ventral side of the hand. The length of the fin-ger was taken from the proximal fold at the base of the fin-ger to the tip thereof, as this measurement procedure has a high replication (Schneider et al., 2006). For this purpose we used digital calipers with an accuracy of 0.01 mm. Two of the three measurements were made directly by two investi-gators, while the third was performed using a scanner for subsequent measurement. The value of the 2D:4D ratio for each hand was obtained by calculating the arithmetic mean of three measurements, as has been done in previous studies (Schneider et al., 2006). Furthermore, as an additional index of androgenisation, we obtained the directional asymmetry ratios of both hands (Dr-l). For this, the value of the 2D:4D ratio of the left hand to the right hand was subtracted (Rahman & Wilson, 2003).

Trait psychological variables

The empathy quotient (EQ) consists of 60 items that are distributed on a Likert scale from 0 to 2, 40 of which relate to empathy while the remaining 20 control-items did not count for obtaining the total score. The higher the score the greater the empathy (Baron-Cohen & Wheelwright, 2004).

The systematisation quotient (SQ-R) consists of 75 items distributed so that 55 relate to the systematic and 20 are con-trol items. Correction and interpretation of this survey is similar to those of EQ (Wheelwright et al., 2006).

Cognitive styles were calculated according to the formu-las: E2 = (SQ-R-55,6) / 150; F2 = (EQ-44,3) / 80; G2 = (E2-F2) / 2. According to the value, G2 is classified as 'Ex-treme S' (G2 = .21), 'S Type' (G2 = .04), 'B Type' (G2

= 0); 'E Type' (G2 = - .021) and 'Extreme E' (G2 = - .21) (Waka-bayashi et al., 2006).

The adult autism questionnaire (AQ adults) is composed of 50 items that quantify autistic traits. This self-administered questionnaire is often used to detect autistic features in adults such as Asperger syndrome or individuals with high functioning autism (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001).

The version of the autism questionnaire for adolescents (adolescent AQ) consists of 50 items that quantify autistic traits. The higher the score the greater the severity of the symptoms. This is an adapted version of the autism spectrum coeffi-cient for children and adolescents aged 9 to 16, although it is also valid for older people (Baron-Cohen, Hoekstra, Knickmeyer, & Wheelwright, 2006) and is designed to be completed by parents or carers.

Empathy was assessed from the Spanish version of 'interpersonal reactivity index' (IRI) (Mestre, Frías, & Samper, 2004) which consists of four subscales (perspective taking, empathic concern, fantasy, and personal distress) and is composed of 28 items which are scored on a Likert scale from 1 to 5.

Cooperativity was assessed with the subscale of coopera-tion of the revised Spanish version (Gutiérrez-Zotes et al., 2004), 'temperament and character inventory' (Cloninger, Svrakic, & Przybeck, 1993). It consists of 37 items rated on a Likert scale of 1 to 5 grouped into six subscales: social toler-ance, empathy, altruism, compassion integrity, and fellow-ship, and a final score obtained from the sum of the above.

Anger was assessed using the Spanish adaptation of 'anger expression inventory state-trait STAXI-II' (Miguel-Tobal, Casado, Cano-Vindel, & Spielberger, 2001). We assessed either trait anger or anger expression. The question-naire consists of 49 items distributed into six scales: two for trait anger (temperament and angry reaction), and four for the expression of anger (expression-in, expression-out, control-in and control-out). A general index of anger expression (AEI) was extracted from the last four scales.

Hormonal analyzes (C and T)

The basal levels of hormones were obtained from two samples of saliva. The C was collected using a Salivette (Sarstedt, Rommersdolf, Germany) and

T directly by salivation through a glass tube. The samples were frozen at -20°C until analysis by radioimmunoassay and LIA, respectively.

The reactives used for C were count-a-count Cortisol (DPC, Siemens Medical Solutions Diagnostics). The samples were measured in duplicate and all those belonging to the same participant were included in the same assay. The coef-ficient of variation inter-duplicate maximum considered for the repetition of the determination was set at 8%. Assay sen-sitivity was 0.5 ng/dl. The coefficients of intra-and inter-assay variation were 2.8 and 5.3% respectively. All values are expressed in nmol/L.

The reactives used for the T were the 'saliva testosterone ELISA Kit' testosterone (dbc-Diagnostics Biochem Canada Inc.). Chemiluminescence inmunoassay (LIA) was used for its determination. Assay sensitivity was 1 pg/ml. The coefficients of variation intra-and inter-assay were 3.98 and 7.98% respectively. All values are shown in pmol / l. Due to an insufficient volume of saliva from seven subjects, 37 experimental subjects (14 males and 23 females) and 41 control subjects (19 males and 22 females) were used in the data.

Data Analysis

Univariate ANOVAs were performed with between-subject factors 'group' and 'gender' for the 2D:4D ratio and the anthropometric variables (age and BMI), psychological (aggression, empathy and cooperativeness), and hormonal (T and C). For the analysis of the frequencies of the sociodemographic and cognitive styles, chi-square statistics were used it having been previously found that they are normally distributed, using the Kolmogorov-Smirnov statistic ($p < .001$).

The post-hoc analysis was performed using the Bonferroni correction. Linear regressions were used to study the association between the 2D:4D ratio and androgenisation variables and the severity of symptoms of the children.

Data analyses were carried out using SPSS 17.0 software (SPSS® Statistics). Statistical significance was accepted for p values $\leq .05$; whereas a tendency to significance was considered as p values $\leq .08$. Average values in the tables are expressed as mean$\pm$SD.

Results

Sample characterisation

Although there were no differences between groups in BMI, there was a significant effect of 'group', and the interaction 'group x gender' for age [F (1, 84) = 9.73, p <.05, F (1, 84) = 4.30, p <.05, respectively], so men in the control group were younger than other subjects (all p <.05). Socio-demographic groups differed in educational levels [χ^2 (3) = 9.56, p <.05], where most of the control subjects had university studies while the experimental group had secondary studies. However, the groups did not differ in the phase of the menstrual cycle (for women), medication, use of cigarettes, marital status, source of income, and total number of children living in the family home. Therefore, both age and educational levels were included as covariates in subsequent analyses. The values (M, SD) for anthropom-etric and sociodemographic variables as a function of group and gender of the participants are summarised in Table 1.

Table 1. Anthropometric and socio-demographic variables in terms of group interaction (parents of ASD or controls) and gender (men or women). * p < .05

		ASD		**CG**	
		Men (n=16)	**Women (n=27)**	**Men (n=19)**	**Women (n=23)**
Age (years)*		46.31±5.80	46.15±7.66	39.32±4.70	44.74±5.01
BMI (kg/m2)		28.79±4.77	26.44±5.13	27.96±2.72	25.06±4.92
Left 2D:4D ratio		0.97±0.04	0.97±0.03	0.97±0.04	0.97±0.03
Right 2D:4D ratio		0.97±0.04	0.98±0.03	0.98±0.03	0.98±0.03
Dr-l		0.002±0.02	0.008±0.03	0.007±0.02	0.007±0.02
Phases of the menstrual cycle	Luteal (1-14)		12 (44.4%)		6 (26.1%)
	Follicular (15-menstrual period)		8 (29.6%)		11 (47.8%)
	AMENORRHEA (>6months)		7 (26%)		6 (26.1%)
Marital status	Single	-	1 (3.7%)	1 (5.3%)	1 (4.3%)
	Married	16 (100%)	24 (88.9%)	18 (94.7%)	20 (87%)
	Divorced	-	2 (7.4%)	-	-
	Widowed	-	-	-	2 (8.7%)
	Others	-	-	-	-
Educational level	None	-	-	-	-
	Basics	3 (17.6%)	7 (25.9%)	-	2 (8.7%)
	Advanced	6 (35.3%)	4 (14.8%)	-	5 (21.7%)
	University	7 (41.2%)	15 (55.6%)	18 (94.7%)	16 (69.6%)
	Others	-	1 (3.7%)	1 (5.3%)	-
Source of income	Pension	-	1(3.7%)	-	1 (4.3%)
	Job	13 (76.5%)	22 (81.5%)	18 (94.7%)	17 (73.9%)
	Unemployment	1 (5.9%)	-	-	2 (8.7%)
	Others	2 (11.8%)	4 (14.8%)	1 (5.3%)	3 (13%)
Number of children	1	4 (23.5%)	6 (22.2%)	9 (47.4%)	5 (21.7%)
	2	11 (64.7%)	16 (59.3%)	8 (42.1%)	13 (56.5%)
	3	1 (5.9%)	3 (11.7%)	2 (10.5%)	4 (17.4%)
	4	-	1 (3.7%)	-	-
	5	-	-	-	1
	6 or more	-	1 (3.7%)	-	-

Differences between parents with and without children diagnosed with ASD

There were no significant effects for the 2D:4D ratio and D_{r-l} (Table 1). The empathy quotient (EQ) had a significant effect of factor 'group' [F (1, 84) = 3.87, p <.05], so that parents of people with ASD had lower EQ than the control subjects. For the systematisation quotient (SQ-R) there was a significant effect of 'gender' [F (1, 84) = 14.16, p <.001] showing a higher ratio of men than women. These results were also found in the case of adult autism quotient (AQ adults) [F (1, 84) = 4.24, p <.05] (Table 2).

Although the groups differed only in the ratio of empathy, we observed a differential distribution of the same, since in the experimental group subjects were similarly distributed between cognitive styles E (34.88%), B (41.86 %) and S (23.25%). However, in the control group most of the subects were concentrated in the type B (61.90%) (Table 2). Moreover, the gender factor was shown to have a significant effect on the distribution of the styles [χ^2 (2) = 8.07, p <.05], the main differences being in the types S and E, predominantly among men (37.14%) and type E among women (30%).

Table 2. Distribution of cognitive styles and psychological trait-variables in terms of group and gender. * p < .05 (t) p < .07

		ASD		CG	
		Men (n=16)	**Women (n=27)**	**Men (n=19)**	**Women (n=23)**
Cognitive style	Extreme S	-	-	-	-
	S type	7 (43.75%)	8 (29.63%)	6 (31.57%)	3 (13.04%)
	B type	8 (50%)	10 (37.03%)	12 (63.15%)	14 (60.87%)
	E type	1 (6.25%)	9 (33.33%)	1 (5.30%)	6 (26.09%)
	ExtremeE	-	-	-	-
EQ*		36.69±10.37	42.70±11.33	44.53±7.36	45.26±7.18
SQ-R*		67.69±21.35	49.37±19.75	65.05±21.04	54.78±15.30
AQ adults*		18.88±6.30	15.41±6.29	14.58±4.93	13.69±4.02
IRI	Perspective taking	24.93±4.17	25.11±4.96	24.11±3.38	25.91±4.10
	Personal distress	16.56±5.42	17.19±4.78	14.11±2.71	15.78±3.98
	Empathic concern (t)	26.93±3.79	28.07±3.99	23.32±4.11	28.00±4.49
	Fantasy*	21.81±5.99	20.89±6.95	18.37±3.70	21.39±5.24
STAXI-2	Anger expression out*	11.12±3.18	11.37±3.41	9.68±2.21	10.09±3.32
		12.94±4.48	11.74±3.17	11.26±2.66	11.13±4.26
	Anger expression in	17.63±4.68	15.63±4.99	16.68±3.38	17.49±3.88
	Anger control out*	12.31±4.63	12.25±3.80	15.26±4.44	16.08±4.18
	Anger control in*	30.13±10.13	31.22±10.02	22.00±8.41	23.69±9.84
	AEI*	2.38±2.68	2.96±3.06	1.89±2.05	1.83±1.85
	Temperament Reaction	7.00±3.77	6.70±2.93	5.95±2.91	6.43±3.26
TCI-R	Friendship*	27.06±4.63	27.93±3.74	25.11±4.94	31.22±2.35
	Altruism*	30.94±3.56	31.48±3.66	29.11±3.98	32.08±3.23
	Integrity*	29.88±4.99	31.56±3.23	28.84±4.51	31.78±4.81
	Empathy*	17.13±2.58	18.29±2.49	18.16±2.00	19.13±2.36
	Tolerance	30.50±4.19	31.74±3.57	30.74±3.16	32.39±3.70
	Total	27.10±3.17	28.20±2.08	26.39±2.31	29.32±1.91

With regard to empathy, there was a trend towards significance for interaction 'gender x group' on the fantasy scale [F (1, 84) = 3.31, p < .07] so that the male ASD group scored higher than the rest of the subjects. On the empathic concern scale either 'group' factor or 'group x gender' interaction showed a trend to statistical significance while the 'gender' factor had a statistically significant effect [F(1, 84) = 3.11, p < .08, F(1, 84) = 3.41, p < .07, F(1, 84) = 9.72, p < .005, respectively]. Parents of people with ASD showed more empathic concern than controls, and women generally scored higher on this scale than men. In addition, men in the control group received the lowest scores, the differences be-ing statistically significant compared to other subjects (all p < .05).

With regard to cooperation, there was a significant effect of the interaction 'group x gender' for the fellowship scale [F (1, 84) = 8.14, p < .05], so that women in the control group had higher scores than the other groups (p < .05). The factor 'gender' was significant for all subscales except for tolerance and the total questionnaire score [F (1, 84) = 14.50, p < .001, F (1, 84) = 3.75, p < .05, F (1, 84) = 5.45, p < .05, F (1, 84) = 4.81, p < .05, and F (1, 84) = 13.90, p < .001] for altruism, integrity, empathy and fellowship with higher scores in women.

Although there were no significant effects for trait aggression, the factor 'group' was statistically significant in the case of external expression, external control, internal control, and the rate of expression of anger [F (1, 84) = 6.07 , p<. 05, F (1, 84) = 4.41, p < .05, F (1, 84) = 11.79, p < .001, and F (1, 84) = 11.11, p < .001, respectively]. Parents of peo-ple with ASD had higher scores for expression-out and the rate of expression of anger; while control subjects scored higher on the control-out and the control-in of anger. In ad-dition, there was a significant effect of 'gender' for external control of anger [F (1, 84) = 5.13, p < .05] and men had higher scores than women (Table 2).

Although there were no differences between groups, the factor 'gender' showed a significant effect for T and C [F (1, 77) = 56.50, p < .001, F (1, 84) = 4.27, p < .05] with levels higher in both cases in men. The average values for both hormones according to the group and gender are presented in Table 3.

Table 3. Hormonal levels of Tsal and Csal in terms of group and gender.

	ASD		CG	
	Men (n=16)	**Women (n=27)**	**Men (n=19)**	**Women (n=23)**
T (pmol/l)	64.45±28.22 (n=14)	26.17±20.29 (*n*=23)	59.29±29.50 (*n*=19)	25.36±35.17 (*n*=22)
Csal (nmol/l)	2.06±1.95 (*n*=16)	1.59±0.91 (*n*=27)	2.47±2.20 (*n*=19)	1.55±1.06 (*n*=23)

Patterns of prediction of the 2D:4D ratio in terms of being a parent or not of offspring with ASD

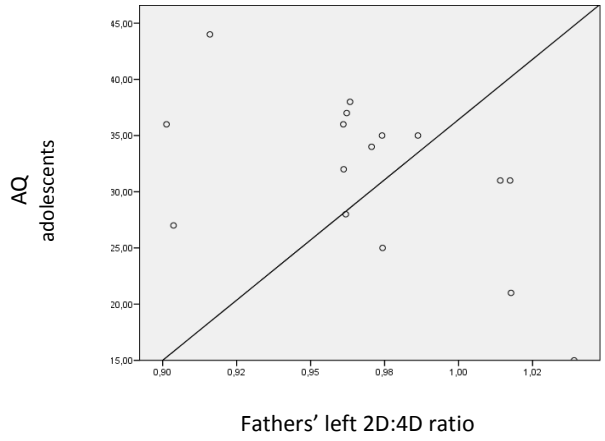
In the case of parents of offspring with ASD, the 2D:4D ratio of the left hand explained 12.4% and 10.3% of the total variability of the scores of the empathy questionnaire (EQ) and autism (AQ adults) [$\beta = 0.352$, $p < .05$, $\beta = -.321$, $p < .05$, respectively]. It also explained 11.8% and 12.9% of the level of integrity and TCI total score [$\beta = .344$, $p < .05$, $\beta = .359$, $p < .05$], respectively, and the 10.6% of basal C [$\beta = -.325$, $p < .05$]. This pattern of relationship was not significant in the control group. The ratio 2D:4D of the right hand explained 12.9% and 9.3% of the expression-out and the control-out of anger [$\beta = -.360$, $p < .05$ and $\beta = .305$, $p < .05$, respectively and 10.2% of the level of integrity of TCI [$\beta = .319$, $p < .05$]. By using the index as a predictor D_{r-l} asymmetry was not explained by any of the variables of androgenisation.

In controls, the left 2D:4D ratio predicted 9.2% of the IRI fantasy scale [$\beta = .303$, $p < .05$] while the right explained 12% and 13% of the scores on the perspective-taking and fantasy subscales in this questionnaire [$\beta = .346$, $p < .05$, $\beta = .361$, $p < .05$, respectively]. The asymmetry index D_{r-l} explained 9.2% of the cognitive styles [$\beta = 0.303$, $p < .05$] and 12% of the expression-out of anger [$\beta = .346$, $p < .05$].

The 2D:4D ratio as a predictor of the severity of ASD

As shown in Figure 1, the 2D:4D ratio of the left hand in men explained 32.6% of the variability of the values of AQ for their children [$\beta = -.571$, $p < .05$].

Figure 1. 2D:4D as predictor of the severity of the symptoms of their children



Discussion

Parents of offspring with ASD show similar values for the 2D:4D ratio, cognitive styles, and basal hormone levels as the control group. However, they are less empathic and have less control over the expression of anger. The 2D:4D ratio of this population predicts cognitive variables (EQ and AQ), behavioural (cooperativeness and expression of anger) and hormonal (C) of androgenisation. It is also a sensitive predictor of the severity of ASD for their children.

The values of the 2D:4D ratio for parents of individuals with ASD are similar to the control group, unlike the finding in a previous study conducted in this population (Manning et al., 2001). In general, this index is 0.25 SD higher in women than in men (Manning et al., 2000), but our study does not reveal these differences (as we had hypothesised). Although the 2D:4D ratio cannot be considered as a deterministic parameter of prenatal androgenisation, it is a useful indicator when analysing its relationship with other variables. In this regard, it has proven useful in explaining human behaviour and cognition (van-den-Bergh & Dewitte, 2006). This study has shown that the 2D:4D ratio of parents of ASD predicts a greater number of variables related to the androgenisation than the control group. It has been suggested that the right-hand 2D:4D ratio is more sensitive to the effects of prenatal testosterone, and so it has a greater ability to predict cognitive variables, while behavioural and physiological variables are somewhat androgenised (Höne-kopp & Watson, 2010). The 2D:4D ratio predicts more left than the right indicators on parents of offspring with ASD, and that includes empathy, the ratio of autism, cooperation and cortisol. No conclusive results affirm that the left-hand 2D:4D ratio is not sensitive to the effects of prenatal androgens. Thus, our results reinforce those found in other stud-ies

that show relationships or predictions associated with the left indicator (Bull & Benson, 2006).

The ratio of both hands was useful in predicting coop-eration variables, although the results are not consistent with those described in a previous study (Millet & Dewitte, 2006), since in our study a higher 2D:4D ratio is related in cooperative strategies. These differences may be partly due to the type of measurement used to evaluate cooperation, because in the work mentioned previously the social dilemma game 'repeated public good game' was used, whereas in our work a questionnaire evaluation was used. In addition, while the left-hand 2D:4D ratio predicted empathy and autism spectrum quotient in adults, the right predicted anger expression. A higher 2D:4D ratio meant more empathy and less autistic traits. Although the ratio does not predict trait anger, a lower value for this indicator is associated with the expression-out and the control-out of anger. These results reinforce those described in previous studies which associated a lower value of the 2D:4D ratio with increased physical aggression in men (Bailey & Hurd, 2005) and reactive aggression in women (Benderlioglu & Nelson, 2004).

It is proposed that the 2D:4D ratio could be used as an indirect indicator of a predisposition to certain diseases such as autism, heart disease, or certain types of cancer (Manning & Bundred, 2000). But the fact that the 2D:4D ratio for the male parent is a predictor of the severity of the symptoms of children suggests that greater prenatal exposure to androgens in men may make them more susceptible to developing autism and increase the likelihood that their offspring will develop ASD. In this way, the 2D:4D ratio is characterised by high heritability through the male line, which increases the possibility that genes related to the Y chromosome may influence its expression (Voracek & Dressler, 2009). However, further studies would be necessary to employ more variable androgenisation to replicate these results.

Cognitive styles in the entire sample was divided into types S, B, and E, whereas there are no subjects in the extreme types. However, the percentage of S-type is slightly higher in ASD parents than in controls. These results are consistent with expectations because they are not individuals with ASD but first-degree relatives. Although the parents of persons with ASD show a systematic quotient similar to the controls, their empathy quotient is lower. A previous study conducted in this population showed less activation of the amygdala and the fusiform gyrus in a test of empathy consisting of recognition of emotional expression in the eyes (Greimel et al., 2010).

However, the analysis of empathy through the IRI subscales in our data indicates that there is more imagination and empathic concern in parents of people with ASD than in control subjects. Although a priori these findings may seem contradictory, it should be noted that empathy is a multidimensional construct (Derntl et al., 2010), so the differences may be specific and refer to specific aspects.

Although there is no scientific proof that parents of people with ASD have higher levels of aggression, our results indicate that they do not show more aggressive traits but do show less control and greater expression of anger. According to the above, the same people may behave aggressively in some situations; but also express empathic behaviour. As a current theory holds, very empathetic people can also be very aggressive and vice versa, although they cannot manifest both behaviours at the same time, due in part to activating similar neuroanatomical structures (Moya-Albiol, Herrero, & Bernal, 2010). It is therefore fitting that people who have trouble controlling their anger, can also express concern for others.

Androgenisation has often been associated with socially undesirable behaviour such as violence, but it has also been claimed that a strategy to gain status characterised by high androgenisation is cooperation (Millet & Dewitte, 2006). Cooperation as it has been evaluated in our study did not differentiate between groups, but has been revealed that women, primarily those in the control group, showed higher scores.

When compared by gender, men were more systematic, show more autistic traits, and were less empathetic and cooperative, as described in previous studies (Moya-Albiol et al., 2010). Our data does not reveal differences between parents of offspring with ASD and control hormone levels, although as expected there are gender differences for T. There are also differences for C, and this result could be explained by differences in age, menstrual cycle phase in women, or that some subjects are postmenopausal (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). Another possibility is a differential pattern of cortisol for each gender linked to the situation of caring for people with ASD, something that would be worth examining in fu-ture studies.

The main limitation of this study is that it is cross-sectional, so that individual differences may overlap other effects to be evaluated in a single moment in time in the life of such persons. However, given that the personal

situation of the participants it is extremely difficult to conduct longitudinal studies.

In order to characterise the idiosyncrasies of the sample it would be interesting to extend the number of variables taken into account. This would mean making an analysis from a holistic perspective, in line with the work we have been doing in our laboratory (de Andrés-García et al., 2011, González-Bono, de Andrés-García, & Moya-Albiol, 2011, Serrano, Moya-Albiol, & Salvador, 2009). It would also be useful to analyse other samples of parents in which prenatal androgenisation had neuroprotective effects when developing the disorder in children, as in the case of eating disorders (Klump et al., 2006). Thus, the 2D:4D ratio is a good indica-tor to characterise the idiosyncrasies of the parents of people with varying degrees of androgenisation.

In conclusion, this study has shown that the 2D:4D ratio and asymmetry by themselves are not significant predictors of a genetic trait of autism in the clinical setting. However, they have considerable predictive value in explaining the behavioural and cognitive variables that characterise the par-ents of offspring with ASD, and serve as a bridge between idiosyncrasy and severity of ASD children. However, given the relatively low percentage of variance explained, an inte-grated approach should be used in which the 2D:4D ratio is another element in a continuous interaction with the various elements that describe the androgenisation. Furthermore, the subjects are not individuals with ASD, but direct ancestors which mean that the effects and differences in androgenisation parameters described throughout the study are subtle and require further analysis.

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Chapter 3

Study 2: Masculinization in Parents of Offspring with Autism Spectrum Disorders Could Be Involved in Comorbid ADHD Symptoms

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Introduction

A considerable percentage of children with autism spectrum disorder (ASD) also present ADHD-related symptoms (Sucksmith, Roth, & Hoekstra, 2011). Both disorders exhibit male-biased prevalence rates (Ames & White, 2010), and a plausible biological risk factor for both disorders may be elevated testosterone (T) levels. Indeed, it was reported that high intrauterine T levels may be partially involved in the development of both disorders (James, 2008). For ASD in particular, it was suggested that T may contribute to develop an "extreme male brain" (Auyeung, Taylor, Hackett, & Baron-Cohen, 2010). A study by De Bruin, Verhiej, Weigman, and Ferdinand (2006) showed that high prenatal T (defined as a smaller 2D:4D ratio) was more often present in children diagnosed with ADHD compared with those diagnosed with anxiety disorders. Also, animal research showed that early exposure to high T during the development is related to impairments in cognitive function (King, Barkley, Delville, & Ferris, 2000; Li & Huang, 2006).

A peripheral indicator of the exposure to prenatal T in the central nervous system (CNS) is the 2D:4D finger ratio the quotient between the lengths of the second and the fourth digits. Accordingly, the greater the exposure and sensitivity to prenatal T and corresponding reductions in estrogens, the greater the likelihood of developing a lower 2D:4D ratio (Breedlove, 2010). Interestingly, a smaller or masculinized 2D:4D ratio has been related to ASD and ADHD disorders (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001; Martel, 2009). Thus, it might be that families with high masculinization, and consequently low 2D:4D ratio, show greater likelihood toward developing autistic symptoms (Manning et al., 2001). Indeed, parents and siblings of people with ASD and ADHD usually show slightly autistic or ADHD traits, which may point to shared endophenotypes (Bernier, Gerdts, Munson, Dawson, & Estes, 2012; Trujillo-Orrego, 2011). A previous study revealed that 2D:4D ratio of ASD parents predicted the degree of autistic traits of their offspring (Romero-Martínez et al., 2013).

The current study examined the involvement of masculinization in the development of ADHD comorbid symptoms in offspring of a carefully selected sample of parents of people with ASD. Masculinization was operationalized by the 2D:4D finger ratio, as well as by salivary T levels in the parents, while inattention was measured with behavior questionnaires. We propose a theoretic model that includes the mediation between all these variables. A masculinized 2D:4D ratio has been related to high current T

levels in a clinical population (infertile men; Manning, Scutt, Wilson, & Lewis-Jones, 1998). Also, high sensitivity to current T levels has been associated with social cognition (van-Honk et al., 2011) and ADHD symptoms (De Bruin et al., 2006). Thus, we examined whether salivary T levels in ASD parents might mediate the relationship between a masculinized 2D:4D ratio and inattentive symptoms of ASD parents. As ADHD is highly heritable (Polderman et al., 2007), we also analyzed whether inattentive symptoms of ASD parents might mediate the relationship between the 2D:4D ratio of ASD parents and ADHD symptoms in their offspring. We hypothesized that T of ASD parents would mediate the relationships between a masculinized 2D:4D ratio of ASD parents and ADHD symptoms of themselves and their offspring, respectively. Hence, high current T levels will be related with a masculinized 2D:4D ratio and more inattentive symptoms in ASD parents. And finally, high inattentive symptom scores in ASD parents will be related to a masculinized 2D:4D ratio and increased ADHD symptoms in their offspring. Moreover, the relationship between masculinization and ADHD symptoms will be stronger with inattentive than hyperactivity symptoms as revealed by previous research (McFadden, Westhafer, Pasanen, Carlson, & Tucker, 2005).

Method

Participants

The sample consisted of 32 participants, all fathers (n = 13) and mothers (n = 19) of offspring with ASD, aged between 33 and 63 years (45.00 ± 5.9). They participated voluntarily in the study and signed an informed consent in accordance with ethical standards for human research. Anthropometrical and demographical variables of ASD parents are summarized in Table 1. The ages of the offspring with ASD ranged from 5 to 30 years (13.60 ± 5.46), with a gender distribution of 28 men and 4 women, in accordance with the expected gender distribution in this disorder. The inclusion criteria for participating in the study were as follows: being a first-degree family of an ASD patient with a clinical diagnosis, living at home with the patient, and being the main provider of first-needs for at least 2 years before the study.

Procedure

Participants visited the laboratories of the psychology faculty at the University of Valencia, without having food or drinks (except water, brushing

teeth, or chewing gum) during the 2 hr period before arrival at the laboratory. The experimental sessions lasted approximately an hour and were carried out between 4:00 and 7:00 p.m. After arrival in the laboratory, participants were taken to a room with a constant temperature (22°C±1°C) where weight, height, 2D:4D ratio, and demographic variables were recorded. Two samples of saliva were collected for determining T; the first sample was taken at the end of the collection of anthropometric data and the second sample was taken 20 min after the first. Participants then completed three questionnaires: one self-report and two assessing hyperactivity and/or inattention symptoms in their offspring.

2D:4D Ratio

To calculate the 2D:4D ratio, three separate measurements of the length of the index finger (2D) and the ring finger (4D) of the right hand were made (Martel, 2009). Measurements were made on the ventral side of the hand. The length of the finger was taken from the proximal fold at the base of the finger to the tip thereof. For this purpose, we used digital calipers with an accuracy of 0.01 mm. Two of the three measurements were made directly by two investigators, while the third was performed using a scanner for subsequent measurement. The value of the 2D:4D ratio was obtained by calculating the average of three measurements, as has been done in a previous study (Schneider, Pickel, & Stalla, 2006). The interobserver reliability was .98, and the reliability between the average of direct and scanned measure was .99.

Inattention Measures for ASD Parents

Attention Switching Subscale of the Autism Spectrum Quotient (AQ) for Adults. For this study, the "Attention Switching" subscale of the AQ for adults (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001) was used as a measure of difficulty in attention switching or deficient inhibitory processing (Polderman et al., 2012). We will refer to this measure as AQ-Switch of Parents. It consists of 10 items that are rated on a 4-point scale with *definitely agree, slightly agree, slightly disagree*, and *definitely disagree*. Ratings were recoded as 1 if the person reports inattentive symptoms and as 0 if they do not show any symptom. So scores could range from 0 to 10, obtained by adding the frequency of inattentive symptoms, with a high score corresponding to more symptoms.

ADHD and Autistic Symptoms Questionnaires for Offspring

The SNAP-IV "Teacher and Parent Rating Scale" is an adapted Spanish revision of the original questionnaire (Swanson, Sandman, Deutsch, & Baren, 1983). Parents completed this questionnaire. Items from the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM-IV*; American Psychiatric Association, 1994) for ADHD are used to measure symptoms of inattention (Items 1-9) and symptoms of hyperactivity/impulsivity (Items 10-18). There are four response options: "not at all" = 0, "just a little" = 1, "often" = 2, and "very often" = 3. Subscale scores (inattention and hyperactivity) on the SNAP-IV are calculated by summing the scores of a subscale and dividing this sum by the number of items in the subscale. We will refer to these measures as SNAP-IV Inattention of Offspring and SNAP-IV Hyperactivity of Offspring.

We used the Attention Switching subscale of the AQ for adolescents as a measure of difficulty in attention switching or deficient inhibitory processing in offspring. This is an adapted version of the AQ for children and adolescents aged 9 to 16, although it is also valid for older people (Baron-Cohen, Hoekstra, Knickmeyer, & Wheelwright, 2006) and is designed to be completed by parents or caretakers. The interpretation is the same as in the Attention Switching subscale for adults. We will refer to this measure as AQ-Switch of Offspring.

T Analyses

Basal levels of T were obtained from two samples of saliva, directly collected by salivation through a glass tube. However, as far as spurious alterations in salivary T levels have been reported using the salivette method of collection (Granger, Schwartz, Booth, & Arentz, 1999), direct and spontaneous salivation was preferred for salivary collection in the present study. The samples were frozen at −20°C until analysis by enzyme-linked immunosorbent assay (ELISA). The reactive used for the T was the "saliva testosterone ELISA Kit" (Diagnostics Biochem Canada Inc.). Assay sensitivity was 1 pg/ml. The coefficients of variation intra- and interassay were 3.98% and 7.98%, respectively. All values are shown in pmol/L Cronbach's alpha for the two T measures was .75.

Data Analysis

It was tested whether data were normally distributed (using the Kolmogorov–Smirnov statistic). To analyze gender differences for anthropometric (age and body mass index [BMI]), psychological (AQ adults), and hormonal (T) variables, *t* tests were performed. Cohen's *d* for independent groups was calculated to estimate the effect size of the different measures (Cohen, 1988). For the analysis of the frequencies of the demographics, chi-square statistics were used. All posterior analyses were conducted with and without including gender as a covariate.

We used linear regression models to investigate whether the 2D:4D ratio of the right hand predicted inattention (AQ adults) and circulating T of ASD parents. Subsequently, it was tested whether the 2D:4D ratio of ASD parents predicted ADHD symptoms (SNAP-IV and AQ adolescents) of their offspring. As recommend by Preacher, Rucker, and Hayes (2007), we confirmed the association between the mediation variable (T) and the independent variable (2D:4D ratio of ASD parents), and inattentive symptoms in ASD parents (dependent variable). Second, we investigated the relationship between the mediation variable (AQ-Switch of Parents) and the independent variable (2D:4D ratio of ASD parents), and ADHD symptoms in their offspring (AQ-Switch of Offspring and SNAP-IV measures of Offspring).

Data analyses were carried out using SPSS 17.0 software (SPSS Statistics). Statistical significance was accepted for p values $< .05$.

Results

Descriptive Characteristics

Values ($M \pm SD$) for T levels, and the anthropometric and demographic variables for males and females are summarized in Table 1. There were no gender differences in BMI, age and demographic variables, and AQ adult scores. However, as expected, men had higher T and smaller right 2D:4D ratio than women, $t(21.93) = 5.79$, $p < .01$; $t(30) = -2.47$, $p < .05$, respectively, with high effect sizes ($d = 2.12$; $d = .90$, respectively).

Table 1. Anthropological and Sociodemographical Variables of ASD Parents.

		ASD parents	
		Men (n=13)	**Women (n=19)**
Age (years)		44.85±4.47	45.16±6.96
BMI (Kg/m2)		29.43±5.75	25.99±4.82
Right 2D:4D ratio		0.96±0.04	0.99±0.03
T levels (pmol/L)		65.21±22.00	22.87±17.53
Phases of the menstrual cycle	Luteal (1-14)	-	7 (36.8%)
	Follicular (15-menstrual period)	-	9 (47.4%)
	Amenorrhea (>6months)	-	3 (15.8%)
Marital status	Single	-	-
	Married	13 (100%)	17 (89.5%)
	Divorced	-	2 (10.5%)
	Widowed	-	-
	Others	-	-
Educational level	None	-	-
	Basics	3 (23.0%)	4 (21.1%)
	Advanced	4 (30.8%)	2 (10.5%)
	University	6 (46.2%)	13 (68.4%)
	Others	-	-
Source of income	Job	1 (7.7%)	
	Unemployment	11 (84.6%)	1 (5.3%)
	Others	1 (7.7%)	16 (84.2%)
			2 (10.5%)
Number of children	1	8 (30.8%)	4 (21.1%)
	2	4 (61.5%)	12 (63.2%)
	2	1 (7.7%)	3 (15.8%)
	3	-	-
	4 or more		

Descriptives of SNAP-IV Offspring measures and AQ-Switch measures of parents and offspring are presented in Table 2.

Table 2. *M*±*SD* for the SNAP-IV Measures of Offspring, AQ-Switch of Offspring, and AQ-Switch of Parents.

	M±SD	**Skewness**	**Kurtosis**
Offspring (n=32)			
Inattention (SNAP-IV)	0.34±0.29	0.17	-0.46
Hyperactivity (SNAP-IV)	0.37±0.28	0.55	-0.39
AQ-switch of Offspring	7.21±2.08	0.59	0.50
ASD parents (n=32)			
AQ-switch of Parents	4.56±2.23	0.47	0.50

Masculinized 2D:4D Ratio of ASD Parents as a Predictor of ADHD Symptoms in Parents and in Their Offspring

Question 1: Is a more masculinized right 2D:4D of ASD parents associated with (a) more inattentive symptoms and (b) high T levels in ASD parents?

The 2D:4D ratio of the right hand of ASD parents predicted 18.3% of AQ-Switch of Parents ($\beta = -.457$, $p < .01$) and 14.3% of the basal T levels ($\beta = -.413$, $p < .05$).

Question 2: Is a more masculinized right 2D:4D of ASD parents associated with (a) more ADHD symptoms in their offspring?

The 2D:4D ratio of the right hand of ASD parents predicted 8.9% of SNAP-IV Inattention of Offspring ($\beta = -.345$, $p < .05$) and 7.2% of AQ-Switch of Offspring ($\beta = -.320$, $p < .05$). The 2D:4D ratio of ASD parents did not predict SNAP-IV Hyperactivity of Offspring ($\beta = -.229$, $p > .05$).

Question 3: Are high current T levels of ASD parents associated with more inattentive symptoms in ASD parents?

The current T levels of ASD parents predicted 6.5% of the AQ-Switch scores of parents ($\beta = .308$, $p < .05$).

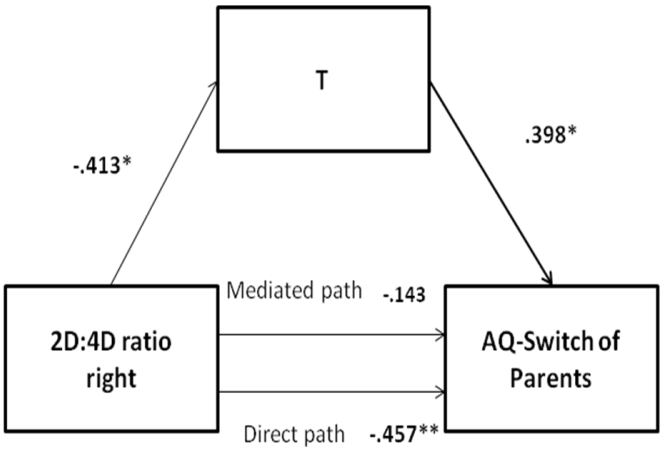
Question 4: Are high inattentive symptoms of ASD parents associated with more ADHD symptoms in their offspring? The AQ-Switch of Parents predicted 32.7% of the AQ-Switch of Offspring ($\beta = .592$, $p < .01$). However, AQ-Switch of Parents did not predict SNAP-IV Inattention of Offspring ($\beta = .155$, $p > .05$) or SNAP-IV Hyperactivity of Offspring ($\beta = .125$, $p > .05$).

Mediation Models

Model 1: Does high T mediate the association between more masculinized right 2D:4D and inattentive symptoms in ASD parents?

Current T levels emerged as a possible mediator of both 2D:4D ratio and AQ-Switch of Parents. This was tested with a mediation model in which the 2D:4D ratio and T were both entered as predictors of AQ-Switch of Parents. The results showed that 2D:4D ratio no longer significantly predicted AQ-Switch of Parents ($\beta = -.143$, $p > .05$), but T remained a significant predictor ($\beta = .398$, $R2$ for full model = .32, $p < .05$). Thus, high T mediated the relationship between 2D:4D ratio and AQ-Switch of Parents (Figure 1).

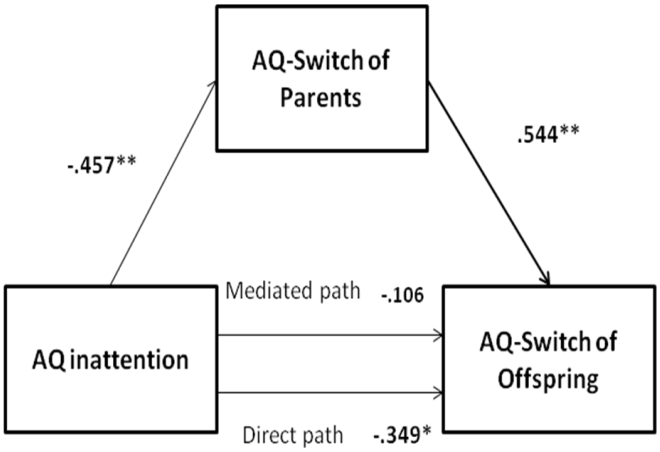
Figure 1. T mediates the relationship between masculinized 2D:4D ratio and AQ-Switch of ASD parents. ** p < .01 * p < .05



Model 2: Does AQ inattention in ASD parents mediate the association between more masculinized right 2D:4D of ASD parents and ADHD symptoms of their offspring?

When the 2D:4D ratio and AQ-Switch of Parents were both entered as predictors, 2D:4D ratio no longer significantly predicted AQ-Switch of Offspring ($\beta = -.106$, $p > .05$). However, AQ-Switch of Parents remained a significant predictor ($\beta = .544$, R^2 for the full model = .33, $p < .01$). Thus, high AQ inattention symptoms in ASD parents mediated the relationship between 2D:4D ratio and AQ-Switch of Offspring (Figure 2).

Figure 2. AQ-Switch of parents mediates the relationship between masculinized 2D:4D ratio and AQ-Switch of their offspring. ** p < .01 * p < .05



When gender of ASD parents and their offspring were entered as a covariate, the same results were obtained.

Discussion

This study examined in a carefully selected clinical sample the effects of direct and indirect T levels on attention deficits in parents of ASD-affected children. Furthermore, indirect effects of T levels (via the parents) on attention deficits and hyperactivity in their offspring were investigated. Our results revealed that a masculinized 2D:4D ratio of the right hand of ASD parents is associated with inattention symptoms in ASD parents, and with inattentive symptoms but not hyperactive symptoms in their offspring. However, in ASD parents, current T levels mediated the association between a masculinized 2D:4D ratio and AQ inattention symptoms, while inattention symptoms in ASD parents mediated the association between the 2D:4D ratio of ASD parents and inattentive symptoms in offspring. Thus, our results confirmed that masculinization is related to ADHD symptoms (De Bruin et al., 2006) and that the involvement is stronger in inattentive ADHD symptoms than hyperactivity symptoms (McFadden et al., 2005). However, a population-based study of dizygotic female twins reported opposite findings (Attermann et al., 2012). That study examined the hypothesis that sex of the co-twin influences the level of fetal exposure to T, and may therefore relate to ADHD symptoms (evaluated by means of the Attention Problem scale of the Child Behavior Checklist, a questionnaire complemented by parents). They found that girls with a twin brother (i.e., high prenatal T levels) had lower ADHD trait scores than those with a twin sister (i.e., low prenatal T levels). However, in this study, ADHD traits in clinically diagnosed ADHD girls were examined, whereas our study explored cognitive and hormonal characteristics of parents of offspring (both girls and boys) with ASD.

A masculinized 2D:4D ratio may point to the influence of prenatal T exposure (Manning, Bundred, Newton, & Flanagan, 2003). Given the presence of slightly (nonclinical) autistic traits in parents of ASD-affected children (Sucksmith et al., 2011), and our current findings of attention deficits in the parents, our results may indicate a shared genetic liability, associated with (prenatal) T levels to develop autistic disorders and comorbid ADHD symptoms.

A limitation of our study is the cross-sectional and nonexperimental design with which causality could not be addressed. Moreover, "AQ-Switch" was used as a measure of inattentive ADHD symptoms, while it is developed as an ASD measure. In addition, our relatively small sample had limited statistical power; future work might replicate these findings in a larger sample.

Another limitation is that the masculinization of parents may have influenced their selfreports and the way they reported on their children. Multiple informants would have optimized our study design. Moreover, as masculinization may affect parenting style, descendants of people with ASD could also partially learn this cognitive style from their progenitors, which, in turn, may reinforce the development of those disorders.

The present study focused on biological factors related to comorbid ASD and ADHD symptoms, but further studies should also consider other educative and social aspects such as parenting styles. Future research could also include additional variables such as neuropsychological tests related to multiple domains of executive functions, or other sex hormones such as estrogens. Our data are relevant and novel as no laboratory studies have analyzed masculinization in ASD parents and its involvement in their offspring disorders. Further analyses are required to determine its involvement and its relationship with other important parameters for ASD and ADHD.

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Chapter 4

Study 3: High Testosterone Levels and Sensitivity to Acute Stress in Perpetrators of Domestic Violence with Low Cognitive Flexibility and Impairments in Their Emotional Decoding Process: A Preliminary Study

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Introduction

Between 15% and 71% of women from different populations have been victims of physical or sexual violence at some point in their lives (WHO, 2011). Therefore, it is necessary to analyze the aggressors to help eradicate this kind of violence while also developing prevention mechanisms. Many researchers have studied aggressors and most used a psychological approach, with little interest in the possible influence of biological variables (Pinto et al., 2010). Due to the limited results obtained in psychotherapeutic programs developed for these individuals, it is worthwhile exploring a different approach based on biopsychosocial models (Babcock, Green, & Robie, 2004) that include psychobiological variables such as hormonal and neuropsychological parameters. This approach enables the wider acknowledgment of intimate partner violence as a complex phenomenon.

One of the most analyzed hormones in violent males is testosterone (T) (Archer, 2006) as an indicator of the activity of the hypothalamic‑pituitary-gonadal axis (HPG). Some violent males, including rapists, alcoholic aggressors, and antisocial personality disorder (ASD) criminals, show higher basal T levels: but the results are not unanimous (Moya-Albiol, 2010). The studies mentioned used the basal T levels as a feature factor, due to their high temporal stability (Liening, Stanton, Saini, & Schultheiss, 2010). Nevertheless, in the same way as with every hormone, there are fluctuations in levels related to diverse causes, such as behavior or the environment and oscillations may be related to mood swings (Liening & Josephs, 2010). Additionally, modifications in T levels may produce mood changes. In this sense, T replacement treatments increase anger, hostility, and/or irritability (van Honk & Schutter, 2007). The mechanism behind the relationship between T and violent behavior is not totally determined. Nevertheless, modulatory functions for this hormone have been conferred in interaction with alcohol or drug use (Soler, Vinayak, & Quadagno, 2000). These include empathy, neuropsychological skills (Pinto et al., 2010), experience, environmental interaction, and/or cortisol (C) levels (Moya-Albiol, 2010). Indeed, Cmay work as amodulator of the effect of T on behavior (Terburg, Morgan, & van-Honk, 2009) because T is closely linked to aggressive behavior only in those participants with low C (Popma et al., 2007).

The interaction between hormonal parameters is established as part of a complex system in which neurocognitive factors must be considered. There

may be some deficits whose interaction with hormonal parameters such as T may either facilitate or inhibit violent behavior. When comparing IPV perpetrators with nonviolent males, the IPV perpetrator group was more likely to misidentify signals given by their partners and react in a hostile and/or violent manner (Babcock, Green, & Webb, 2008). These detection errors were restricted to neutral and dislike facial expressions that were usually interpreted as hostile signals. Errors decoding facial expressions (which constitute an initial stage of social information processing) may produce deficits in the "theory of mind" (ToM). For this reason, hostile biases in the decoding process, especially in partners, may make it difficult to understand a partner's perspective or feelings (Babcock et al., 2008) and may lead to social inadequacy and even cause the adoption of inappropriate behavior that predisposes violent action (Tirapu-Ustárroz, Pérez-Sayes, Erekatxo-Bilbao, & Pelegrín-Valero, 2007). The ToM is similar to the concept of "cognitive empathy," although empathy is a multidimensional construct which also has an affective component that can be analyzed with two scales of the interpersonal reactivity index (IRI). One of these scales assesses feelings of sympathy for the misfortune of others (empathy or concern), and the other scale measures interior feelings of unease and discomfort in reaction to the emotions of others (personal distress).

Additionally, using the IRI, four types of aggressors were obtained, reflecting reductions in cognitive and/or affective empathy capacities (Covell, Huss, & Langhinrichsen-Rohling, 2007). As shown in several neuropsychiatric disorders such as alexithymia, schizophrenia, and Asperger syndrome, deficits in cognitive empathy and/or ToM may be related to greater difficulties in dealing with emotional and negative interpersonal situations. For this reason, IPV perpetrators may experience higher levels of personal distress because they may misunderstand how they are evaluated by others (Moriguchi et al., 2007; Rogers, Dziobek, Hassenstab, Wolf, & Convit, 2007; Smith et al., 2012).

Psychopathic traits are positively related to perspectivetaking or ToM; and inversely related to affective empathy (Mullins-Nelson, Salekin, & Leistico, 2006). T may have activator effects in the cognitive - affective system because high levels hinder emotional facial recognition (van Honk & Schutter, 2007). So when added to an emotional recognition deficit, increases in T may notably reduce the capacity to detect and/or misinterpret affective stimuli.

Diverse neuropsychological functions also show certain diminished levels in males charged with domestic violence. The affection lies in the executive functions, attention, and verbal abilities (Teichner, Golden, Van Hasselt, & Peterson, 2001). As far as we are aware, the relationship between these deficits and the T levels of men charged with domestic violence has not been analyzed. These deficits seem to be related with déficits observed in patients with prefrontal cortex (PFC) damage revealing in many cases a high level of irritability and/or physical aggressiveness (Farmer & Aman, 2011). However, there is not a solid indication that enables a statement to be made about cerebral damage in aggressors in terms of PFC.

High levels of prenatal androgens may be associated with an excessive masculinization of the central nervous system (CNS). Masculinization has a relatively stable anthropometric indicator throughout lifewhich is the ratio between the length of the index finger and the ring finger, or the 2D:4D ratio (Manning & Bundred, 2000). Smaller ratios imply a higher prenatal masculinization (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001) that has been linked to male physical aggression (Bailey & Hurd, 2005).

As far as we know, no studies have analyzed the hormonal response to a laboratory stressor in adult males charged with domestic violence. In other studies, a conflictive marital discussion was employed as a stressor, but only psychophysiological variables were analyzed (Pinto et al., 2010). Stress response might be elicited in a laboratory through various tools, such as the trier social stress test (TSST), which simulates a highly stressful evaluation scenario (Kirschbaum, Pirke, & Hellhammer, 1993). Studies about the affect under endocrine parameters focused on non-aggressive males in whom C levels increase while T levels decrease and mood worsens (Wolf, 2011).

The main aim of this paper is to confirm whether men charged with domestic violence show specific features that differentiate them from the general population, using several hormonal, cognitive-affective, and neuropsychological parameters that stress the T response. For this purpose, we assume an integrated psychobiological view that is in consonance with the works we are carrying out in our laboratory (De Andrés-García et al., 2011; González-Bono, De Andrés-García, & Moya-Albiol, 2011; Moya-Albiol et al., in press). We analyzed the relationship between hormonal and psychological responses to the laboratory stressor using psychological and neuropsychological variables focusing on T and its possible relationship with

psychological response and the feature variables. We hypothesized that IPV perpetrators would show higher basal T levels (Soler et al., 2000) and lower C levels (Moya-Albiol, 2010) than controls. IPV perpetrators would also present cognitive dysfunctions such as poor cognitive flexibility (Teichner et al., 2001) and poor recognition of emotions or thoughts (Holtzworth-Munroe & Smutzler, 1996), especially for neutral expressions (Babcock et al., 2008). Finally, we hypothesized that IPV perpetrators should report lower affective empathy (Covell et al., 2007) and a masculinized or smaller 2D:4D ratio (Bailey & Hurd, 2005). The 2D:4D ratio could explain the high levels of sensitivity to T effects (van Honk et al., 2011) and may predict T changes, especially in IPV perpetrators. As supported in a previous study, heightened feelings of anger are related to high T levels (Herrero, Gadea, Rodríguez-Alarcón, Espert, & Salvador, 2010). For this reason, we expect that in both groups anger feelings will be related with high T levels although this relationship could be stronger in IPV perpetrators than controls due to their violent past.

Additionally, for the reversal relationship between T and C and its effects on the neuropsychological and empathic performance, we hypothesized that the neuropsychological performance (Wisconsin card sorting test [WCST]) would be positively related with T levels (Muller, Aleman, de Haan, & van der Schouw, 2005) and negatively related to C levels (Egeland et al., 2005). Moreover, emotional recognition (reading the mind in the eyes) will be negatively related to T levels (van Honk & Schutter, 2007) and positively related to C levels (Smeets, Dziobeck, &Wolf, 2009). The analysis of these variables and their relationships may offer a wider explanation of the complex phenomenon of domestic violence, and may delimit differences between categories of IPV perpetrators.

Method

Participants

The final sample was composed of 40 healthy men (19 IPV perpetrators and 21 controls) who participated voluntarily in the study. IPV perpetrators were recruited from the community and the psychoeducational treatment program CONTEXTO at the University of Valencia in Spain where they were serving as mandatory participants in male abuser programs at the Department

of Social Psychology of the University of Valencia (Lila, Oliver, Galiana, & Gracia, 2013). The selection criteria and assessment of candidates have been described in detail previously (Romero-Martínez, González-Bono, Lila, & Moya-Albiol, 2013). The average age of the participants was 37.55±1.67 years old; and body mass index (BMI) 27.37±0.55 kg/m2. The inclusion criteria for controls included: having no organic or mental illnesses, and having similar anthropometrical and demographic characteristics to the IPV perpetrators; as well as not having perpetrated severe violence (defined as assaulting a partner or other individual outside the home, or engaging in any severely violent act). Control individuals were required to provide criminal record certificates to check that they had no history of violence.

Further, all participants were interviewed by trained researchers (with extensive experience treating IPV perpetrators) to assess their mental health. Cohen's kappa, used to assess inter-rater agreement between qualitative interviewers in the nine psychopathological dimensions evaluated (the same dimensions as the SCL-90-R), ranged from .67 to .84. Regardless of the objective SCL-90-R results, subjects were considered not to have any psychopathological signs and symptoms if they scored less than the mean for their age for each dimension. Candidates were eligible to participate if the qualitative interviews and SCL-90-R scores confirmed they were free of mental illness; three IPV perpetrators and four controls were excluded because their results suggested psychological disorders. All participants were right-handed and healthy, lived in Valencia (Spain), and gave written informed consent. The experiment was performed in accordance with the Helsinki Declaration and approved by the University of Valencia Ethics Committee.

Procedure

Each subject participated in three sessions that were carried out at the psychobiology laboratories at the University of Valencia. In the first session participants were interviewed in order to identify (and subsequently reject) participants who suffered from organic or psychological diseases. The second session took place between 4 pm and 7 pm in order to control diurnal variations of C secretion (Dickmeis, 2009). Participants were instructed to abstain from eating or brushing their teeth, as well as caffeine, alcohol, or exercise, and any drug 2 hr before arriving at the laboratory. All participants were informed about the fact that they would provide saliva for hormonal analyses, and that they would be asked to perform several behavioral tasks.

After arriving at the laboratory participants were conducted to a room where they signed an informed consent to participate in the study, and anthropometrical (height, weight, and 2D:4D digit ratio) and substance consumption variables (tobacco, drug, alcohol abuse, and medication) were registered. Before stress exposure started, two saliva samples for evaluating C and T levels and three questionnaires for measuring psychological states (STAI-T, STAXI, and POMS) were completed.

Participants were then conducted to another noiseinsulated room with a constant temperature 22±1°C where they carried out an adapted version of the TSST (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). The TSST is a standardized psychosocial laboratory stressor that consists of preparing a speech about a specific argument during a brief preparation period followed by a test period in which participants deliver a specific argument and then perform mental arithmetic tasks. In these cases, all participants had to express their opinion about IPV during 2.5 min and interviewers asked a set of related questions during the final minutes of the participant's presentation.

Participants then performed an arithmetic task consisting of subtracting numbers in a constant series, or calculating simple arithmetical operations such as additions, subtractions, multiplications, and divisions. To increase the evaluative threat perception, four evaluators of both genders carried out the TSST and a video camera was connected during the test (false registration). The whole procedure included the following periods: baseline, preparation, anticipatory, task and post-task; and all the periods lasted 15 min. Between public speaking task instructions and the preparation period, two saliva samples were taken. Furthermore, when the stressor finished, four saliva samples were collected (+0, +15, +30, and +45 min). A second psychological state measurement was obtained after the psychosocial stressor, and researchers evaluated the performance appraisal, perceived stress, and internal and external attribution. The third laboratory session took place 1 day after the previous session, and between 10 am and 2 pm to avoid a fatigue effect due to the working day. In this session, two neuropsychological tests were administrated, the WCST and the reading the mind in the eyes (eyes test).

Finally, participants completed a battery of questionnaires for evaluating psychological trait profiles. Data of C for two IPV perpetrators was not determined due to insufficient saliva volume (not enough salivation).

Moreover, three participants reported personal problems (one IPV and two controls) and could not complete the eyes test and the WCST.

Appraisal and State Self-Reports

After the TSST, four questions were assessed to evaluate task appraisal using a list of four items based on previous studies (Baggett, Saab, & Carver, 1996; Carrillo et al., 2001; Moya-Albiol et al., in press). The first question refers to satisfaction with performance in the test. The second concerns self-appraised performance, and the third evaluated internal (e.g., personal effort and physical and technical abilities) and external (e.g., luck) attribution of the outcome. The final question measured the degree of stress perceived during the TSST. All of these questions were answered using a 10-point Likert type scale.

State anxiety was assessed using the "State-Trait Anxiety Inventory" (STAI-S) (Spielberger, 1999) suitably adapted, which contains 20 items, ranked on a 4-point Likert scale. The reliability coefficient was 0.62. A Spanish version of the "State-Trait Anger Expression Inventory-2" (STAXI-2) (Miguel-Tobal, Casado, Cano-Vindel, & Spielberger, 2001) was employed for measuring state anger. It contained 15 items ranked on a 4-point Likert scale and distributed into three subscales: feelings, verbal, and physical expression. To reduce the number of tests, increase power for effect size, and aid interpretation within a conceptual framework, state anger subscales were combined into the single variable (S-Ang). Cronbach's alpha ranged from .67 to .89.

Mood states were measured using the abbreviated version of the profile of mood states (POMS) suitably validated (Fuentes, Balaguer, Meliá, & García-Merita, 1995). This questionnaire is composed of 29 Likert-point items grouped into five subscales (tension, depression, anger, vigor, and fatigue) with a Cronbach's alpha higher than 0.80. All the scales apart from vigor stated negative mood. A total score was calculated by adding all the negative scales and subtracting vigor (POMS-t).

Psychological Trait Profiles

The interpersonal reactivity index (IRI) assesses four aspects of empathic response (Davis, 1983). We used the Spanish adaptation (Mestre, Frías, & Samper, 2004), which includes four subscales (perspective taking, fantasy,

empathic concern, and personal distress) ranked in a 5-point Likert scale with reliability coefficients ranging from .56 to .70.

Anger and its expression were measured by an adapted version (Miguel-Tobal et al., 2001) of the State-Trait Anger Expression Inventory-2 (STAXI-2) (Spielberger, 1999). This test is distributed into six subscales: two for evaluating trait anger (temperament and reaction) and four for anger expression (anger expression out, anger expression in, anger control out, and anger control in). To reduce the number of tests, increase power for effect size, and aid interpretation within a conceptual framework, trait anger subscales were combined into a single variable (T-Ang). Moreover, a general anger expression index (AEI) is calculated by adding the scores of the two expression subscales and subtracting the scores of the two control scales, and finally adding 36 units to avoid negative scores. The Cronbach's alpha ranged from .67 to .89.

Neuropsychological Measures

The revised version of reading the mind in the eyes (eyes test) was administered. This task is considered an advanced theory of mind test that contains 36 black and white photographs of the eye region of the face of different actors and actresses. Subjects must attribute the mental state of the actors. Participants were instructed to choose which of four words best described what the person in the photo was thinking or feeling. Scores are calculated as the total number of correct choices for all 36 photographs (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001).

The revised version of the WCST (Heaton, 1993) was used to measure cognitive flexibility. Cards must be sorted until six categories are matched or until all 128 cards are sorted. Cards are matched according to different criteria such as color, form, and number. After 10 consecutive correct cards are sorted, a new criterion is instituted without warning.

2D:4D Digit Ratio

The 2D:4D ratio was obtained by taking photocopies of the palms and fingers of both hands and measuring the length of the second to the fourth digit from the most proximal wrinkle in each finger to the tip using a calliper for this purpose. The ratio was calculated by dividing the length of the second by the fourth digit (Manning et al., 2001).

Hormonal Determination

Saliva was directly collected from the mouth to a glass tube for T determination using a salivette (Sarstedt, Rommersdolf, Germany) for C. All saliva samples were collected in the same order: firstly C, and then T immediately afterwards. Participants were informed about the necessity of following the instructions for saliva sampling in order to obtain valuable data. The samples were frozen at □ 20°C until analyzed by enzimoimmunoassay in the case of T, and radioimmunoassay (RIA) in the case of C.

The salivary T (T) appropriate reactive was the "saliva T Elisa kit" T (Diagnostics BiochemCanada Inc, Canada, Dorchester, Ontario Canada). Enzimoimmunoassay was used in its determination. The assay sensitivity was 1 pg/mL and it was expressed in pmol/L. Good precision was obtained, with intra and inter-assay variation coefficients of 3.98% and 7.98%, respectively.

Salivary C (C) levels were determined by RIA using an appropriate reactive count-a-count C (DPC-Siemens Medical Solutions Diagnostics, Bad Nauheim, Germany) with 0.5 ng/dL sensitivity. All samples were analyzed in duplicate and the samples of the same subject were included in the same assay. Although the variation coefficient necessary for replication was fixed at 8%, the maximum intra- and inter-assay variation of the coefficients obtained were 4.3% and 5.2%, respectively. All the values were expressed in nmol/L.

Data Analysis

It was previously established using the Kolmogorov–Smirnov statistic ($p < .001$) that the data was normally distributed. T-tests were carried out to check significant differences between groups (IPVand control men) in age, BMI, appraisal scores, psychological trait profiles (STAXI-2 and IRI), performance in the neuropsychological test (WCST and eyes test), and in the 2D:4D ratio.

Effect sizes for the between-group differences were calculated using Cohen's d (Cohen, 1988). Chi square analyses were performed for alcohol, tobacco, and drugs.

For psychological state responses, repeated-measures ANOVAs with "moment" (pre and post) as the within subject factor and "group" as the between-subject factor were performed. To analyze T and C responses repeated ANOVAs were applied, with "moment" (at six levels: baseline,

preparation period, 0, 15, 30, and 45 min after TSST) as a within-subject factor, and "group" (IPV and control) as a between-subject factor. Greenhouse-Geisser adjustments for degrees of freedom were employed. Partial eta squared was reported as a measurement for effect size. To control for potential baseline differences in psychological variables, or in the 2D:4D digit ratio, ANCOVAs were carried out using baseline values, psychological traits, as well as drug, and tobacco consumption as covariates to see if there were any differences between the groups. Analysis of the area under the curve (AUC) enabled a quantification of the individual's hormonal response to the stressor to be calculated according to the widely used trapezoid formula (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Pruessner, Kirschbaum, Meinlschmidt, & Hellhammer, 2003; Stalder, Evans, Hucklebridge, & Clow, 2011). Partial AUC were performed considering only the different times before the final measurement. To achieve this, differences between each of the five hormonal values (preparation period and 0, 15, 30, and 45 min after TSST) and the baseline level were added and this resulted in one AUC.

To show possible psychological manifestations of hormonal differences/changes that may be indicative of risk for IPV Pearson or Spearman correlation tests were performed where appropriate to examine relationships between. Moreover, a stepwise regression following the Aiken and West (1991) methodology was used. The interaction effects between the group and the psychological state variables for T or C response to stress were tested. We entered T AUC as a dependent variable, in Step 1 we entered group as a covariate, in Step 2 we entered a psychological baseline, and in Step 3 we entered the interaction between both. The same procedure was repeated for T baseline, C AUC and C baseline.

Data analyses were carried out using SPSS 17.0 software (SPSS® Statistics). Statistical significance was accepted for p values $\leq .05$; whereas a tendency to significance was considered as p values .08. Average values in the tables are expressed as mean$\pm$SEM.

Results

Participant Characteristics and Psychological Trait Profiles

Descriptive characteristics and psychological trait profiles for the IPV and controls are presented in Table 1. Groups did not differ in age or BMI. The IPV men showed a lower left-hand 2D:4D digit ratio than the controls ($t = -1.93$, $p < .06$, $d = .80$), although these differences were not replicated in the right hand. Differences in tobacco consumption, alcohol, and drug abuse (χ^2 (1) = 16.85, $p < .05$; χ^2 (1) = 7.80, $p < .01$, and χ^2 (1) = 4.91, $p < .05$, respectively) were recorded with more IPV than control men using these substances. There were no differences in drug use.

Regarding psychological trait profiles, IPV perpetrators showed lower scores in perspective taking and higher scores in the IRI personal distress scales ($t = -2.10$, $p < .05$ and $t = 2.99$, $p < .01$, respectively) and in anger control in the STAXI-2 subscale ($t = 2.15$, $p < .05$) than controls, whose effect sizes were .78 and .79, respectively.

Table 1. Mean ± SEM of descriptive characteristics, and psychological trait profiles for IPV and control men. * p < 0.05 ** p < 0.01 (t) p < 0.06

	IPV men (n=19)	**Control (n=21)**
Age (years)	38.00±3.13	35.80±1.47
BMI (Kg/m²)	26.97±0.80	27.55±0.63
Left 2D:4D ratio (t)	0.96±0.04	0.99±0.01
Right 2D:4D ratio	0.96±0.03	0.96±0.04
Stroke	Yes 0 (0%) No 19 (100%)	Yes 0 (0%) No 21 (100%)
Diabetes	Yes 0 (0%) No 19 (100%)	Yes 0 (0%) No 21 (100%)
Thyroid illnesses	Yes 0 (0%) No 19 (100%)	Yes 0 (0%) No 21 (100%)
Smoker*	Yes 15 (79 %) No 4 (21 %)	Yes 3 (14 %) No 18 (86 %)
Drug abuse**	Yes 6 (32 %) No 13 (68 %)	Yes 0 (0 %) No 21 (100%)
Alcohol abuse*	Yes 4 (21 %) No 15 (79 %)	Yes 0 (0 %) No 21 (100%)
Drug		
Non-psychiatric	Yes 3 (16 %) No 16 (84 %)	Yes 4 (19 %) No 17 (81 %)
Anxiolytic	Yes 3 (16%) No 16 (84%)	Yes 0 (0 %) No 0 (0 %)
STAXI-2 anger expression in	11.00±0.70	10.19±0.47
STAXI-2 anger expression out	9.05±0.54	9.10±0.48
STAXI-2 anger control in*	17.42±1.06	14.24±1.03
STAXI-2 anger control out	18.37±0.86	19.48±0.92
STAXI-2 AEI	20.26±10.00	21.57±8.01
STAXI-2 T-Ang	3.82±2.09	3.24±2.27
IRI perspective taking*	16.00±1.00	25.75±0.76
IRI empathic concern	24.21±0.71	23.05±0.61
IRI personal distress**	16.00±0.93	12.50±0.70
IRI fantasy	17.58±0.92	18.35±0.82

Neuropsychological Variables

Data from the eyes test and WCST is presented in Table 2. IPV perpetrators obtained a worse performance in the eyes test than the controls ($t = -2.08$, $p < .05$, $d = .87$). When the eyes test was distributed by gender, IPV perpetrators obtained lower scores in eye recognition ($t = -3.22$, $p < .01$, $d = 1.04$), but groups did not differ in recognition of women's eyes. When classified by emotional meaning, IPV perpetrators obtained lower scores in neutral emotions than the controls ($t = -2.31$, $p < .05$, $d = .94$).

Regarding WCST performance, IPV perpetrators completed fewer categories ($t = -4.92$, $p < .01$, $d = 1.60$) and committed more total ($t = 4.99$, $p < .01$, $d = 1.62$), perseverative ($t = 2.63$, $p < .01$, $d = 0.85$), percentage of perseverative ($t = 2.52$, $p < .01$, $d = 0.82$) and non-perseverative errors ($t = 3.52$, $p < .01$, $d = 1.14$) and had lower learn to learn scores ($t = -5.10$, $p < .01$, $d = 1.65$) than controls. Additionally, IPV perpetrators used more trials than control participants ($t = 4.43$, $p < .01$, $d = 1.44$).

Table 2. Mean±SEM of neuropsychological variables (eyes test and WCST). * $p < 0.05$ ** $p < 0.01$

	IPV men (n=17)	**Control (n=19)**
Eyes test*	21.5±1.37	24.89±0.88
Eyes test (men eyes)**	10.44±0.66	13.27±0.57
Eyes test (women eyes)	11.07±0.92	11.63±0.49
Eyes test (positive emotions)	4.67±0.36	5.11±0.45
Eyes test (negative emotions)	7.50±0.82	8.63±0.39
Eyes test (neutral emotions)*	9.33±0.60	11.16±0.51
WCST total trials**	118.28±4.45	88.68±4.08
WCST correct answer	70.89±2.99	70.73±2.21
WCST total mistakes**	48.00±5.24	17.89±2.15
WCST perseverative mistakes**	13.78±4.37	1.95±0.36
WCST non perseverative mistakes**	34.16±4.24	16.26±2.03
WCST perseverative mistakes (%)**	16.22±5.79	2.05±0.47
WCST failure to maintain set	1.57±0.34	0.79±0.34
WCST trials to complete the first category	14.57±1.81	15.05±2.17
WCST number of categories**	3.56±0.47	6.00±0.00
WCST conceptual level	7.50±0.77	6.89±0.48
WCST learn to learn**	3.22±0.56	6.00±0.00

Stress Responses

Appraisal scores and psychological state profiles

IPV perpetrators scored similar appraisal scores to controls in satisfaction (5.01±1.73 and 5.78±1.83, respectively), internal (5.41±1.45 and 5.47±2.19, respectively), and external control index (4.58±1.45 and 4.52±2.19, respectively); but IPV perpetrators perceived the TSST as less stressful than controls (3.41±2.49 and 5.57±2.38, respectively; $p \leq .01$). For this reason, it was covariate in the ulterior analysis.

Basal and post-test psychological state scores are presented in Table 3. For anxiety, a significant effect of "moment" and "group x moment" interaction was found ($F(1, 38) = 22.01$, $p < .01$, $\eta_p^2 = .37$); $F(1, 38) = 10.36$, p

< .01, η_p^2 = .21), respectively). Although both groups increased their states of anxiety after the task, it was lower in the case of IPV perpetrators. No additional effects were obtained after including perceived stress, or tobacco consumption and drugs as covariates.

As in the case of anxiety, the laboratory stressor was shown to be efficient for eliciting mood alterations, since the factor "moment" was significant in tension and anger subscales, as well as in the total score ($F(1, 38) = 5.41$, $p < .05$, $\eta_p^2 = .13$; $F(1, 38) = 4.01$, $p < .05$, $\eta_p^2 = .10$; $F(1, 38) = 23.99$, $p < .01$, $\eta_p^2 = .39$, respectively). Additionally a tendency to significance for the vigor subscale was found ($F(1, 38) = 3.28$, $p < .08$, $\eta_p^2 = .08$). All participants experienced worse moods after the task. Moreover, a significant effect for the "group x moment" interaction was found in the total score ($F(1, 38) = 4.70$, $p < .05$, $\eta_p^2 = .09$) and a tendency to significance in the anger subscale ($F(1, 38) = 3.17$, $p < .08$, $\eta_p^2 = .07$). Although both groups increased their scores in anger and total mood after the task, it was lower in the case of IPV perpetrators. The same results were obtained after including the perceived stress or alcohol, drug, and tobacco consumption during the TSST as a covariate, although an additional effect for the "group x moment" interaction was found in the depression subscale ($F(1, 38) = 4.98$, $p < .05$, $\eta_p^2 = .12$). In this case, whereas IPV perpetrators decreased depression after the TSST, control participants increased their scores in this subscale.

When analyzing anger, a significant effect for the "group x moment" interaction in the feeling subscale was found ($F(1, 38) = 7.83$, $p < .01$, $\eta_p^2 = .17$) that decreased in IPV perpetrators and increased in controls. Furthermore, the factor "group" proved to be significant in the case of physical expression of anger ($F(1, 38) = 4.05$, $p < .05$, $\eta_p^2 = .09$), and almost significant in verbal expression ($F(1, 38) = 3.64$, $p < .06$, $\eta_p^2 = .15$) with IPV perpetrators presenting higher scores in both subscales. No additional effects were obtained after including perceived stress or alcohol, drug, and tobacco consumption as covariates.

Table 3. Mean±SEM of psychological states before and after task. *p < 0.05 ** p < 0.01 (t) p < 0.06

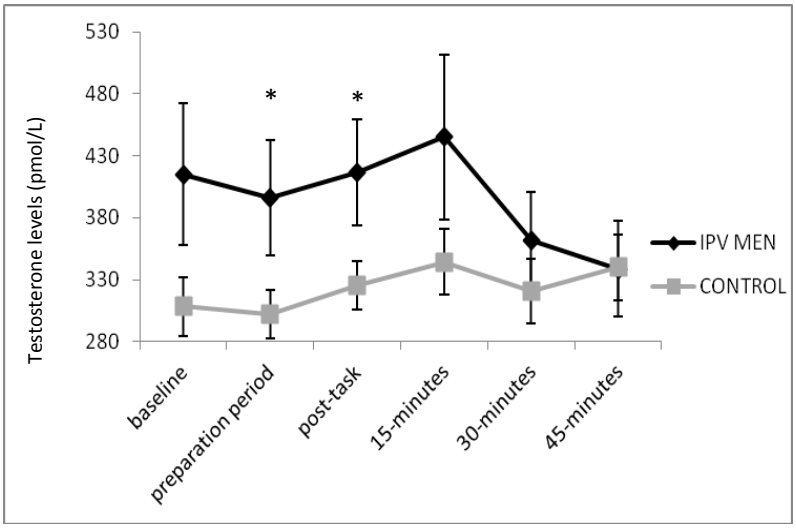
	IPV (n=19)		CONTROL (n=21)	
	Before Task	After Task	Before Task	After Task
STAI-S*	18.16±1.79	19.84±1.58	14.48±1.62	23.52±2.40
POMS depression	1.68±0.71	1.26±0.53	0.57±0.32	0.71±0.29
POMS Tension	4.95±0.96	5.42±0.73	4.57±0.75	6.48±0.81
POMS(t) Anger	1.32±0.65	1.37±0.69	0.33±0.16	1.24±0.40
POMS Vigor	13.26±1.09	12.68±1.16	13.48±0.76	12.57±0.93
POMS Fatigue	1.37±0.54	1.37±0.51	1.38±0.36	1.62±0.43
POMS* Total	-3.94±2.37	0.68±1.39	-6.62±1.39	4.09±1.09
STAXI-2 S-Ang**	5.53±0.17	5.31±0.69	5.08±0.18	5.30±0.54

Hormonal responses

For T, the laboratory stressor proved to be efficient for eliciting T changes, since the factor "moment" showed a tendency towards significance ($F(2.86, 108.59) = 2.47$, $p < .07$, $\eta_p^2 = .06$). After dividing the sample by groups the factor "moment" was significant for IPV perpetrators and controls ($F(2.40, 43.26) = 2.70$, $p < .05$, $\eta_p^2 = .13$; $F(3.03, 60.60) = 2.89$, $p < .05$, $\eta_p^2 = .13$, respectively). Additionally, a tendency towards significance for the "group x moment" interaction was found; $F(2.86, 108.59) = 2.55$, $p < .06$, $\eta_p^2 = .07$). Differences between IPV perpetrators and controls in T baseline values were not found and so there was no reason to include covariates. After including perceived stress during the TSST as a covariate, the interaction reached statistical significance ($F(2.83, 104.79) = 2.68$, $p < .05$, $\eta_p^2 = .10$). Post-hoc analyses showed that IPV perpetrators had higher T levels in speech preparation and post-task periods than controls (for both $p < .05$; Fig. 1). Although groups did not differ in the total T AUC in both partial AUCs (from baseline to anticipatory and from baseline to 0 min after the stressor) significant differences between groups were found when perceived stress was

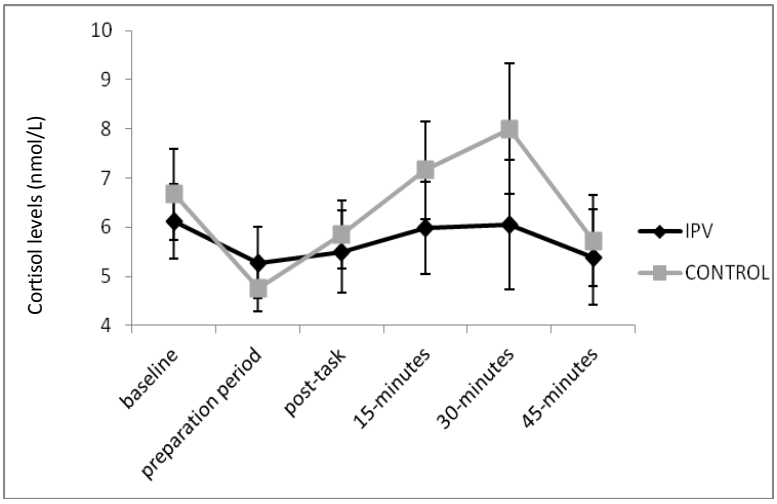
included as a covariate ($F(1, 40) = 5.16$, $p < .05$, $\eta_p^2 = .12$; $F(1, 40) = 4.16$, $p < .05$, $\eta_p^2 = .10$, respectively). IPV perpetrators obtained lower T AUC. The same results were obtained after including alcohol, drugs, and tobacco consumption as covariates.

Figure 1. T levels for IPV perpetrators and control men. * $p < .05$



In the C case, the laboratory stressor proved to be efficient in eliciting C changes, but only the "moment" was significant in controls ($F(1.56, 31.28) = 2.65$, $p < .05$, $\eta_p^2 = .12$). No differences were found between groups in C baseline values and so there was no reason to introduce covariates. No significant effects in the case of C levels or the C AUCs were found in any case. Although not significantly, IPV perpetrators showed a less pronounced increase in C levels after the task and until 30 min post-task than the controls (Fig. 2). When C response was analyzed separately, only in the case of controls was there a significant decrease in C levels between baseline and the preparation period ($F(1, 20) = 11.47$, $p < .05$, $\eta_p^2 = .29$); while an increase between the preparation period and 15 min post-task was found ($F(1, 20) = 6.97$, $p < .05$, $\eta_p^2 = .21$). The same results were obtained after including alcohol, drug, and tobacco consumption as covariates.

Figure 2. C levels for IPV perpetrators and control men



Relationships between variables

Relationships between WCST, eyes test, IRI, psychological state scores, and 2D:4D ratio for all participants

The relationships between the variables were summarized in Table 4. In addition, only in the case of IPV perpetrators were the right 2D:4D ratios negatively correlated to T AUC ($r = -.521$, $p < .05$) and positively to the eyes test ($r = .571$, $p < .05$).

Relationships between WCST, eyes test, IRI and psychological state scores with T and C baseline and AUCs for IPV perpetrators and controls

The relationships between these variables were summarized in Table 5 for IPV perpetrators and controls. Thus, significant correlations between these variables were formally tested in hierarchical multiple regressions separately in order to analyze the existence of specific relationships between these variables in both groups. Nonetheless, no significant and specific models for those interactions were found. Moreover, alcohol, drug, and tobacco consumption included as covariates did not show significant effect on the hierarchical models or predictions.

Table 4. Correlations between eyes test, WCST, IRI and psychological state scores for IPV perpetrators and controls. *p < 0.05 ** p < 0.01 (t) p < 0.06

	WCST perseverative mistakes (%)		WCST number of categories		Eyes test		IRI perspective taking		IRI empathic concern		IRI personal distress		IRI fantasy		STAI baseline		STAXI-2 S-Ang		POMS total baseline	
	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls	IPV	Controls
WCST perseverative mistakes (%)																				
WCST number of categories	-.565*	-.598*																		
Eyes test	-.479*	-.078	.467*	.143																
IRI perspective taking	-.164	-.043	.159	.014	.558*	-.295														
IRI empathic concern	.153	.336	.096	.012	.009	-.213	.241	.121												
IRI personal distress	-.336	-.090	.327	.278	-.016	.232	-.166	.260	.106	.361										
IRI fantasy	.074	.276	-.015	.103	-.380	.094	.241	-.282	-.213	.321	.041	.444*								
STAI baseline	-.220	-.095	-.022	-.036	-.022	-.146	-.104	-.379(t)	.248	.138	-.335	.522*	.355	.041						
STAXI-2 total baseline	.410*	.152	.106	.200	-.150	.207	-.181	-.693**	.229	-.180	.132	.142	.400	.031	.404(t)	.282				
POMS total baseline	-.131	-.052	-.133	-.044	-.134	.063	-.150	-.447*	.128	-.044	.029	.168	.503*	.007	.798**	.587**	.668**	.451*		

Table 5. Correlations between WCST, eyes test, IRI and psychological state scores with T and C baseline and AUCs for IPV perpetrators and controls *p < 0.05 ** p < 0.01 (t) p < 0.06

	WCST perseverative mistakes (%)		WCST number of categories		Eyes test		IRI Perspective taking		IRI empathy concern		IRI Personal distress		IRI fantasy	
	IPV	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**
T baseline	-.050	.311	-.120	-.192	-.566*	.119	-.174	-.209	.071	-.106	-.129	-.124	.278	.078
T AUC	-.111	-.180	.166	-.039	.293	-.035	.109	.062	.143	.067	-.409*	-.123	-.256	.055
C baseline	.188	.301	.227	.002	.190	.165	-061	.028	.016	-.188	.052	.012	-.184	-.275
C AUC	-.077	-.281	-.185	.106	.134	-.134	.220	-.018	-.043	.070	.049	-.131	-.180	-.137

	STAI Baseline		STAI (post-basal)		STAXI-2 S-Ang baseline		STAXI-2 S-Ang (post-baseline)		POMS total baseline		POMS total (post-baseline)	
	IPV	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**	**IPV**	**controls**
T baseline	.308*	.126	.057	.339(t)	.462**	.266	-.274	.145	.413*	.378(t)	-.118	.100
T AUC	-.336*	-.311	.032	-.011	-.515**	-.085	.120	-.284	-.457**	-.363	.201	.076
C baseline	-119	-.040	.248	.141	.107	.224	.188	.217	-.063	.228	.167	.272
C AUC	-.158	-.297	-.082	-.286	.063	-.145	-.101	-.383(t)	-.006	-.276	-.185	-.284

Discussion

After presenting the TSST, IPV perpetrators experienced decreases in T levels, a moderate worsening of mood, slight anxiety, and a C level increase. Moreover, when basal Twas higher, it was related with high levels of anger and anxiety and worse mood. However, that basal mood does not significantly alter T levels in response to stress. Furthermore, only in controls high cognitive empathy was associated with a better baseline mood and low anger and anxiety. On the other hand, poor executive performance was related with poor empathic skills.With respect to neuropsychological and cognitive empathic features, IPV perpetrators showed poor executive performance and emotional recognition. For emotional empathy, IPV perpetrators showed higher personal distress than controls. Nonetheless, only in controls better emotional decoding process was related to better cognitive empathy. The 2D:4D ratio was lower in IPV than in controls. In addition, in IPV perpetrators a smaller 2D:4D ratio was related with large increases in T in response to stress and poor emotional recognition.

The stressor increased anxiety and C levels, diminished T levels, and produced a worsening of mood in both IPV and controls. This emphasizes that differences are not due to a different emotionally charged topic of the TSST. In addition, controls experienced more perceived stress than IPV perpetrators and this indicates that they were emotionally involved in the task.

In the general population, the TSST usually induces decreases in T levels, increases in C levels and anxiety, and a worsening of mood (Dickerson, Gruenewald, & Kemedy, 2004; Heinz et al., 2003). However, the relationship between acute psychosocial stress and changes in T levels has not been frequently studied.

This diminution is clear in competitive situations or during physical stress (Schoofs & Wolf, 2011) but not in other contexts. No studies on this subject have been carried out on IPV perpetrators. In the present test, controls and IPV perpetrators showed these modifications after the TSST.

Our result does not refute the data in the literature that is mainly based on the analysis of baseline T. Those males with higher levels of androgens demonstrated greater increases in anger and aggressive response to threatening stimuli than non-violent men (George et al., 2001; Moya-Albiol, 2010). Moreover, increases in T levels and in feelings of anger after an

exposition to an anger-induction procedure was described in a nonviolent population (Herrero et al., 2010). Although our data does not support T differences at baseline, IPV perpetrators with high levels of anger and anxiety and worse mood showed high basal T levels. Thus, when basal Twas higher a high anger response to stress did not significantly alter T levels in response to stress. For this reason, IPV perpetrators could perceive the TSST as threatening or hostile in itself. This, in turn, could mean that they adopt a defensive position with smaller T responses. For that, the negative emotional reactivity of the IPV perpetrators drives most of the differential responding to stress.

Changes in T levels and the magnitude of response were similar as both groups showed a negative response to acute stress. However, IPV perpetrators experienced greater decreases in their T levels between the baseline and preparation period than control participants. This result may explain their anticipatory response to psychosocial stress. Moreover, during the preparation period and 0 min after task, IPV perpetrators showed greater T levels than controls. Therefore, IPV perpetrators and control menmay have the same fluctuations in T levels—but occurring in different ways. Nevertheless, it would be necessary to conduct more studies to assess whether these participants have an abnormal metabolism of T (George et al., 2001), higher T levels (Soler et al., 2000), or more sensitivity to activation T effects (Pope et al., 2000). Indeed the 2D:4D ratio could be an indicator of this and so exposure to high T levels in the womb could increase the sensitivity to androgen in adulthood (van Honk et al., 2011) and facilitate physical aggression in men (Bailey & Hurd, 2005). In IPV perpetrators, the 2D:4D ratio of the left hand was smaller than controls. For this reason, our results support previous research because the 2D:4D ratio of both hands has been used as index of masculinization (Bull & Benson, 2006; Romero-Martínez et al. 2013). Therefore, IPV perpetrators may have been exposed to high prenatal T levels (Manning et al., 2001) and may also present an increased genetic sensitivity (Manning et al., 2003). As described in clinical populations for the case of men with fertility problems, the 2D:4D ratio is related to circulating levels of T (Manning, Scutt, Wilson, & Lewis-Jones, 1998). In our study, the 2D:4D ratio predicted substantial changes in T and poor emotional recognition only in IPV perpetrators which may reinforce the sensitivity to T.

The TSST produced significant increases in C levels in controls but not in IPV perpetrators. This response is frequent and has been replicated in many

populations, which could indicate that IPV perpetrators are less reactive to psychological stress when related to domestic violence. The slight changes in C levels in IPV perpetrators in comparison with controls may suggest hypoactivity of the HPA axis, which has been associated with aggression and other behavioral problems in violent participants (Gordis, Granger, Susman, &Trickett, 2006). These results may be explained by a habituation effect (Kudielka et al., 2006) due to the fact that IPVperpetrators reported a low level of perceived stress, anxiety, and a slight worsening of mood. This issue could be the product of repeated exposures to psychosocial stressors during discussions with their partners.

Although it has been suggested that the interaction between high T and low C may facilitate aggressive behavior (Carré & Mehta, 2011), in our study T and C were not directly related. It is therefore important to consider the interactions between hormonal parameters and the cognitive system and howthese interactions could predispose to violent behavior (Pinto et al., 2010).

Our data does not support that IPV perpetrators may have a more hostile temperament. Indeed, they tend to describe themselves in interviews and questionnaires in a correct and socially acceptable way (Saunders, 1991). They also describe themselves as people with high levels of external control of anger. In our study, IPV perpetrators showed high levels of control of anger, possibly because their anger reactions are reduced to the domestic domain. In addition, it has been hypothesized that there are different types of offenders according to their empathetic capacities (Covell et al., 2007). The IPV perpetrators analyzed in our study have an impaired capacity to understand the thoughts and emotions of others (cognitive empathy) but are able to feel compassion for others (emotional empathy). Behind the reduced capacity to understand thoughts and emotions and predict the behavior of others may lay deficits in cognitive components involved in empathy (Tirapu-Ustárroz et al., 2007). Our results lead us to believe that the deficit could be in emotional stimuli decoding, especially in those stimuli with a neutral value.

In this sense, a previous study suggested that a significant percentage of IPV perpetrators may have these déficits (Babcock et al., 2008). IPV perpetrators may misunderstand and attribute hostile connotations to neutral stimuli and this increases the likelihood of behaving aggressively (Holtzworth-Munroe & Smutzler, 1996) if they believe that their partners maligned or blamed them. Our study revealed that IPV perpetrators lacked cognitive

empathy but not emotional empathy. However, the eyes test (emotional decoding process) was statistically related to the perspective taking (cognitive empathy) score. That result, although was found only in controls, reinforced the importance of the decoding process for the ToM. As proposed by Covell et al. (2007), this type of IPV perpetrator may feel remorse after perpetrating violent acts. This effect and empathic abilities may be modulated by endogenous T levels. The administration of exogenous T in non-violent men produced a higher emotional salience of stimuli associated with such hostility; increasing the probability of displaying antisocial behavior (van Honk & Schutter, 2007). Rises in endogenous levels resulted in a decrease in empathic abilities (Hermans, Putman, & van - Honk, 2006). Hence, those results were supported by our results as high T was related to low empathic abilities. Impairments in empathy were analyzed separately and the relationship with other neuropsychological alterations has not yet been studied. ToM deficits in several diseases such as autism and Asperger syndrome may occur with deficits in executive functions (Baron-Cohen, 2010). Nevertheless, patients with organic frontal lobe damage presented impairments in both functions, and both executive and empathic deficits were relatively independent (Rowel, Bullock, Polkey, & Morris, 2001) and could coexist without apparent causal connection. However, our results supported the hypotheses that both deficits may be related. Hence, this kind of deficits could difficult the socialization and reintegration processes of IPV perpetrators.

Mental flexibility and/or problem - solving abilities in terms of WCST revealed that IPV perpetrators may have a reduced ability to learn from their mistakes and a certain degree of mental rigidity, a result that has already been obtained (Pinto et al., 2010). These potential cognitive characteristics could explain the persistence of sexist stereotypes that frequently appear in IPV perpetrators (Ministry of Interior, Technical General Secretary, 2010). Behind the relationship between worse WCST performance and the increase in physical and verbal hostility may lay impairments in the PFC that are involved in anger and frustration regulation (Koenings et al., 2007). Sexist cognitive schemas combined with potential empathic deficits could constitute an idiosyncratic processing system for ambiguous stimuli that induces violent behavior. However, the activation effects of androgens on cognition should be considered. Decreases in endogenous levels of T may underlie impairment of several cognitive abilities. Nevertheless, studies analyzing this aspect were conducted in men with symptoms and signs of andropause, and/or endocrine

disorders (Beauchet, 2006). To our knowledge, no studies have analyzed this relationship in violent adult men. However, our results did not reveal a direct relationship between T changes and WCST performance. It seems then that the relationship is clearer between T and empathy and its cognitive processes.

Finally, we must point out that alcohol, cocaine, and marijuana may contribute to the observed differences between groups. A smaller or masculinized 2D:4D ratio, such as that of our IPV perpetrators, could be an indicator of the adoption of reckless behavior, such as alcohol or other drug abuse (Kornhuber et al., 2011). Despite the fact that not all men involved in domestic violence use alcohol or other toxics, a large percentage of men attack their partners under the effects of these substances (Pinto et al., 2010). Alcohol consumption has been linked with both the maintenance and facilitation of domestic violence (Hines & Douglas, 2011) and could act as an accelerator of violence (Fals-Stewart, 2003); or as a negative reinforcement that mitigates the negative emotions and so entails the perpetuation of violence (Simons, Gaher, Jacobs, Meyer, & Johnson-Jiménez, 2005) given that the perpetrators analyzed in this study might express remorse after committing a violent act. The current model for this phenomenon goes by the name of the Myopic Model (Steele & Josephs, 1990) which states that alcoholic intoxication worsens attention capacities and/or information processing. Therefore the quantity of stimuli the subject is able to process decreases and this facilitates a violent reaction (Giancola, Duke, & Ritz, 2011). This means that other cognitive-affective and neuropsychological factors, in direct or indirect interaction with T, should be considered in order to offer an explanation for domestic violence.

One limitation of the study was the small sample size, and so our study can be considered as a preliminary approach to the analyzed subject. Although the groups differed in several variables such as alcohol, drug, and tobacco consumption, these variables were statistically controlled. Nevertheless, this research was interesting enough to offer some valuable results. Future research may replicate these findings after evaluating a larger sample of IPV perpetrators. Additionally, other variables such as immunity or cardiovascular responses should be included. Our data is relevant and novel as no studies have previously analyzed the psychological and hormonal responses to psychological stress as related to domestic violence in a laboratory context. It would be useful to analyze other subsamples of IPV perpetrators as this

would enable a better understanding of a phenomenon as complex as domestic violence.

In conclusion, this study shows that T could be an indirect modulator of aggressive behavior through its effects on the processing of cognitive-affective information. Furthermore, the psychological response might be better explained by cognitive variables than by fluctuations in hormonal levels in IPV perpetrators. Moreover, the negative emotional reactivity of IPV perpetrators drive most of the differential responding to stress. Our data must be considered together with previous reports related to mental health that were assessed qualitatively by trained researchers, the objective reports obtained by SCL-90 scores, and the participants' court records to assess any history of violence. We suggest that endocrine, psychological, and neuropsychological specific biases should be included in the "family-only" (FO) type, as proposed by Holtzworth-Munroe and Stuart (1994), for individuals whose violent behavior is confined to the home. IPV perpetrators normally behave in a nonaggressive way in non-domestic contexts. Babcock et al. (2004) proposed that a biopsychosocial model may increase our knowledge of violent behavior in IPV perpetrators. These results could benefit rehabilitation programs designed for abusers that employ a communitarian and psychotherapeutic perspective focused primarily on changing beliefs, biases, and/or cognitive distortions of offenders. Hormonal parameters and neuropsychological variables indicate the need to use cognitive-affective functions in impaired rehabilitation programs, and even raise the possibility of admission criteria to these programs for participants who may change or learn new behavioral patterns.

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Chapter 5

Study 4: Testosterone/cortisol ratio in response to acute stress: A possible marker of risk for marital violence

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Introduction

Intimate partner violence (IPV) perpetrators use physical and/or psychological abuse to control their partners (Antai, 2011). This behavior may indirectly contribute to improving their health. Although results in humans are unclear, animal research has recently revealed that dominance is related with a low risk of disease and rapid recovery from illness (Archie, Altmann, & Alberts, 2012). Moreover, in many mammals, dominance can affect the hypothalamic–pituitary–gonadal (HPG) axis by increasing testosterone (T) levels (Zilioli & Watson, 2012). A high level of self-esteem has been related to better mental health in healthy subjects (Mann, Hosman, Schaalma, & de Vries, 2004). In IPV perpetrators, however, high self-esteem has been associated with aggression (Bushman et al., 2009).

High T levels facilitate the development of violent behavior in men (Moya-Albiol, 2010). This effect could be related to low sensitivity to punishment or fear, and risky behavior. Cortisol (C), on the other hand, may inhibit this behavior by increasing sensitivity to social punishment. Specifically, C may reduce T production by inhibiting the HPG axis, and block T effects in target tissues, including the prefrontal cortex. In turn, T may affect the hypothalamus–pituitary–adrenal axis by reducing C levels through the hypothalamus (van Honk, Harmon-Jones, Morgan, & Schutter, 2010). Indeed, the relationship between T and violence may be stronger in men with low C levels (Popma et al., 2007). Both hormones together with trait and/or state anger have been related to the development and maintenance of aggression. IPV perpetrators report moderately higher levels of anger and hostility than nonviolent men (Norlander & Eckhardt, 2005).

Moreover, anger mood induction usually increases anger and T levels in nonviolent young adults and children (Herrero, Gadea, Rodriguez-Alarcón, Espert, & Salvador, 2010; Sánchez-Martín et al., 2011). Nevertheless, only severely violent IPV perpetrators were observed to exhibit more anger after marital conflict discussions, although T changes were not analyzed in this context (Babcock, Green, Webb, & Yerington, 2005). Although the basal T/C ratio has been proposed as a marker for proneness to social aggression in reactive and proactive aggressors (Terburg, Morgan, & van Honk, 2009), no studies have analyzed whether the ratio is related to violence by IPV perpetrators.

Moreover, as far as we know, the association of the T/C ratio with self-esteem and mental health has not been explored in IPV perpetrators. The most related research has focused on T (Pinto et al., 2010). Nevertheless, as IPV is a complex phenomenon, biological factors should be analyzed together with psychological characteristics, to broaden our perspective on violence (Babcock, Green, & Robie, 2004). Often single saliva samples are used for assessing T and C because both hormones tend to be temporally stable when measured at the same time of day.

Thus, it has been proposed that the basal levels of these hormones are themselves traits (Denson, Ronay, von Hippel, & Schira, 2012; Liening, Stanton, Saini, & Schultheiss, 2010). Nevertheless, some researchers have measured these hormones at different times, a more valid approach that enables calculation of the area under the curve (AUC) in response to acute stress (Stalder, Evans, Hucklebridge, & Clow, 2011). The magnitude of the response has been related to traits (van Santen et al., 2011; Zoccola, Dickerson, & Yim, 2011).

The aim of this study was to assess whether IPV perpetrators have higher T/C ratios than non-violent control men, using several measurements of T and C in response to stress. Specific time points for measuring the hormones were carefully chosen following previous recommendations (Costa & Salvador, 2012; De Andrés-García, Moya-Albiol, & González-Bono, 2012; Moya-Albiol et al., 2001). Further, we explored the relationships of T/C ratios with state anger (related to stress responses), and with self-esteem and mental health in IPV perpetrators. The results indicate how combining hormonal and psychological indicators may improve the identification of perpetrators. It has been hypothesized that IPV perpetrators, as dominant men who are highly prone to aggression, show higher T/C ratios (Terburg et al., 2009) and higher self-esteem (Bushman et al., 2009) than controls which could lead to a better mental health (Mann et al., 2004).

Method

Participants

The final sample was composed of 37 healthy men volunteers (16 IPV perpetrators and 21 controls). One IPV perpetrator was excluded from the analysis involving T/C ratios because his hormone levels were more than 3.5 SD from the means in the rest of the sample. Three controls could not complete the Rosenberg Self-Esteem Scale (RSES) and the Symptom

Checklist 90-Revised (SCL-90-R). IPV perpetrators were recruited from the community and from among the participants in the CONTEXTO psycho-educational treatment programme (mandatory for male abusers) at the Department of Social Psychology, University of Valencia (Lila et al., 2010).

Requirements for participating included: having being jailed for IPV; not having been convicted for assault outside the home; and no mental illness (assessed with the SCL-90-R). We advertized in the University of Valencia for male volunteers for the control group, establishing contact by email before screening applicants in interviews. The inclusion criteria for controls included: having no organic or mental illnesses, and similar anthropometrical and demographic characteristics to the IPV perpetrators as well as not having perpetrated severe violence, defined as assaulting a partner or other individual outside the home, or engaging in any severely violent act. Control individuals were required to provide criminal record certificates, to check that they had no history of violence.

Further, all participants were interviewed by trained researchers (with extensive experience treating IPV perpetrators) to assess their mental health. Cohen's kappa, used to assess the inter-rater agreement between qualitative interviewers in the nine psychopathological dimensions evaluated (the same dimensions as the SCL-90-R), ranged from .67 to .84. Regardless of the objective SCL-90-R results, subjects were considered not to have any psychopathological signs and symptoms if they scored less than the mean for their age for each dimension. Candidates were eligible to participate if the qualitative interviews and SCL-90-R scores confirmed they were free of mental illness; three IPV perpetrators and four controls were excluded because their results suggested psychological disorders. All participants were righthanded and healthy, lived in Valencia (Spain), and gave written informed consent. The experiment was performed in accordance with the Helsinki Declaration and approved by the University of Valencia Ethics Committee.

Procedure

Each subject participated in three sessions in the psychobiology laboratories of the University of Valencia. In the first sessions, participants were interviewed to exclude any individuals with organic diseases. The second sessions all took place between 4 and 7 pm to control for diurnal variations in cortisol secretion (Dickmeis, 2009). Participants were instructed to abstain from food, caffeine, alcohol, brushing their teeth, exercise, and any

medication in the 2 h before these afternoon sessions. It was explained that they would be asked to provide saliva samples for hormonal analysis and perform several behavioral tasks.

After arriving at the laboratory, participants were taken to a room where they signed informed consent forms and anthropometric data (height and weight) were obtained. Before stress exposure, two saliva samples were collected (for assessing T and C levels) and a Spanish version of the State-Trait Anger Expression Inventory (STAXI-2, see below) was administered.

Subsequently, participants were taken to a soundproofed room with a steady temperature (22±1◦C) for the Trier Social Stress Test (TSST) (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). This is a standardized psychosocial laboratory stressor that involves preparing a speech about a specific topic followed by a test period in which participants deliver their speech and perform a mental arithmetic task in front of an audience. All participants had to express their opinion about IPV and its consequences for society and themselves and, then, perform an arithmetic task. To increase the perceived social evaluative threat, there was an audience of four evaluators (two men and two women), and a video camera was turned on (though only as a decoy). Lasting 15 minutes, the procedure included the following periods: baseline, preparation, anticipatory, task, and post-task. Between the public speaking task instructions and the preparation period, an additional saliva sample was collected.

After the test, the STAXI-2 was completed for a second time, with another four saliva samples being taken at +0, +15, +30, and +45 minutes, and finally, one day after the laboratory task participants completed the RSES, as well as repeating the SCL-90-R.

Psychological state and trait characteristics

State anger was measured using an adapted versión (Miguel-Tobal, Casado, Cano-Vindel, & Spielberger, 2001) of the STAXI-*2* (Spielberger, 1999). The inventory is divided into three subscales for state anger (S-Ang: feelings, verbal, and physical expression), all being rated on a 4-point Likert-type scale (1 = "not at all" to 4 = "very much so"). Cronbach's alpha ranged from .67 to .89. To reduce the number of tests, increase power for effect size, and aid interpretation within a conceptual framework, S-Ang subscales were combined into single variable. Hence, we considered a score (S-Ang).

Self-esteem was measured using the Spanish translation of the RSES (Echeburúa & Corral, 1998). The RSES consists of ten items and one factor (selfesteem) ranked on a 5-point Likert scale. A higher score indicates greater self-esteem. Cronbach's alpha was then .90.

To assess a broad range of psychological problems and psychopathological features, as well as provide an overview of patient symptoms and their intensity at specific points in time, a Spanish versión of the Symptom Checklist 90-Revised (SCL-90-R) was used (González de Rivera et al., 1989).

This self-report scale consists of 90 items divided into nine subscales: somatization (SOM); obsessivecompulsive (OBS); interpersonal sensitivity (INT); depression (DEP); anxiety (ANX); hostility (HOS); phobic anxiety (PHOB); paranoid ideation (PAR); and psychoticism (PSY). We also calculated three summary scores: global severity index (GSI); positive symptom distress index (PSDI); and positive symptom total (PST). Participants indicated the extent to which they had been bothered by the symptom described in the previous week on a scale from 0 (not at all) to 4 (very often). A higher score represents more psychological symptoms. Cronbach's alpha ranged from .72 to .86.

Hormone measurements

Saliva was directly collected from the mouth using Salivettes for C (Sarstedt, Rommelsdorf, Germany) and sterile glass tubes for T measurements. In all cases, participants were informed about the need to follow the instructions for saliva sampling to obtain meaningful data and samples were collected in the same order, C then T, and frozen at –20◦C until analysis.

Salivary T levels were assessed by chemiluminescence immunoassays using testosterone saliva ELISA kits (Diagnostics Biochem Canada). Intra- and interassay coefficients of variation were 3.98% and 7.98%, respectively, indicating good reproducibility. Salivary C levels were determined by radioimmunoassay using Coat-to-Count cortisol kits (DPC-Siemens Medical Solutions Diagnostics) with 1.4 nmol/L sensitivity.

All samples were analyzed in duplicate and those from the same subject were included in the same assay. Though the criterion for measurement replication was a coefficient of variation between duplicates of 8%, the

maximum intra- and inter-assay coefficients of variation obtained were 4.3% and 5.2%, respectively.

Data analysis

t-Tests with Levene's test for equality of variances were used to check for significant differences in age, BMI, RSES, and SCL-90-R between the groups (IPV and control men).

After confirming the normality of the data using the Kolmogorov–Smirnov test, the effectiveness of the stressor in the total sample was confirmed by general linear model repeated measures ANOVA with "time" (at six levels: baseline, preparation period, and 0, 15, 30, and 45 minutes after the TSST) as a withinsubjects factor. To examine group effects, repeated measures ANOVA was conducted with "time" as the within-subject and "group" as the between-subject factors.

Analysis of the AUC enabled hormonal responses to the stressor to be quantified, using the trapezoid formula (Pruessner, Kirschbaum, Meinlschmidt, & Hellhammer, 2003; Stalder et al., 2011). Partial AUC values were calculated by only considering the time points before the previous measurement. To achieve this, differences between each of the five hormonal values (preparation period and 0, 15, 30, and 45 minutes after TSST) and the baseline level were summed to obtain a single AUC.

For S-Ang responses, repeated ANOVAs with "moment" (pre and post) as the within-subject factor and "group" as the between-subject factor were performed.

Greenhouse–Geisser corrections for degrees of freedom and Bonferroni corrections for multiple comparisons were applied where appropriate. For significant results, partial eta squared (η^2_p) is reported as a measure for effect size. Pearson correlation tests were performed where appropriate to examine relationships between T/C ratios and anger state, RSES, and SCL-90-R scores.

Data analyses were performed using SPSS 17.0 (SPSS IBM). All reported p-values are two-tailed except for one-way ANOVAs, and $p \leq .05$ was considered significant. Average values are expressed as mean±SEM.

Results

Sample characteristics, T/C ratio and anger

Groups did not differ significantly in age, BMI and SCL-90-R. However, IPV perpetrators showed lower scores in the RSES than controls ($t(32) = -15.30$, $p < .00$, $d = 5.41$) (Table 1).

Table 1. Mean±SD of anthropometric, trait and state profiles for IPV perpetrators and controls. (*) p <.05

		IPV men (n=16)	Controls (n=21)
Age (years)		38.31±10.37	35.81±6.76
BMI (Kg/m²)		26.81±3.33	27.55±2.89
STAXI-2 S-Ang baseline**		5.56±1.45	5.07±0.18
STAXI-2 S-Ang post-task		5.35±0.75	5.30±0.59
Rosenberg Self-Esteem Scale (*RSES)***		25.00±1.41	36.13±2.45
Symptom Checklist 90-Revised (SCL-90-R)	Somatisation (SOM)	0.65±0.73	0.40±0.32
	Obsessive-compulsive (OBS)	0.65±0.69	0.59±0.37
	Interpersonal Sensitivity (INT)	0.84±0.38	0.49±0.37
	Depression (DEP)	0.89±0.83	0.36±0.29
	Anxiety (ANX)	0.62±0.76	0.32±0.23
	Hostility (HOS)	0.38±0.71	0.50±0.57
	Phobic anxiety (PHOB)	0.34±0.57	0.10±0.13
	Paranoid ideation (PAR)	0.63±0.91	0.64±0.51
	Psychoticism (PSY)	0.41±0.56	0.23±0.30
	Global severity index (GSI)	0.63±0.67	0.46±0.28
	Positive symptom distress ind (PSDI)	26.53±23.18	35.56+24.20
	Positive symptom total (PST)	2.39±4.61	1.11±0.04

Effectiveness of the TSST in eliciting T/C ratio response

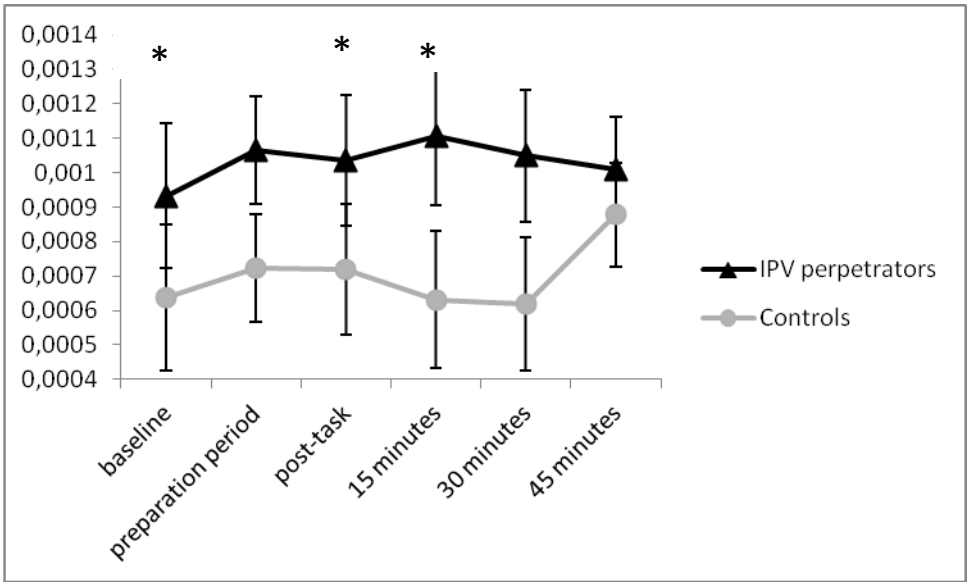
The stressor used in our study, the TSST, was effective and the factor "time" was significant for the T/C ratio of the total sample [$\varepsilon = .53$, $F(2.66, 93.13) = 2.96$, $p < .04$, $\eta_p^2 = .08$]. For all subjects, T/C ratios significantly increased from baseline to the preparation period ($p < .05$) and decreased from preparation to post-task periods, these changes being non-significant; but then increased significantly from 30 to 45 minutes post-task ($p < .05$).

Role of group in the T/C responses to the TSST

There was a significant "time × group" interaction [$\varepsilon = .53$, $F(2.66, 93.13) = 3.19$, $p < .05$, $\eta_p^2 = .08$] with IPV perpetrators having higher T/C ratios than controls in the preparation period, as well as 15 and 30 minutes

post-task (all $p < .05$) (Figure 1). The magnitude of total T/C ratio response did not differ between groups.

Figure 1. T/C ratio levels for IPV perpetrators and control men. (*) p < .05



S-Ang responses to the TSST

A significant effect for the "time × group" was found for S-Ang ($F(1, 35) = 8.17$, $p < .01$, $\eta_p^2 = .19$). In fact, IPV perpetrators had higher baseline S-Ang levels than controls ($p < .05$). Moreover, the stressor provoked a decrease in S-Ang among IPV perpetrators but an increase in controls. No significant correlations were found between S-Anger and T/C AUCs for IPV perpetrators or controls.

Relationship between T/C ratio, psychopathological symptoms, and self-esteem

The total AUC of the T/C ratio was negatively correlated with phobic anxiety, psychoticism, and interpersonal sensitivity (PHOB, $r = -.490$, PSY, $r = -.455$ and INT, $r = -.494$, $p < .05$); and positively with self-esteem (RSES score, $r = .450$, $p < .05$). Partial AUCs from baseline to the preparation and 0-minute post-task periods were negatively correlated with obsessive-compulsive symptoms (OBS; $r = -.490$ and $r = -.445$, respectively, both $p < .05$). However, there were no significant correlations between the AUC of the T/C ratio and the RSES and SCL-90-R in controls.

Discussion

IPV perpetrators had higher T/C ratios than controls in response to acute stress, particularly for the preparation, and 15- and 30-minute post-task periods. There were, however, no differences between groups in the magnitude of the T/C ratio response to stress. High T/C ratio responses were only significantly associated with high self-esteem and better mental health in IPV perpetrators.

The validity of the stress protocol was demonstrated by the significant increase in T/C ratios in all subjects. Indeed, in an earlier phase of this research, we found that T and C levels decreased significantly from baseline to the preparation period in response to the TSST in IPV perpetrators. Subsequently, perpetrator T and C levels increased from that period to post-task, and T then decreased from that point to 45 minutes post-task. However, C levels increased from post-task to 30 minutes post-task. Finally, C decreased from 30 to 45 minutes post-task. Regarding controls, T and C levels followed a similar pattern from baseline to post-task periods, but T increased from 30 to 45 minutes post-task. These findings confirm that differences between the groups are not due to the emotionally-charged topic of the TSST. Popma et al., 2007, hypothesized that men with high aggression scores have higher basal T and lower C levels than controls. However, our earlier research did not support this because the baseline T and C levels in IPV perpetrators and controls were not significantly different. We did, however, find that IPV perpetrators have higher T levels in preparation and post-task periods than controls, and although C levels increase in all subjects, the rise is more pronounced in controls. This increase in C levels explains why, as seen in the present study, controls have larger increases in T/C ratios, specifically from 30 to 45 minutes post-task. Further, at this stage, T and C levels decreased in IPV perpetrators, remaining low to the end of the post-task period. Thus, high T levels in IPV perpetrators and high C levels in controls would lead to higher T/C ratios in the former.

Overall, these data suggest that the T/C ratio may be useful for identifying men who are violent or prone to violence, and that the three key time points are the preparation and the 15 and 30 minute post-task periods. Compared to non-violent individuals, violent individuals may have a faster anticipatory response to stress, which would prepare them for fighting, and would maintain this level more effectively after the stress stopped. Nonetheless, an imbalance between hormones could explain greater proneness to aggressive

behavior among healthy as well as violent individuals (Montoya, Terburg, Bos, & van Honk, 2012). This idea has been demonstrated in our findings; control men with high responsivity showing high T/C ratio increases with respect to baseline and an increase in hostile feelings when the stress ended. Although IPV perpetrators had higher T/C ratios and baseline feelings of anger, these feelings decreased after the stress. This could explain why, although increases in anger feelings in IPV perpetrators are limited to the domestic domain, they tend to express anger more frequently than controls.Moreover, only severely violent IPV perpetrators experience a pronounced increase in anger after marital conflict discussion tasks (Babcock et al., 2005).

Psychological characteristics such as high self-esteem combined with high T/C ratios increase the likelihood of aggressive behavior in IPV perpetrators (van Honk & Schutter, 2007). Initially, it was assumed that IPV perpetrators were characterized by low selfesteem, but it has been reported that such individuals have high self-esteem (Baumister, Smart, & Boden, 1996). Our data supports low self-esteem in IPV perpetrators. The interpretation of any signal as a threat may play a role in the onset of violent behavior (Bushman et al., 2009). Hence, they may be violent to their partners to reinforce their power. Our results reveal that increases in T/C ratios with respect to baseline are linked to high self-esteem and better psychological health. It has been hypothesized that heterosexual couples with comparable T levels (high or low levels in both the man and the woman) report more physical assaults than those with discordant levels (high-low or low-high). This could be due to the fact that in the first case, both partners may fight to establish dominance (Cohan, Booth, & Granger, 2003). Arguing with another person could be considered an acute stressor and the effects of arguing on the endocrine system and psychological state can be simulated using a standardized psychosocial stress test such as the TSST. Moreover, a high T/C ratio would be a marker of dominance, characterized by high self-esteem and better health. This could, however, impair the health of battered women (Inslicht et al., 2006) who are in the subordinate position.

A limitation of the study is the small sample size, and hence the findings should be considered preliminary. Further research is needed to replicate these findings in larger samples. Another methodological limitation of this type of study is the fact that it not possible to collect saliva during the stressor. In conclusion, our study reveals that the relationship between anger and the T/C ratio is similar in IPV perpetrators and men with no history of violence but also that the T/C ratio, together with other psychological characteristics, may

be a useful trait marker of violence in IPV perpetrators. However, to explain why IPV perpetrators cannot inhibit their violence, neuropsychological variables need to be explored to assess executive functioning and impulse control (as well as their interactions with hormonal markers). Our data are relevant and novel as no laboratory studies have examined T/C ratio responses to psychological stress in the context of IPV. In particular, they could benefit rehabilitation programs for abusers involving community-based psychotherapy focused on changing beliefs, biases, and/or the cognitive distortions of offenders. It would be useful to analyze other subsamples of offenders as this could improve our understanding of this complex phenomenon.

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Chapter 6

DISCUSSION

General discussion

Our results with ASD parents and male perpetrators of intimate partner violence lead us to believe that high levels of prenatal masculinized traits exist in both populations. Nevertheless, the degree of masculinization differs between them. In both cases, the 2D:4D ratio was a good indicator for characterizing high cognitiva and behavioral masculinization, as previously demonstrated (van-den-Bergh & Dewitte, 2006). Our study did not reveal differences between the ASD parent and control groups in the 2D:4D ratio. However, it was a good marker for explaining cognitive hypermasculinization in ASD parents – characterized by high anger expression, low empathy and high autistic and systemizing traits. Thus, our results support the hypotheses that early high T prenatal exposition could be involved in the development of an extreme male brain (Auyeung, Baron-Cohen, Ashwin, Knickmeyer, Taylor, & Hackett, 2009; Auyeung, Baron-Cohen, Chapman, Knickmeyer, Taylor, & Hackett, 2006; Saenz & Alexander, 2013).

IPV perpetrators showed lower or more masculinized 2D:4D ratios than controls. The high masculinization is associated with low empathy and larger increases in T levels in response to acute stress. These results supported previous findings that early high T exposition is associated with aggressive behavior, or proneness to aggressive behavior in men (Bailey & Hurd, 2005; Saenz & Alexander, 2013), and high sensitivity to activational T effects during adulthood (van Honk et al., 2011). Hence, masculinization was presented in a different way in both populations (ASD parents show a hypermaculinized brain, and IPV perpetrators show proneness to aggressive behavior due to high sensitivity to changess in T levels). Nevertheless, in both populations the relationship between high early T exposure and aggression was similar. Hence, future research should consider the relationship between T, autistic traits, and aggressive behavior in order to offer a wider explanation of the facilitation of aggressive behavior by analyzing the modulator variables.

Parents of people with autism spectrum disorders

Our results support the existence of a BAP in ASD parents as characterized by higher autistic traits than normative population (Sucksmith et al., 2011; Wheelwright, Auyeung, Allison, & Baron-Cohen, 2010). Moreover, as postulated in empathizing-systemizing theory, ASD parents show less empathy than controls. Based on the postulate of 'extreme male brain' theory, these traits may be associated with high fetal testosterone exposure (Auyeung

et al., 2006; 2009). It could be expected that there is a high T exposure in ASD parents as they present a smaller or masculinized 2D:4D ratio (a peripheral indicator of fetal T exposure) than the normative population (Manning et al., 2001). However, no differences were found between groups or gender in the first study. This can be explained because the sample was composed of parents of people with classic autism, asperger syndrome, as well as pervasive developmental disorder – and excessive masculinization can only explain a few of these disorders. This hypothesis was supported by previous findings because children with Asperger syndrome presented higher 2D:4D ratios than those with classic autism – although in both cases they presented lower 2D:4D ratios than controls (Manning et al., 2001). Moreover, the second study reinforced the importance of the homogenous diagnostic subsamples of ASD as gender differences were found in the 2D:4D ratio of parents of offspring with Asperger and ADHD comorbid symptoms. The relationship between the 2D:4D ratio and current T levels isinconsistent because only one study with men with fertility problems has found a negative relationship between both variables (Manning et al., 2004). Nonetheless, no association in the normative population of both genders was found between the 2D:4D ratio and current T levels (Hönekopp et al., 2007). In our first study, we did not find an association between the 2D:4D ratio and basal salivary T levels; although in the second study, involving ASD parents with offspring with inattentive ADHD comorbid symptoms, we found a negative association between these masculinization markers. This reinforces the previous hypothesis that the 2D:4D ratio will be more useful in sub-samples of people with ASD and their offspring. Hence, our second study supports that in individuals with excessively masculine brains, the 2D:4D ratio is related to autistic traits, as well as current T levels (Bejerot, Eriksson, Bonde, Carlström, Humble, & Eriksson, 2012).

In this sub-sample of ASD parents, the 2D:4D ratio was related with high levels of inattention (also among ASD individuals) as measured by high levels of T. Thus, it was suggested that the high intrauterine T exposure of ASD parents would make them more likely to show the same high current T levels during adulthood as their offspring (Ingudomnukul, Baron-Cohen, Wheelwright, & Knickmeyer, 2007). However, our study only reveals differences by gender and not group. Only two baseline hormonal samples were employed – rather than T response to stress or changes produced during the day by secretions of the HPG axis.

Regarding the sexual dimorphism of the 2D:4D ratio (Manning et al., 2000), the second study reinforced the importance of sub-samples among ASD samples because in this study only sex differences in the 2D:4D ratio were obtained. It has been suggested that the right-hand 2D:4D ratio is more sensitive to the effects of prenatal T (Hönekopp & Watson, 2010), although another study showed that 2D:4D ratio of the left hand is a good marker of such a condition (Bull & Benson, 2006). In the first study the left ratio dominated, but the right 2D:4D ratio was employed in the second study because it has greater predictive power. Our results suggest the importance of using both markers for defining cognitive hypermasculinization. Moreover, in our first study, fathers presented higher C levels than mothers. Nonetheless, women tend to show higher C levels than men, although there were studies that reported the opposite (Gusenoff et al., 2001; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Laughlin & Barrett-Connor, 2000; Zhao, Lu, Xie, Fu, Bogdan, & Touitou, 2003). Hence, gender differences in our study could be partially explained by the masculization affecting C levels – as has been reported in our studies with IPV perpetrators.

Cognitive features presented by ASD parents as a result of high prenatal T exposure have been defined as BAP, which is important for the etiology of the ASD. In our first study, the high masculinization in ASD parents, particularly in fathers, was associated with greater severity in autistic traits by offspring. Meanwhile, in the second study, the high prenatal masculinization of both parents (inattentive related symptoms of parents) was associated with increased inattention traits in offspring. Therefore, these studies provide new results that highlight the importance of T in the prenatal context of ASD and ADHD. Thus, following the results of our first study we can conclude that in ASD, the masculinization of the fathers is important; but that the masculinization of both parents is important for inattentive ADHD comorbid symptoms of ASD. This confirms the initial hypothesis that the 2D:4D ratio could be used, together with other parameters, as a valid indicator of predisposition to autism (Manning & Bundred, 2000).

The main limitation of our research is that the cross-sectional and non-experimental design meant that causality could not be addressed. Furthermore, the ASD parents are first-degree relatives and did not present ASD. The masculinization described throughout the study is subtle and requires further analysis in order to correctly define the BAP. Moreover, the experience of caregivers of offspring with ASD may have considerable effects

on the physiology of parents (specific T and C levels). Hence, this variable should be considered in future research. However, the comparative lack of studies analyzing masculinization in parents of individuals with ASD makes our results relevant to the scientific literature and of preventive value. Moreover, the personal situation of the participants makes conducting longitudinal studies extremely difficult, and so our study is relevant for studies of ASD etiology.

Our research in this area has two objectives. Firstly, analyse the current T response to several cognitive tasks and how those changes affect the cognitive performance (Romero-Martínez, de Andrés-García, Ruiz-Robledillo, González-Bono, & Moya-Albiol, under review). Secondly, it would be useful to compare the sample of ASD parents with other populations in which prenatal androgenisation may of had neuroprotective effects – as in the case of eating disorders (Klump, Gobrogge, Perkins, Thorne, Sisk, & Breedlove, 2006; Romero-Martínez, González-Bono, & Moya-Albiol, in preparation).

The main benefit of these studies lies in the prevention of ASD – as the emotional and financial cost is very high for parents. The use of relatively cheap masculinization markers has enabled the targeting of future genetics with respect to the synthesis of T and its receptors. We must not neglect the fact that masculinization does not seem to be homogenous in the spectrum of autism disorders. To achieve homogeneity, it would be necessary to clarify the different disorders that constitute ASD – although in the DSM-V it is considered as a unitary disorder with different levels of severity (Wing, Gould, & Gillberg, 2011).

Male perpetrators of intimate partner violence

IPV perpetrators presented a smaller or masculinized 2D:4D ratio than controls. Thus, IPV perpetrators may have been exposed to high prenatal T levels (Hönekopp et al., 2007) and so an increased genetic sensitivity to T fluctuations during adulthood (van-Honk et al., 2011) may also be present, which could be explained by a high level of androgenic receptor sensitivity (Manning, Bundred, Newton, & Flanagan, 2003). In the first study, the 2D:4D ratio was related to large T increases in response to acute stress only in IPV perpetrators. These changes in T response were associated with high negative affect (high anger and anxiety and worse mood). Furthermore, a smaller 2D:4D ratio was related with worse emotional decoding processes. This tempory lack of empathy and increase in negative affect could explain the

perpetration of violence, as previously reported (Carr & Lutjemeier, 2005). The high sensitivity of the HPG axis in IPV perpetrators affects the normal fuctioning of the HPA axis because T inhibits the C levels. T facilitates the attribution of hostile connotations; meanwhile C reduces T levels and diminishes the likelihood of behaving aggressively by heightening sensitivity to social punishment (Terburg et al., 2009). Our first study revealed that slight changes in C levels in IPV perpetrators in comparison with controls may suggest hypoactivity of the HPA axis, which has been associated with aggression and other behavioral problems in violent participants. These results support the imbalance between the HPG and HPA axis, or between the T and C levels that characterize violent individuals (Popma et al., 2007). The quotient between T and C could be defined as the T/C ratio and used as an indicator of proneness to violence (Terburg et al., 2009). In our second study, we concluded that the T/C ratio is a marker of aggressive behavior in this population. We then hypothesized that the imbalance between T and C facilitates aggressive behavior in men with specific cognitive characteristics, such as low cognitive flexibility and an emotional decoding process that could be associated with low cognitive empathy, as well as attributing hostile connotations to neutral stimuli. Furthermore, the low self-esteem of IPV perpetrators increases the greater the imbalance between the HPG and HPA axes, or the greater the predisposition for violence towards their partners. Partner submission improves the self-esteem of IPV perpetrators, as well as their health or psychopathological symptoms.

As suggested in ASD parents, the major limitation is the cross-sectional design that limits causality. In addition, the small sample size limits external validity. In this sense, although structural equation modeling could improve the quality of the results, the sample size makes it impossible to employ these statistical analyses. Thus, it should be considered in future research with larger sample sizes. However, our data is relevant and novel as no studies have previously analyzed the psychological and hormonal responses to psychological stress as related to domestic violence in a laboratory context. Our current studies in this field analyze the autonomic nervous system and immune correlates of IPV perpetrators (Romero-Martínez, Lila, Conchell, González-Bono, & Moya-Albiol, second review; Romero-Martínez, Lila, Williams, González-Bono, & Moya-Albiol, in press) and so facilitate a wider understanding of the complex phenomenon of intimate partner violence. Because of the promising results obtained in the neuropsychological variables, it would be desirable to use more complex assessment batteries that enable an

analysis of possible cognitive deficits that might underlie those discussed in these studies. The role of toxics such as alcohol could explain both the facilitation and maintenance of violent behavior by acting as a catalytic factor (Romero-Martínez, Lila, Catalá-Miñana, Williams, & Moya-Albiol, 2013; Romero-Martínez & Moya-Albiol, in press).

The main benefit of these studies lies in reproducing the response of offenders to an acute stressor (as could be a discussion between partners). The imbalance in the neuroendocrine axes with certain cognitive biases, and/or emotional factors, facilitates the perpetration of violence. Therefore, an analysis of hormonal, psychological, and neuropsychological parameters enables the creation of categories for the identification of violent people. This would result in improved intervention programs, based mainly on psychological parameters, whose main objective is reintegration. Such an analysis would also elucidate the factors that contribute to poor adherence to treatment programs and medium and long term recidivism.

This Ph.D work is applicable in clinical and therapeutic cases. It may help in the development of new therapeutic strategies for the social reintegration of individuals charged with domestic violence. Psychobiological analysis provides the opportunity for a wider understanding of a complex phenomenon such as IPV, and thereby improve current therapies that show limited benefits. It may be possible to detect psychoendocrine and contextual profiles of individuals with a high likelihood of ASD developing in their offspring and develop preventative strategies. Moreover, it would contribute to the detection of predispositional factors for inappropiate behavior.

On a scientific level, it would be necessary to analyze the consequences of masculinization in CNS and its modulating effects on behavior. This could improve the quality of life of parents of individuals with ASD as caregivers, and help establish more effective therapies for IPV perpetrators.

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Chapter 1 (Spanish)

INTRODUCCIÓN

Introducción general

Los esteroides sexuales como la testosterona (T) producen una organización y/o modificaciones relativamente permanentes de la estructura y función del Sistema Nervioso Central (SNC) (Filová, Ostatníková, Celec, y Hodosy, 2013; Lust, Geuze, Van de Beek, Cohen-Kettenis, Bouma, y Groothuis, 2011). Una alta exposición prenatal a la T podría estar relacionada con una mayor sensibilidad a los efectos activadores de dicha hormona durante la edad adulta (van-Honk, Schutter, Bos, Kruijt, Lentjes, y Baron-Cohen, 2011). Por otra parte, las fluctuaciones en los niveles endógenos de T pueden modificar transitoriamente las capacidades socio-cognitivas (Celec, Ostatnikova, Putz, y Kudela, 2002; Ostatníková, Laznibatová, y Dohnányiová, 1996; Ostatníková y cols., 2007; van-Honk y cols., 2011; van Rooij y cols., 2012; Wirth, y Schultheiss, 2007). Así, la conducta y los procesos cognitivos podrían ser el resultado de la interacción entre los efectos hormonales organizadores y activadores. Sin embargo, los estudios que abordan las implicaciones de esta interacción son escasos.

En cuanto a los efectos organizadores, se ha asociado una elevada androgenización prenatal a perfiles cognitivos concretos con implicaciones sobre variables fisiológicas y de salud como una alta sistematización y una baja empatía (Nakayama, Takahashi, Wakabayashi, Oono y Radford, 2007), a la facilitación de comportamientos agresivos (Bailey y Hurd, 2005; Hampson, Ellis y Tenk, 2008), a la falta de empatía (Von-Horn, Bäckman, Davidsson y Hansen, 2010) y a la búsqueda de sensaciones (Hampson, Ellis, y Tenk, 2008). Debido a la dificultad de analizar la androgenización a nivel del SNC resulta de gran utilidad emplear indicadores periféricos, como la ratio interdigital D2:D4. Se trata del cociente entre la longitud de los dedos índice (D2) y anular (D4), el cual se relaciona inversamente con los niveles prenatales de T. Por tanto, una menor ratio D2:D4 indicaría una alta androgenización antes del nacimiento (Bull, Davidson, y Nordmann, 2010). Sin embargo, su relación con los niveles de T en la etapa adulta es débil o incluso nula (Hönekopp, Bartholdt, Beier y Liebert, 2007). Una población en la que una posible androgenización prenatal excesiva desempeña un papel importante son las personas con Trastornos del Espectro Autista (TEA), específicamente aquellas con autismo de alto funcionamiento (Scheeren y Stauder, 2008; De Bruin, Verhiej, Weigman, y Ferdinand, 2006), así como otros trastornos con una alta comorbilidad con los TEA, como los Trastornos por Déficit de Atención con Hiperactividad (TDAH) (Ames y White, 2010; Sucksmith,

Roth, y Hoekstra, 2011). Ha sido hallada una relación positiva entre la ratio D2:D4 de los niños con TEA y la ratio D2:D4 de sus familiares. Por tanto, se hipotetizó que los progenitores (tanto los padres como las madres) con una ratio D2:D4 masculinizada podrían proporcionar ciertos factores biológicos, tales como una alta T prenatal, que incrementarían la probabilidad de que su descendencia desarrollara TEA (Manning, Baron-Cohen, Wheelwright y Sanders, 2001).

Las personas que padecen estas alteraciones, con una elevada heredabilidad, están aquejadas de alteraciones conductuales y cognitivas que conllevan un solapamiento de efectos. El estudio de sus progenitores puede arrojar luz a esta cuestión en la medida en que presentan ciertos patrones cognitivos androgenizados, aunque sin llegar a desarrollar completamente dichos trastornos (Kose, Bora, Erermiş, Ozbaran, Bildik, y Aydın, 2013). Sin embargo, hasta el momento actual escasas investigaciones científicas han analizado los efectos activadores de la T en esta población. Además, las personas con TEA presentan una serie de problemas de conducta comunes tales como altos niveles de agresividad, reactividad emocional ante situaciones estresantes y ansiedad (Kaartinen, Puura, Helminen, Salmelin, Pelkonen y Juujärvi, 2012; Pouw, Rieffe, Oosterveld, Huskens y Stockmann, 2013; Saenz y Alexander, 2013; Schwichtenberg y cols., 2013). Sin embargo, el rol de la T en la relación entre los TEA y la agresividad no se encuentra tan bien estudiado y establecido como en los hombres penados por violencia contra la mujer en el ámbito doméstico.

Respecto a los efectos activadores, un elevado número de estudios tanto en animales como en humanos han relacionado la T con la agresión y la violencia. En este sentido, los andrógenos disminuyen el umbral para la irritabilidad, la hostilidad y la violencia. Aunque la hipótesis clásica establecía una relación causal entre la T y la agresión, en la actualidad es considerada un factor modulador que, junto a muchos otros (genéticos, hormonales, neuroquímicos, ambientales, etc.) incrementaría la probabilidad de que aparezca (Moya-Albiol, 2010). Una población específica en la que los efectos activadores de la T podrían ejercer una influencia sobre la conducta agresiva es la de hombres penados por violencia contra la mujer en el ámbito doméstico. Sin embargo, no hay evidencia empírica que avale si los efectos organizadores de los andrógenos en esta población podría facilitar la adopción del comportamiento agresivo ante estímulos específicos.

A la vista de todo lo expuesto, el objetivo principal de esta tesis doctoral es abordar los efectos organizadores y activadores de los andrógenos en dos poblaciones específicas, empleando variables comunes en todos los estudios tales como la ratio D2:D4 y los niveles en circulación de T y C, una asociada a una elevada androgenización prenatal, en concreto progenitores de personas con TEA, y otra relacionada con una mayor activación de la conducta agresiva, como es el caso de los hombres penados por violencia contra la mujer en el ámbito doméstico.

Progenitores de personas con TEA

Altos niveles de T antes del nacimiento han sido asociados a comportamientos típicamente masculinos (Knickmeyer y Baron-Cohen, 2006). Además, un exceso prenatal de andrógenos podría incrementar la probabilidad de desarrollar los TEA, caracterizados por poseer cerebros "extremadamente masculinos" (Baron-Cohen, 2010a). Las personas afectadas por los TEA poseen déficits de empatía, puesto que son incapaces o tienen graves dificultades para inferir estados emocionales propios y/o ajenos (Baron-Cohen, 2010b). En cambio, poseen una buena capacidad de sistematización (Baron-Cohen, 2010a), aunque sus intereses son muy restringidos y presentan cierta rigidez para fijar su atención en nuevos objetivos. En el caso de sus progenitores de personas con TEA, se han detectado indicios sutiles de androgenización cognitiva. De este modo, poseen rasgos similares a los de sus descendientes, aunque no lo suficientemente marcados como para llegar a un diagnóstico del trastorno, por lo que han sido englobados en el denominado Fenotipo Autista Amplio (FAA) (Scheeren y Stauder, 2008). Este fenotipo no está sólo caracterizado porque los progenitores manifiesten más rasgos autistas y sistematizadores y menos empáticos que la población general, sino por la existencia de déficits neuropsicológicos. En este sentido, los déficits principales son de tipo ejecutivo (Sucksmith, Roth y Hoekstra, 2011), aunque también se han descrito déficits atencionales, que podrían plasmarse en síntomas o cuadros subsindrómicos de inatención e hiperactividad. Este resultado es avalado por recientes estudios que han revelado la alta comorbilidad entre los TEA y el TDAH (Sucksmith y cols., 2011), así como por la implicación de los esteroides sexuales en el desarrollo de ambos (James, 2008).

Teniendo en consideración que los progenitores de personas con TEA podrían presenter un endofenotipo idiosincrásico, el objetivo principal de estos estudios es estudios si los padres de personas con TEA presentan ciertos rasgos que los diferencien de la población general. Para ello, se emplearán distintos indicadores de androgenización, con especial énfasis en la ratio D2:D4. Además, otro objetivo de los estudios es el de estudiar si la ratio D2:D4 podría ser utilizado como marcador de otras variables psicobiológicas de masculinización en esta población (especialmente los niveles circulatorios de T), y la gravedad de los TEA y síntomas de TDAH comórbidos en su descendencia. La elevada androgenización en progenitores de personas con TEA se plasma en el hecho de que poseen una menor ratio D2:D4 que la población general (Manning y cols., 2001). A su vez, esta alta androgenización conduciría a que presenten una mayor sensibilidad a los efectos activadores de la T (Moffat y Hampson, 1996; van Honk y cols., 2011). Por todo ello, el análisis del endofenotipo específico de los progenitores de TEA o FAA, conduciría a una mejor comprensión de la etiología de estos trastornos, así como el rol que en ellos desempeñan los esteroides sexuales.

Hombres penados por violencia contra la mujer en el ámbito doméstico

La T en hombres, como un indicador de la actividad del eje hipotálamo-hipofiso-gonadal (HHG), no está únicamente relacionada con el desarrollo de diversos trastornos neuropsiquiátricos, sino que también desempeña un rol fundamental en la modulación del comportamiento violento. Además, una mayor exposición prenatal a la T (definida por un ratio D2:D4 masculinizada) ha sido relacionada con una mayor probabilidad de expresar la ira físicamente (Bailey y Hurd, 2005). En este sentido, se ha descrito niveles basales elevados de T en diversas tipologías de varones violentos, como los violadores, agresores con dependencia del alcohol y criminales con personalidad antisocial (Moya-Albiol, 2010).

El cortisol (C), producto del eje hipotálamo-hipofiso-adrenal (HHA), podría actuar como freno del comportamiento agresivo modulando los niveles de T, por el efecto recíproco e inverso entre los ejes HHG e HHA (Terburg, Morgan, y van Honk, 2009). En este sentido, los sujetos más violentos han mostrado tener menores niveles de C (Popma y cols., 2007). Por ello, un eje HHA hipoactivo facilitaría la expresión de la agresión. El cociente entre

ambas hormonas, T y C, ha sido empleado como indicador de agresión social (Terbug y cols., 2009). La actividad recíproca de ambos ejes modula la expresión de la violencia, e interacciona con déficits neuropsicológicos, tanto en las funciones ejecutivas (Teichner, Golden, Van Hasselt, y Peterson, 2001), como la empatía (Babcock, Green, y Webb, 2008) y en diversos factores de personalidad como la ira y la hostilidad rasgo (Norlander y Eckhardt, 2005). Dichos déficits contribuyen a la adopción de comportamientos inapropiados que predispondrían a la violencia (Tirapu-Ustárroz, Pérez-Sayes, Erekatxo-Bilbao, y Pelegrín-Valero, 2007).

La T podría ser considerada como un predictor de rasgos masculinizados tales como los rasgos autistas y la alta agresividad (Saenz y Alexander, 2013). Por tanto, analizamos el efecto de la exposición prenatal a T en dos poblaciones específicas, estudiando la ratio D2:D4, los niveles en circulación de T y sus cambios en respuesta a un estresor agudo de laboratorio, y la relación de estas variables con el resto de variables que evalúan la androgenización. A raíz de todo lo comentado, esta Tesis Doctoral se enmarca en el análisis de la androgenización en dos poblaciones específicas, empleando para ello los niveles basales de T y los cambios en dicha hormona en respuesta a estresores de laboratorios y tareas cognitivas y/o sociales. Además, se han utilizado indicadores periféricos de los efectos organizadores como la ratio D2:D4 y los estilos cognitivos, con el objetivo último de determinar factores predisposicionales y moduladores de la conducta. Para ello, se ha considerado una perspectiva integradora que incluye parámetros de estudio de diverso tipo, como los psicológicos, neuropsicológicos y hormonales, que modulan la relación entre la T y la cognición y el comportamiento en sujetos con distintos niveles de androgenización.

En base a lo comentado, los principales objetivos de esta Tesis Doctoral, así como las hipótesis de trabajo son los que se detallan a continuación:

1. Caracterizar a los progenitores de personas con TEA mediante indicadores de androgenización (la ratio D2:D4 y la T basal), hormonales (C basal) y variables psicológicas (agresión rasgo, empatía, cooperación, sistematización y grado de autismo). Analizar además la relación de estos marcadores con los TEA de su descendencia. Se ha hipotetizado que los progenitores de personas con TEA podrían estar más androgenizados que la población normal. Por tanto, dichas

características podrían ser descritas como pertenecientes al FAA y la ratio D2:D4 podría ser un buen indicador de dicha condición (Manning y cols., 2001). Además, en línea con los resultados de investigaciones previas a nivel cognitivo (Baron-Cohen, 2010b), hipotetizamos que la ratio D2:D4 no será distinta en progenitores de personas con TEA, debido a su alta androgenización. Finalmente, la ratio D2:D4 será un buen predictor de la gravedad de los síntomas de TEA de su descendencia. De modo que una ratio D2:D4 menor o masculinizada se asociará con síntomas más severos (Manning y Bundred, 2000).

2. Analizar el papel de la androgenización en progenitores de personas con TEA sobre la comorbilidad de síntomas de TDAH en los descendientes. Hipotetizamos que los niveles de T de los progenitores de personas con TEA mediarán la relación entre una ratio D2:D4 masculinizada y los síntomas de inatención de los propios progenitores. Por tanto, altos niveles circulatorios de T se asociarán con una ratio D2:D4 masculinizada y con más síntomas de inatención en los progenitores. Finalmente, altos síntomas de inatención se asociarán con una menor ratio D2:D4 de los progenitores y con más síntomas de TDAH en sus hijos. Además, la relación entre la masculinización y los síntomas de TDAH serán más fuertes en los síntomas de inatención que en los de hiperactividad tal y como se ha demostrado en un estudio previo (McFadden, Westhafer, Pasanen, Carlson y Tucker, 2005).

3. Estudiar la respuesta de la T y el C de hombres penados por violencia contra la mujer en el ámbito doméstico a un estresor en comparación con sujetos control, así como su relación con otras variables neuropsicológicas y psicológicas. Se pretende analizar el papel modulador de la T en el comportamiento agresivo. Hipotetizamos que los hombres penados por violencia contra la mujer en el ámbito doméstico presentarán niveles altos de T basal (Soler y cols., 2000) y bajos niveles de C (Moya-Albiol, 2010) que los controles. Además, los hombres penados por violencia contra la mujer en el ámbito doméstico también presentarán altos niveles de déficits cognitivos tales como una pobre flexibilidad cognitiva (Teichner y cols., 2001) y un pobre reconocimiento de las emociones y los pensamientos (Holtzworth-Munroe y Smutzler, 1996), especialmente para aquellas con valencia neutra (Babock y cols., 2008). Finalmente, también hipotetizamos que los hombres penados por violencia contra la mujer en el ámbito

doméstico deberán presentar bajos niveles de empatía emocional (Covell y cols., 2007) y una ratio D2:D4 masculinizada (Bailey y Hurd, 2005). La ratio D2:D4 podría explicar la alta sensibilidad a los efectos de la T circulatoria (van Honk y cols., 2011) y podría predecir los cambios en los niveles de dicha hormona, especialmente en hombres penados por violencia contra la mujer en el ámbito doméstico. Tal y como ha sido obtenido en estudios previos, altos niveles de ira se han relacionado con altos niveles de T (Herrero, Gadea, Rodríguez-Alarcón, Espert y Salvador, 2010). Por tanto, esperamos que en ambos grupos los niveles de ira se asocien con altos niveles de T, aunque esta relación será más fuerte en hombres penados por violencia contra la mujer en el ámbito doméstico debido a su historial de violencia. Además, debido a la relación inversa entre la T y el C y sus efectos sobre le ejecución en tareas neuropsicológicas y de empatía. Hipotetizamos que la ejecución neuropsicológica, que fue evaluada mediante el test de clasificación de cartas de Wisconsin, se asociarán de forma positiva con la T (Muller, Aleman, de Haan y van der Schouw, 2005) y de forma negativa con los niveles de C (Egeland y cols., 2005). Además, el reconocimiento de las emociones, que fue evaluado mediante el test de la lectura de la mente en la mirada, se asociará de forma negativa con la T (van Honk y Schutter, 2007) y positiva con el C (Smeets, Dziobeck y Wolf, 2009).

4. Validar la utilización del cociente entre la T y el C, junto a otros parámetros, como indicador de la propensión hacia la violencia en hombres penados por violencia contra la mujer en el ámbito doméstico. Se ha hipotetizado que los hombres penados por violencia contra la mujer en el ámbito doméstico, como individuos dominantes y que presentan una alta propensión hacia la violencia, presentarán una alta ratio T/C (Terburg y cols., 2009) y autoestima (Bushman y cols., 2009) que los controles. Además, estos resultados en hombres penados por violencia contra la mujer en el ámbito doméstico se asociarán con una mejor salud mental (Mann, Hosman, Schallma y de Vries, 2004).

Chapter 6 (Spanish)

DISCUSIÓN

Discusión general

Con los resultados obtenidos hasta el momento tanto en progenitores de personas con TEA como en hombres penados por violencia contra la mujer en el ámbito doméstico, podríamos concluir que existen indicios de una alta androgenización prenatal en ambas poblaciones. No obstante, la manifestación en cada una de ellas es diferente. En ambos casos la ratio D2:D4 fue un indicador eficaz para explicar la alta androgenización cognitiva y comportamental, tal y como se había apuntado previamente (van-den-Bergh y Dewitte, 2006). Los progenitores de personas con TEA no mostraron diferencias en la ratio D2:D4 respecto a los participantes de control. Sin embargo, dicho indicador predijo en mejor medida la alta androgenización cognitiva caracterizada por una alta expression de la ira, una baja empatía y una alta sistematización y rasgos autistas. Por tanto, nuestros resultados apoyan la hipótesis de que la elevada exposición prenatal a la T tiene como consecuencia el desarrollo de un cerebro excesivamente masculino (Auyeung, Baron-Cohen, Ashwin, Knickmeyer, Taylor, y Hackett, 2009; Auyeung, Baron-Cohen, Chapman, Knickmeyer, Taylor, y Hackett, 2006; Saenz y Alexander, 2013).

Por otro lado, los hombres penados por violencia contra la mujer en el ámbito doméstico presentaron una menor ratio D2:D4 o más androgenizada que los sujetos control. Además, esta mayor androgenización se asoció con una menor empatía, así como con un mayor incremento en los niveles de T en respuesta a un estresor. Estos resultados apoyan los estudios previos, en los que una mayor exposición prenatal estaba relacionada con la propensión hacia el comportamiento agresivo en hombres (Bailey y Hurd, 2005; Saenz y Alexander, 2013), así como con una mayor sensibilidad a los efectos de los andrógenos durante la vida adulta (van Honk y cols., 2011). Por tanto, la androgenización, aunque de forma diferencial, se ha manifestado de manera evidente en ambas poblaciones. En el caso de progenitores de personas con TEA como un cerebro excesivamente masculino, mientras que en el caso de los hombres penados por violencia contra la mujer en el ámbito doméstico por la facilitación del comportamiento agresivo debido a la alta sensibilidad a las fluctuaciones en los niveles circulantes de T. Sin embargo, en ambas poblaciones la relación entre la alta exposición prenatal a la T y la agresividad fue similar. Por tanto, los próximos estudios deberían considerar la relación entre la T, los rasgos autistas y el comportamiento agresivo para poder ofrecer

una explicación más amplia de la facilitación del comportamiento agresivo analizando las variables moduladoras.

Progenitores de personas con TEA

Nuestros resultados apoyan la existencia del FAA en progenitores de personas con TEA, caracterizado a nivel cognitivo por mayores rasgos autistas que la población general (Sucksmith y cols., 2011; Wheelwright, Auyeung, Allison, y Baron-Cohen, 2010). Además, tal y como sugiere la teoría de la empatía-sistematización (Baron-Cohen, 2010a), éstos presentaron una menor empatía cognitiva que los sujetos control. Según la hipótesis del cerebro excesivamente masculino, estas características de los progenitores de personas con TEA podrían ser debidas a la excesiva exposición pretanatal a la T (Auyeung y cols., 2006; 2009). Cabría esperar entonces que los progenitores de personas con TEA presentaran una menor ratio D2:D4 que la de los sujetos control, tal y como fue obtenido en una investigación previa (Manning y cols., 2001). No obstante, no fueron halladas tales diferencias al comparar por grupos o género en el primer estudio. Esto podría ser explicado porque la muestra estuvo compuesta de progenitores de personas con autismo clásico, síndrome de Asperger y trastornos generalizados del desarrollo, por lo que, la excesiva masculización prenatal podría circunscribirse a un grupo reducido de ellos. Estos resultados coinciden con los descritos previamente en los propios afetados, en concreto en niños con síndrome de Asperger, quienes presentaron una ratio mayor que la de los niños con autismo clásico, siendo además en ambos casos menor que la de los controles (Manning y cols., 2001). Además, el segundo estudio refuerza la importancia de las submuestras más homogéneas de TEA debido a que sí que fueron halladas diferencias de género en la ratio D2:D4 de progenitores de personas con asperger y síntomas de TDAH comórbidos.

La relación entre la T y la ratio D2:D4 en adultos es inconsistente, puesto que sólo ha sido hallada una relación negativa entre dicho indicador y los niveles de T de hombres con problemas de fertilidad (Manning y cols., 2004). Sin embargo, en población no clínica de ambos géneros no fue hallada ninguna relación entre ambas variables (Hönekopp y cols., 2007). Aunque en nuestro primer estudio no observamos una relación significativa entre los niveles de T y la ratio D2:D4, el estudio posterior con una submuestra de progenitores de personas con TEA y síntomas comórbidos de TDAH de predominancia inatenta, mostró una relación negativa entre ambas variables. Reforzando la hipótesis anterior de que la ratio D2:D4 sería más útil en

submuestras de personas con TEA, así como en sus propios progenitores. Por tanto, este segundo estudio apoya que en las personas con un cerebro excesivamente masculino, la ratio D2:D4 no sólo está asociado con elevados rasgos autistas, sino también con los niveles circulantes de T (Bejerot, Eriksson, Bonde, Carlström, Humble, y Eriksson, 2012). De este modo, en dicha submuestra de progenitores la ratio D2:D4 se relacionó con elevados rasgos de inatención (compartidos por los TEA), siendo esa relación mediada por altos niveles de T. En línea con estos resultados, se hipotetizó que la androgenización intrauterina de los progenitores de personas con TEA los predispondría a presentar altos niveles en circulación de T durante su vida adulta, como presentaban su descendencia (Ingudomnukul, Baron-Cohen, Wheelwright, y Knickmeyer, 2007). Pero nuestro estudio no reveló dichas diferencias por grupo, únicamente las evidentes diferencias de género. No obstante, sólo se emplearon dos muestras basales de T y no su respuesta a un estresor o los cambios que se producen a lo largo del día en las secreciones del eje HHG.

Respecto al dimorfismo sexual de la ratio D2:D4 (Manning y cols., 2000), el segundo estudio apoya la idea de la importancia de las submuestras dentro del grupo de TEA, ya que en el primero no se obtuvieron diferencias de género, mientras que en el segundo sí. Por otro lado, la mayor parte de los estudios han sugerido que la ratio D2:D4 de la mano derecha es un marcador más sensible a los efectos de los andrógenos (Hönekopp y Watson, 2010), aunque también existe literatura que afirma que la ratio D2:D4 izquierda es un buen indicador de dicha condición (Bull y Benson, 2006). En el primero de nuestros estudios existió una predominancia de la ratio izquierda. No obstante, en el segundo estudio la ratio derecha fue seleccionada por su mayor capacidad predictiva. Por tanto, nuestros resultados plantean la necesidad de emplear ambos indicadores indistintamente para definir la elevada androgenización cognitiva. Además, en nuestro primer estudio los padres presentaron niveles más altos de C que las madres. No obstante, las mujeres tienden a presentar niveles más altos que los hombres, aunque hay estudios que han hallado lo contrario (Gusenoff y cols., 2001; Kudielka, Buske-Kirschbaum, Hellhammer y Kirschbaum, 2004; Laughlin y Barrett-Connor, 2000; Zhao, Lu, Xie, Fu, Bogdan y Touitou, 2003). Por tanto, las diferencias de género halladas en nuestro estudio podrían ser parcialmente explicadas por la masculinización, que podría afectar a los niveles de C como fue hallado en nuestro estudio con hombres penados por violencia contra la mujer en el ámbito doméstico. Las características cognitivas que presenta esta población

derivadas de la androgenización prenatal, tal y como se ha afirmada anteriormente, están amparadas bajo la denominación de FAA, cuya importancia radica en el papel etiológico en los trastornos que desarrolla posteriormente su descendencia. En el primero de los estudios, la elevada androgenización en los progenitores, particularmente en varones, se asoció con una mayor gravedad de los rasgos autistas que presentaron los hijos. Mientras que en el segundo, la elevada androgenización prenatal de ambos progenitores (relacionada con los síntomas de inatención de los progenitores) se asoció con mayores rasgos de inatención en su descendencia. Por tanto, estos estudios ofrecen nuevos resultados que resaltan la importancia que desempeña la T en el contexto prenatal de los TEA y de los TDAH. De este modo, a raíz de los resultados de nuestro primer estudio podemos concluir que en los TEA la androgenización en los progenitores varones desempeña un papel importante, mientras que lo hace tanto en padres como en madres en los TEA con comorbilidad de TDAH de predominancia inatenta. Se confirma así la hipótesis inicial planteada según la cual la ratio D2:D4 podría ser utilizado, junto a otros parámetors, como un indicador válido de predisposición hacia el autismo (Manning y Bundred, 2000).

La principal limitación de nuestras investigaciones es que se son de tipo transversal, por tanto, limita la causalidad de los resultados. Además, los sujetos objeto de estudio son familiares de primer grado y no los propios afectados por el trastorno, por lo que los efectos de la androgenización son sutiles, y sería necesario mayor número de variables con las que definir el FAA. Además, también debería ser considerado el efecto de la experiencia de ser un cuidador de una persona con TEA, que podría tener efectos considerables sobre la fisiología de los progenitores (específicamente sobre los niveles de T y C). Por tanto, dicha variable deberá ser considerada en las próximas investigaciones. Sin embargo, debido al escaso o casi inexistente número de estudios que analicen la androgenización en los progenitores de personas con TEA los convierte en estudios relevantes para la literatura científica y con un alto valor preventivo. Ello junto a la dificultad de esta población de disponer del tiempo necesario para participar en estudios de laboratorio llevados a cabo fuera de su ámbito doméstico dota a nuestros resultados de una gran valía. A la luz de estos resultados, nuestra investigación actual en este temática va dirigida hacia dos objetivos principales. Por un lado, analizar la respuesta de los niveles circulantes de T al realizar diversas tareas cognitivas, así como estudiar cómo afectan los cambios en los niveles de T a la ejecución en dichas tareas (Romero-Martínez, de Andrés-García, Ruiz-

Robledillo, González-Bono, y Moya-Albiol, en revisión). Por otro lado, sería conveniente comparar la muestra de progenitores de personas con TEA con otras poblaciones en el que la elevada androgenización prenatal pudiera tener un papel neuroprotector como los trastornos de la conducta alimentaria (Klump, Gobrogge, Perkins, Thorne, Sisk, y Breedlove, 2006; Romero-Martínez, González-Bono, y Moya-Albiol, en realización).

El principal beneficio de estos estudios reside en la prevención de los TEA cuyo coste emocional y económico es muy elevado para los progenitores. El empleo de indicadores relativamente económicos ha permitido trazar líneas de investigación futuras con marcadores genéticos en los que la síntesis de la T, así como de sus receptores sean los principales objetos de estudio. No debemos descuidar el hecho de que la androgenización no parece ser homogénea en el espectro de los TEA, por tanto, sería necesario clarificar los distintos trastornos que lo componen, a pesar de que en el DSM-V sea considerado como un trastorno unitario en el que varía la gravedad del mismo (Wing, Gould, y Gillberg, 2011).

Hombres penados por violencia contra la mujer en el ámbito doméstico

Los hombres penados por violencia contra la mujer en el ámbito doméstico presentaron una ratio D2:D4 menor que la de los controles. Por tanto, podríamos asumir una mayor exposición prenatal a la T en los primeros (Hönekopp y cols., 2007). Este contexto prenatal condicionaría su respuesta cognitiva y/o comportamental posterior, en función de las fluctuaciones en los niveles de T durante su adultez (van Honk y cols., 2011), posiblemente debido a la mayor sensibilidad en los receptores androgénicos (Manning, Bundred, Newton, y Flanagan, 2003). En el primer estudio, la menor ratio D2:D4 se relacionó con un mayor incremento general en los niveles de T en respuesta a la tarea estresante únicamente, pero únicamente en el caso de los hombres penados por violencia contra la mujer en el ámbito doméstico. Estos cambios en la respuesta de la T se asociaron a un incremento del afecto negativo (incremento de la ira, la ansiedad y un empeoramiento del estado de ánimo). Además, la menor ratio D2:D4 también se asoció con una menor capacidad de decodificación emocional que redundó en una menor empatía cognitiva o en la capacidad para ponerse en el lugar de los demás. Esta disminución temporal de la empatía y el incremento del afecto negativo podría

explicar la perpetración del acto violento, tal y como ha sido revelado previamente (Carr y Lutjemeier, 2005). La mayor sensibilidad del eje HHG observada en los hombres penados por violencia contra la mujer en el ámbito doméstico interferiría en el correcto funcionamiento del eje HHA, puesto que elevados niveles de T inhibirían la producción del C. De esto modo, la T favorecería un sesgo de atribución hostil y/o la saliencia de estímulos hostiles, mientras que el C reduciría los niveles de T disminuyendo a su vez el riesgo de comportarse violentamente e incrementando así a su vez el temor a las contingencias punitivas de dichos comportamientos (Terburg y cols., 2009). Nuestro estudio inicial concluyó que aunque no hubo diferencias en los niveles de C, sí que existió una respuesta diferencial puesto que los hombres penados por violencia contra la mujer en el ámbito doméstico presentaron una respuesta amortiguada en esta hormona. Por tanto, hemos corroborado el desequilibrio entre los ejes HHG e HHA definido por altos niveles de T y bajos de C que caracterizan a distintas poblaciones de sujetos violentos (Popma y cols., 2007). De este modo, el cociente entre ambas hormonas podría ser sistematizado como la ratio T/C y empleado como un indicador de predisposición hacia la violencia (Terburg y cols., 2009). En nuestro segundo estudio, tras calcular la ratio T/C, concluimos que efectivamente sería un indicador útil como indicador de predisposición a la violencia en esta población. Posteriormente, hipotetizamos que el desequilibrio entre ambos ejes facilitaría el comportamiento violento en poblaciones con unas características cognitivas determinadas. Los hombres penados por violencia contra la mujer en el ámbito doméstico se caracterizarían poruna elevada rigidez cognitiva que subyacería al mantenimiento de ideas machistas. También presentarían una baja capacidad de decodificación emocional que supondría una baja empatía cognitiva, así como a un sesgo de atribución hostil ante emociones neutras. Además, este procesamiento cognitivo podría cursar con una baja autoestima que mejoraría cuanto mayor fuese el desequilibrio entre los ejes HHG e HHA o cuanto mayor fuese la predisposición para la violencia hacia su único objetivo específico, en concreto hacia su pareja. En este sentido, la dominación hacia el cónyuge mejoraría su autoestima, así como los síntomas psicopatológicos que presenta esta población, es decir, mejoraría en definitiva su salud y/o bienestar psicológico.

Tal y como se ha sugerido en el caso de los progenitores con TEA, la principal limitación de los estudios proviene del tipo de diseño experimental empleado, transversal, lo que, limita la causalidad de los mismos. Además, el reducido tamaño de la muestra limita su validez externa. En este sentido,

aunque los modelos de ecuaciones estructurales podrían incrementar la calidad de los resultados con el tamaño muestral de los estudios no habría sido posible emplearlos. Por tanto, dichos modelos serán considerados en próximas investigaciones con tamaños muestrales más grandes. Sin embargo, debido al elevado número de variables psicobiológicas empleadas, y el difícil acceso a esta población sobre todo en laboratorio, los convierte en estudios relevantes y útiles socialmente, ya que sugiere su utilización junto a otras técnicas psicoterapéuticas y estrategias de intervención para la prevención de la violencia contra la mujer en el ámbito doméstico. Nuestros estudios actuales en este campo de estudio se dirigen hacia el análisis de correlatos del sistema nervioso autónomo e inmunológico (Romero-Martínez, Lila, Conchell, González-Bono, y Moya-Albiol, segunda revisión; Romero-Martínez, Lila, Williams, González-Bono, y Moya-Albiol, en prensa) que permitan una comprensión más amplia de un fenómeno tan complejo como la violencia contra la mujer en el ámbito doméstico. Por otro lado, debido a los prometedores resultados obtenidos en las variables neuropsicológicas, sería conveniente emplear baterías de evaluación más complejas que permitan el análisis de los posibles déficits cognitivos que podrían subyacer a los analizados en estos estudios, así como el papel de tóxicos como el alcohol que podría explicar tanto la facilitación como el mantenimiento de los comportamientos violentos actuando como factor catalítico (Romero-Martínez, Lila, Catalá-Miñana, Williams, y Moya-Albiol, 2013; Romero-Martínez y Moya-Albiol, en prensa).

El principal beneficio de estos estudios reside en reproducir la respuesta de los maltratadores a un estresor agudo como podría ser una discusión con su pareja. De modo que, el desequilibrio en los ejes neuroendocrinos junto a ciertos sesgos cognitivos y/o emocionales, facilita la perpetración del acto violento. Por tanto, el análisis de los parámetros hormonales, psicológicos y neuropsicológicos permitiría crear categorías de personas violentas para su correcta identificación. Ello redundaría en una mejora de los programas de intervención, basados principalmente en parámetros psicológicos, cuyo objetivo principal es la reinserción. Por tanto, permitirían dilucidar los factores que contribuyen a la baja adherencia al tratamiento y a la reincidencia a medio y largo plazo.

Para finalizar indicar que esta Tesis Doctoral es aplicable en los ámbitos clínico, asistencial y terapéutico. Permitiría desarrollar nuevas estrategias

terapéuticas para la reinserción social de personas penadas judicialmente. El análisis psicobiológico llevado a cabo ofrece la oportunidad de incorporar nuevas variables que permitan una mayor comprensión de un fenómeno complejo como la violencia contra la mujer en el ámbito doméstico, en el que las terapias actuales tienen unos beneficios relativamente limitados. En relación con progenitores de personas con TEA permitiría detectar el perfil psicoendocrinológico y contextual de personas del alto riesgo en el desarrollo de proceso patológicos y/o comportamientos inadecuados para poner en marcha una estrategia preventiva. En el ámbito asistencial contribuiría a la detección de factores predisponentes o precipitantes de distintos comportamientos desadaptativos para sí mismos o para los demás.

A nivel científico, es necesario profundizar en las consecuencias de la androgenización a nivel de SNC y en los efectos sobre la modulación del comportamiento. Ello podría mejorarla calidad de vida de los progenitores de personas con TEA a la hora de afrontar sus problemas, y ayudar a establecer terapias más efectivas en el caso de los hombres penados por violencia contra la mujer en el ámbito doméstico.