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Digit ratio (2D:4D) moderates the relationship between cortisol reactivity and self-reported externalizing behavior in young adolescent males

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ABSTRACT

Although reduced cortisol reactivity to stress and increased circulating testosterone level are hypothesized to be associated with higher levels of externalizing behavior, empirical findings are inconsistent. One factor that may account for the heterogeneity in these relationships is prenatal testosterone exposure. This study examined whether the second-to-fourth digit ratio (2D:4D), a putative marker of prenatal testosterone exposure, moderates the relationships of testosterone and cortisol reactivity with externalizing behavior. Left and right hand 2D:4D and self-reported externalizing behavior were measured in a sample of 353 young adolescents (*M* age = 11.92 years; 178 females; 79.7% African American). Saliva samples were collected before and after a stress task and later assayed for cortisol. Testosterone levels were determined from an AM saliva sample. 2D:4D interacted with cortisol reactivity to predict externalizing behavior in males, but not females. In males, low cortisol reactivity was associated with higher levels of aggression and rule-breaking behavior, but only among subjects with low 2D:4D (i.e., high prenatal testosterone). Findings suggest the importance of a multi-systems approach in which interactions between multiple hormones are taken into account. Furthermore, results demonstrate the importance of considering the organizational influence of prenatal testosterone in order to understand the activational influence of circulating hormones during adolescence.

1. Background

Hormones, including testosterone and cortisol, are frequently studied in relation to externalizing problem behavior (Archer, 1991; Susman et al., 1987, 2010). However, the findings of studies that examine main effects of cortisol and testosterone on behavior problems are somewhat inconsistent. A meta-analysis of child and adolescent studies reported no association between cortisol reactivity and externalizing behavior ($r = -.04$, $p > .05$) and only a small relationship between basal cortisol and externalizing behavior ($r = -.05$, $p < .05$; Alink et al., 2008). Although a meta-analysis showed a stronger association between testosterone and aggression ($r = .13$, $p < .01$), there was significant heterogeneity in effect sizes across studies (Book, Starzyk, & Quinsey, 2001; Book & Quinsey, 2005), suggesting that there may be factors that moderate this relationship. A large body of research has examined social context, including family and peer relationships, as moderators of the relationship between hormones and behavior problems (Booth, Johnson, Granger, Crouter, & McHale, 2003; Rowe, Maughan, Worthman, Costello, & Angold, 2004). Less research has been conducted on non-social, biological factors as moderators, although there is an increasing interest among researchers in incorporating a multi-systems approach to behavior that takes into account multiple biological processes (Mehta & Josephs, 2010; Montoya, Terburg, Bos, & van Honk, 2012; Terburg, Morgan, & van Honk, 2009). This article examines prenatal testosterone as a putative biological moderator of the cortisol-externalizing behavior and testosterone-externalizing behavior relationships.

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We examine prenatal testosterone as a moderator because of its hypothesized organizational influences on the developing fetus. In addition, in contrast to environmental moderators, to our knowledge, no prior research has examined prenatal testosterone as a moderator of the effect of cortisol on behavior. Organizational effects were first discovered through animal research; a seminal study found that female rats who were treated prenatally with testosterone were more likely to exhibit male-typical behaviors than untreated rats, and that this effect persisted even after the termination of testosterone treatment (Phoenix, Goy, Gerall, & Young, 1959). This finding suggested that prenatal testosterone played a role in the masculinization of the brain. Since the publication of Phoenix et al. (1959), evidence has mounted that prenatal testosterone plays a role in the masculinization of the developing brain and nervous system and affects brain structure during critical periods of development (Arnold, 2009; Breedlove, 1994). Given the organizational role of testosterone during prenatal developmental, the purpose of this article is to determine whether the extent of exposure to prenatal testosterone moderates the effects of circulating hormones on behavior during early adolescence.

1.1. Testosterone

Testosterone is the end product of the hypothalamic-pituitary-gonadal (HPG) axis and is the primary androgen, the group of steroid hormones responsible for the development and maintenance of masculine traits (Mazur & Booth, 1998). Testosterone is released prenatally by the gonads and is secreted in much higher levels in males, contributing to the masculinization of the central nervous system. Testosterone is thought to have both organizational effects on behavior—through its effects on neurodevelopment during gestation—and activational effects that occur through the influence of postnatal circulating testosterone (Breedlove, 2010; Mazur & Booth, 1998).

Research on prenatal testosterone is often conducted using indirect biological markers due to the difficulty of measuring hormones prenatally. The second-to-fourth digit ratio of the hand (2D:4D) is thought to be a marker of prenatal testosterone levels, with a lower 2D:4D indicating higher exposure to prenatal testosterone relative to estrogen (Manning, Scutt, Wilson, & Lewis-Jones, 1998; Manning, Kilduff, Cook, Crewther, & Fink, 2014). Several indirect findings are often used to support the validity of 2D:4D as a marker of prenatal testosterone exposure. These include (1) 2D:4D is a sexually dimorphic trait; males tend to have lower 2D:4D than females (Hönekopp & Watson, 2010), and this sex difference is already present during gestation (Galis, Broek, Van Dongen, & Wijnaendts, 2010), (2) males and females with congenital adrenal hyperplasia (CAH), a disorder that results in increased in utero androgen production, have lower 2D:4D ratios than males and females without CAH (Brown, Hines, Fane, & Breedlove, 2002; Okten, Kalyoncu, & Yaris, 2002), and (3) a correlational study of routine amniocentesis samples taken during the second trimester of pregnancy found that 2D:4D ratios were negatively associated with the prenatal testosterone/estrogen ratio at age two years (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). In addition to this indirect and correlational evidence, a recent experimental study in mice found that inactivation of the androgen receptor during gestation resulted in more feminized digit ratios in mice, while inactivation of the estrogen receptor resulted in more masculinized digit ratios (Zheng & Cohn, 2011). Conversely, postnatal doses of androgen and estrogen had no effect on digit ratios, a finding which suggests that there is a critical prenatal period for the determination of digit ratios.

As would be expected based on prenatal testosterone's hypothesized organizational effects, in some early studies low 2D:4D (indicating high prenatal testosterone) was associated with higher levels of male-typical traits, including spatial abilities (Csathó et al., 2003) and sensation-seeking (Hampson, Ellis, & Tenk, 2008). However, later meta-analyses showed that there was not a significant association between a low 2D:4D and these traits (Puts, McDaniel, Jordan, & Breedlove, 2008; Voracek, Tran, & Dressler, 2010). In some studies, low 2D:4D has been associated with aggression, (Hampson, Ellis, & Tenk, 2008), dating violence (Cousins, Fugère, & Franklin, 2009), and traffic violations (Schwerdtfeger, Heims, & Heer, 2010), although results are again inconsistent (Austin, Manning, McInroy, & Matthews, 2002). A recent meta-analysis found a small negative association between 2D:4D and aggression in males (Hönekopp & Watson, 2011). This pattern of a small, negative relationship between 2D:4D and aggression in males but not females was confirmed in a later large-scale study (Butovskaya, Fedenok, Burkova, & Manning, 2013; N = 1452). The presence of only a small, negative relationship between 2D:4D and aggression in males leaves open the possibility that other hormonal factors could account for heterogeneity in this relationship.

1.1.1. Circulating testosterone

Following a dramatic rise in testosterone production in males during gestation, as well as a brief surge in testosterone beginning shortly after birth and lasting until about the sixth month of infancy, testosterone levels return to low levels in both males and females until puberty (Hines, 2004). In males, testosterone levels remain at low levels until the transition to Tanner Stage 3, when testosterone levels begin to increase dramatically. In males, testosterone reaches adult levels by Tanner Stage 4 or 5, which occurs around age 16 years (Kushnir et al., 2010; Sato, Scuhlz, Sisk, & Wood, 2008). In females, the greatest increase in testosterone occurs earlier in life, during the transition to Tanner Stage 2, and adult levels are reached by Tanner Stage 3. Because the brain is a target organ for steroid hormones (Sisk & Zehr, 2005), hormones during puberty are thought to activate steroid receptors in the brain that contribute to behavioral change in adolescents (Sato et al., 2008). Adolescence is also a period when risk-taking behaviors become more frequent (Sato et al., 2008) and offending begins to increase dramatically (Moffitt, 1993). Because of this, it has been hypothesized that the hormonal surges during puberty may in some form contribute to the higher levels of externalizing behavior observed in adolescents. Consistent with this, higher circulating testosterone has been associated with externalizing behavior among older male children transitioning to puberty (ages 9–11 years; Chance, Brown, Dabbs, & Casey, 2000) and adolescent males (ages 11–14 years Fang et al., 2009), although results have varied (Granger et al., 2003).

Postnatal testosterone is thought to influence antisocial behavior by activating the hormone structures established prenatally. It is possible, therefore, that examining *both* prenatal and circulating testosterone is necessary in order to understand the etiology of antisocial behavior. Experimental research in women has shown that the effect of administered doses of testosterone on cognitive empathy (van Honk et al., 2011), cooperation (van Honk, Montoya, Bos, van Vugt, & Terburg, 2012), and moral decision-making (Montoya et al., 2013) are dependent on the 2D:4D ratio. Similarly, a recent study in men found that the effect of administered doses of testosterone on empathy was dependent on the left 2D:4D ratio (Carré et al., 2015). These studies suggest that the prenatal exposure to sex steroids could play an important role in influencing sensitivity to the activational effects of testosterone later in life. Although results should be interpreted with caution given the small sample sizes of these studies (less than 25 subjects in each study), the use of experimental manipulation in these studies provides a more powerful test of 2D:4D as a biological marker than prior correlation studies for which small sample sizes are more problematic. Another small sample study (N = 45) found that white adult males' testosterone levels increased after viewing an aggressive video, but not a control video (Kilduff, Hopp, Cook, Crewther,

& Manning, 2013). Amongst those individuals whose testosterone level increased, low 2D:4D was associated with higher levels of self-reported aggression in response to hypothetical situations. These results suggest that in adult men, the effect of testosterone on behavior may be moderated by 2D:4D, although this remains to be seen in a study with a larger sample of higher risk adolescents.

1.2. Cortisol

The release of cortisol is regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis, which is activated by psychological stressors (Dickerson & Kemeny, 2004). Reduced stress reactivity is thought to be characteristic of individuals with high levels of antisocial behavior (van Goozen & Fairchild, 2008); reduced stress reactivity may make individuals less fearful of the negative consequences of their actions, which could increase the likelihood of externalizing behavior (Raine, 1993, 2002). Consistent with this, low basal cortisol has been associated with conduct disorder (Pager, Gardner, Rubin, Perel, & Neal, 2001) and aggression in adolescents (McBurnett, Lahey, Rathouz, & Loeber, 2000). However, many studies have found no relationship between externalizing behavior and both basal cortisol and cortisol reactivity to stress (Alink et al., 2008).

In order to better understand the effect of cortisol on behavior, it may be necessary to examine interactions between hormone systems. Cortisol, which is the end product of the HPA axis, inhibits the activity of the HPG axis, of which testosterone is the end product. Therefore, the balance between cortisol and postnatal testosterone may be crucial to understanding behavior (Glenn, Raine, Schug, Gao, & Granger, 2011; Mehta & Josephs, 2010; Montoya et al., 2012; Terburg et al., 2009). One study, for instance, found that psychopathy was associated with a higher circulating testosterone-to-cortisol reactivity ratio (Glenn et al., 2011).

Similarly, it may be possible that prenatal testosterone and cortisol interact to predict antisocial behavior. Animal studies suggest an important connection between prenatal testosterone and cortisol secretion, finding that prenatal testosterone contributes to the masculinization of the HPA axis (Seale, Wood, Atkinson, Lightman, & Harbuz, 2005a,b). It has been hypothesized that the combination of high prenatal testosterone and reduced cortisol may predispose individuals toward aggressive, reward-driven behavior (Yildirim & Derksen, 2012). However, to our knowledge, no study has empirically tested whether cortisol and 2D:4D interact to predict antisocial behavior.

1.3. Sex differences

Testosterone levels are dramatically higher during adolescence in males than in females (Kushnir et al., 2010), making it important to examine sex differences in the relationship between testosterone and externalizing behavior. Studies examining testosterone in relation to externalizing behavior in males and female adolescents have detected sex differences in this relationship, with higher testosterone tending to show more consistent associations with increased externalizing behavior in males than in females (Booth et al., 2003; Granger et al., 2003; Susman et al., 1987). This is somewhat unsurprising given that there are important sex differences in the production of androgens in males and females (Burger, 2002). However, many prior studies have not adequately controlled for gender, age, or pubertal development (Granger et al., 2003), which may obscure the nature of the relationship between testosterone and externalizing behavior in females. Similarly, meta-analytic results suggest that the effects of 2D:4D on behavior may only be present amongst males (Hönekopp & Watson, 2011), a possibility that is consistent with a more recent study that found that 2D:4D was related to externalizing behavior problems in male, but not in female children (Liu, Portnoy, & Raine, 2012).

1.4. Current study

The purpose of this article is to examine whether 2D:4D interacts with cortisol and adolescent testosterone level to predict externalizing behavior in a sample of young adolescents. We hypothesize that lower cortisol reactivity will be associated with higher levels of externalizing behavior, and that this effect will also be strongest amongst subjects with low 2D:4D. Similarly, we predict that higher adolescent testosterone will be associated with higher levels of externalizing behavior and that this effect will be strongest amongst subjects with low 2D:4D (indicating high prenatal testosterone). We examine these hypotheses separately for males and females in order to determine whether there are any sex differences in these relationships. We predict that any observed effects will be stronger in males than in females because of prior research showing stronger effects of testosterone on behavior in males. We also examine these hypotheses separately for aggressive and non-aggressive forms of antisocial behavior. This is important given that responses to social stressors like the one used in this study are thought to be differentially related to aggressive and non-aggressive forms of externalizing behavior (Burt & Larson, 2007). We also control for a number of covariates that are thought to be associated with both hormone levels and externalizing behavior.

2. Methods

2.1. Participants

Data for this study come from the Healthy Brains and Behavior study (Liu et al., 2013). The sample for this study consisted of 11 and 12-year old boys and girls living in Philadelphia County, PA or suburbs of Philadelphia. Within the study area, fliers soliciting enrollment were placed in recreation centers, libraries, health clinics, and other community centers. Targeted mailings were also sent to parents of 11–12 year old children living in the geographic catchment area. Youths with a diagnosed psychotic disorder, mental retardation, or a pervasive developmental disorder were excluded. More information about subject recruitment and exclusionary criteria can be found in Liu et al. (2013). The original sample consisted of 454 subjects. Of this original group, eight subjects were later deemed ineligible or withdrew, resulting in a sample of 446 subjects. The sample was 50.6% male. The racial makeup of the sample was 11.9% white, 79.7% African American, and 4.8% multiracial. Less than 1% of the sample identified as Hispanic, Asian, or Native American. The mean age of the sample was 11.92 years ($SD = .59$). 14.2% of subjects had a lifetime diagnosis of conduct disorder and 19.1% had a lifetime diagnosis of oppositional defiant disorder (Liu et al., 2013). All subjects were accompanied to the laboratory with a caregiver, who also completed questionnaires about the child's behavior and demographics.

2.2. Externalizing behavior measures

Self-reported antisocial behavior was assessed using the externalizing behavior scale of the Youth Self-Report (Achenbach and Rescorla, 2001). The externalizing behavior scale consists of rule-breaking and aggression sub-scales, which were also analyzed separately. Parent-reported antisocial behavior was assessed using the rule-breaking and aggression sub-scales of the Child Behavior Checklist, as well as the overall externalizing behavior score (Achenbach and Rescorla, 2001). The CBCL externalizing scale has 35 items in total. Seventeen of the items measure rule-breaking (e.g., "lie or cheat") and eighteen are aggression items (e.g., "gets

in many fights") that are rated by the parent on a 3-point Likert scale, with higher scores indicating higher levels of externalizing behavior. The Cronbach's alpha of both the CBCL rule-breaking and aggression sub-scales in this sample were .97. The YSR has 32 items in total. Fifteen items measure rule-breaking and seventeen are aggression items that are rated by the child on 3-point Likert scale, with higher scores indicating higher levels of externalizing behavior. The Cronbach's alpha of the YSR rule-breaking and aggression sub-scales in this sample were .88 and .85 respectively.

2.3. Digit ratio (2D:4D)

The second and fourth finger digit of each participant was measured directly according to the protocol described in Manning et al. (1998). There is some debate as to whether it is preferable to measure 2D:4D directly or indirectly, such as through the use of scanned copies of the hand (Allaway, Bloski, Pierson, & Lujan, 2009). Although reliability is high when digit ratio is measured indirectly, indirect measurements of 2D:4D measurements tend than be lower than direct measurements and it is not yet fully understood why this is the case (Manning, Fink, Neave, & Caswell, 2005; Xu & Zheng, 2015). Therefore, we opted to use direct finger measurement in this study, which do not result in any distortions in the shift from a three-dimensional digit to a two-dimensional scan. Ultratech digital calipers (General Tools & Instruments Co., New York), which are reliable to .001 millimeters, were used to measure the digits. Researchers instructed participants to fully flex their fingers. The second and fourth digits of the right hand were then measured from the finger's basal crease (crease closest to the palm) to the most distal point of the finger. The second and fourth digits of the right hand were measured twice according to this protocol. The same process was then employed to measure the digits of the left hand. The 2D:4D ratio for each hand was calculated by dividing the average length of the second digit by the average length of the fourth digit. This procedure has been utilized in several digit ratio studies (e.g., Benderlioglu & Nelson, 2004). Subjects' digits were measured at the child's initial visit, a 3-month follow-up, a 6-month follow-up, and a 12-month follow-up. In order to minimize missing data, the average 2D:4D across the available time points was calculated for each subject. Digit measurements across the time points were highly correlated; the reliability (Cronbach's alpha) for the four left 2D:4D measurements was .99 and the reliability for the right 2D:4D measurements was .94.

2.4. Stress task procedure

Subjects completed a modified version of the Trier Social Stress Test, which consists of speech and arithmetic tasks (Kirschbaum, Pirke, & Helhammer, 1993). During the speech task (Raine, Lencz, Bihrle, LaCasse, & Colletti, 2000), subjects were instructed to spend two minutes thinking about the worst or most stressful thing that had ever happened to them. After two minutes, they were told to describe the event to an experimenter for an additional two minutes. In order to increase the level of stress experienced by subjects, a researcher remained in the room with the subject and the task was video recorded. After completing the speech task, subjects completed a cognitive stress task. During the cognitive task, subjects were instructed to count backward from 758 in 7's as quickly as possible without making mistakes. Subjects were given verbal prompts at standard intervals throughout the task to increase the uncontrollability of the task. The combination of a short public speaking task and a cognitive task with elements of uncontrollability and social evaluative threat has been shown to be a reliable way in which to induce a substantial cortisol response in the laboratory (Dickerson & Kemeny, 2004). These stress tasks were embedded in a series of other laboratory tasks; stress tasks were preceded by a resting period, conditioning task, the "oddball" target detection task, and an empathy task. The stress tasks were then followed by a final resting period.

2.5. Collection and determination of salivary analytes

Saliva samples were collected across a single day for each participant. Participants were instructed to refrain from food and drink (except water) prior to sample donation (Granger et al., 2012). Whole un-stimulated saliva was collected by passive drool. A morning saliva sample was collected at an average time of 9:18 AM. Between sample collections, subjects completed behavioral questionnaires. In the afternoon, four saliva samples were collected to assess cortisol reactivity to the stressor at the following times: (1) Immediately prior to the laboratory tasks (mean time = 12:36 PM), (2) 5 min after the end of the stress task (mean time = 1:27 PM), (3) 20 min after the end of the stress task (mean time = 1:42 PM), and (4) 40 min after the end of the stress task (mean time = 2:02 PM). Following collection, samples were store and frozen at −80 °C until assay. Cortisol stress reactivity for each subject was measured by calculating area under curve with respect to ground (AUC_G) using the following formula (Pruessner, Kirschbaum, Meinlschmid, & Helhammer, 2003),

$$AUC_G = \sum_{i=1}^{n-1} \frac{\left(m_{(i+1)} + m_i\right) \times t_i}{2}$$

where m_i denotes cortisol level of sample i, n denotes the total number of samples, and t_i denotes the time interval between samples i and $i+1$ (t_i will be specific to each participant). AUC_G, which captures cortisol reactivity and baseline cortisol, is useful because it combines information from repeated measurements into a single index, which increases statistical power and reduces the need for multiple comparisons, which can lead to type I error (Pruessner et al., 2003).

Testosterone was assayed using the morning saliva sample, and cortisol was assayed using the four samples collected throughout the stress task. On the day of testing, all samples were centrifuged at 3000 r.p.m. for 15 min to remove mucins. Samples were assayed for salivary cortisol using a commercially available enzyme immunoassay (Salimetrics, State College, PA). The test used 25 μl of saliva for singlet determinations and had a range of sensitivity of .007–3 μg/dl. Samples were assayed in duplicate and the averages of cortisol concentrations were used in the current analysis. Coefficient of variation is less than 5% for intra-assay and less than 10% for inter-assay.

All samples were assayed for salivary testosterone in duplicate using a highly-sensitive enzyme immunoassay (Cat. No. 1-2402, Salimetrics LLC, State College, PA). The test used 25 μl of saliva per determination, has a lower limit of sensitivity of 1.0 pg/mL, standard curve range from 6.1 pg/mL to 600 pg/mL, an average intra-assay coefficient of variation of 4.6% and an average inter-assay coefficient of variation of 8.25%.

2.6. Covariates

In regression analyses, we controlled for the time of the saliva sample collection, race (0 = black; 1 = not black), social adversity, body mass index (BMI), age (in years), and pubertal timing. We used the time of collection of the first morning sample for calculations involving adolescent circulating testosterone level. For calculations involving cortisol, we controlled for the time of the first stress sample. We created a social adversity index based on 18 demographic items completed by the parents (e.g., parents unemployed, parents arrested, problems with living accommodation). Each item

was coded as 0 (low adversity) or 1 (high adversity). Item scores were summed with higher scores indicating a higher level of social adversity. More information about this measure can be found in Choy et al. (2015).

Pubertal development was measured using the Tanner Stages of Development (Morris & Udry, 1980). Subjects were shown two sets of drawings of five stages of pubertal development (stage 1 = preadolescent, stage 5 = adult appearance). Males rated their development in genitalia and pubic hair growth, and females rated their development in breast and pubic hair growth. The scores were averaged for each subject. As in prior studies, in order to calculate a measure of pubertal timing, we regressed pubertal development score on age separately for males and females and used the saved residuals as a measure of pubertal timing (Dorn, Susman, & Ponirakis, 2003; Susman et al., 2010). A higher residual indicates that the subject was further along in pubertal development than same-aged peers.

2.7. Statistical analyses

All analyses that follow were conducted separately for males and females (Constantinescu & Hines, 2012). We first examined zero-order correlations between 2D:4D, testosterone, cortisol reactivity, and externalizing behavior. We then used the PROCESS SPSS macro to test whether 2D:4D significantly interacted with cortisol to predict child- and parent-reported externalizing behavior (Hayes, 2013). The OLS regression analyses conducted by the PROCESS macro included the covariates specified above. We plotted significant interactions using the procedures described in Aiken and West (1991) at 1 standard deviation above and below the mean of the moderator and independent variable. We then determined the significance of the simple slopes at these points using PROCESS, which reports the significance of the simple slopes across different levels of the moderator when the Johnson-Neyman technique is invoked. These analyses were repeated to test whether 2D:4D interacted with morning testosterone to predict antisocial behavior.

As in prior cortisol and testosterone research (Gordis, Granger, Susman, & Trickett, 2006; Granger et al., 2003), cortisol reactivity and circulating testosterone outliers three standard deviations or greater from the mean were removed, resulting in the exclusion of 7 testosterone scores and 3 AUC_G scores. Complete data were available for 175 males and 178 females. Among the male and female groups, subjects with missing data did not differ from subjects with complete data on demographic variables, including age, race, and social adversity ($p > .05$).

3. Results

3.1. Bivariate correlations

Descriptive statistics by race and sex are shown in Table 1, and bivariate correlations between the study variables are shown in Table 2. In bivariate correlations, adolescent testosterone and cortisol were not associated with externalizing behavior in males or females ($p > .05$). In females, left and right 2D:4D were not associated with externalizing behavior ($p > .05$). In males, only right 2D:4D was associated with externalizing behavior and aggression ($p < .05$), although these relationships were in the unexpected positive direction. In general, most bivariate correlations between the biological variables and behavioral outcomes were not significant.[1]

3.2. Interactions

3.2.1. Self-reported externalizing behavior

We first examined whether 2D:4D interacted with cortisol reactivity to predict self-reported externalizing behavior. As shown in Table 3, in boys, left 2D:4D interacted with cortisol reactivity to predict self-reported externalizing behavior ($B = 7.92$, $p < .05$), aggression ($B = 5.32$, $p < .05$), and rule-breaking ($B = 2.87$, $p < .05$). In boys right 2D:4D also interacted with cortisol reactivity to predict self-reported externalizing behavior ($B = 7.60$, $p < .05$), aggression ($B = 4.56$, $p < .05$), and rule-breaking ($B = 3.03$, $p < .05$). We probed the interactions as shown in Figs. 1 and 2. For subjects with low 2D:4D (indicating higher prenatal testosterone), low cortisol reactivity was associated with higher levels of externalizing behavior. However, for subjects with high 2D:4D (indicating lower prenatal testosterone), there was no relationship between cortisol reactivity and externalizing behavior. Therefore, the expected relationship between low cortisol and increased levels of externalizing behavior was only present in subjects with low 2D:4D (see Table 3). In females, neither left nor right 2D:4D interacted with cortisol reactivity to predict aggression, rule-breaking, or externalizing ($p < .05$; see Table 4).

We then examined whether 2D:4D and circulating adolescent testosterone interacted to predict child-reported externalizing behavior. In males and females, neither left nor right 2D:4D interacted with testosterone to predict externalizing behavior outcomes ($p > .05$).

3.2.2. Parent-reported externalizing behavior

In both males and females, left and right 2D:4D did not significantly interact with cortisol reactivity to predicting parent-reported externalizing behavior ($p > .05$) The interactions between left and right 2D:4D and adolescent testosterone were also non-significant in predicting parent-reported externalizing behavior in males and females ($p > .05$).[2,3]

4. Discussion

The purpose of this article was to examine whether 2D:4D, a marker of prenatal testosterone, interacts with adolescent circulating testosterone level and cortisol reactivity to predict externalizing

[1] We should note that despite the small age range included in the sample, age was significantly correlated with left and right 2D:4D in females. Because researchers are interested in how 2D:4D changes across the life course, follow-up analyses were conducted that examined 2D:4D among males and females who had complete data across the study visits. Data were analyzed for the initial visit (0 months), 3 month follow-up visit, and 6 month follow-up visit only because only 2 males and 0 females had complete 2D:4D data across all four visits. Supplementary Table 1 reports the mean left and right 2D:4D at 0 months, 3 months, and 6 months for males and females separately. Results of repeated measures ANOVA are also reported that examined whether 2D:4D significantly changed within-individuals over the three measurements. Results are also shown in Supplementary Figs. 1 and 2. We urge caution in the interpretation of these supplemental results, as little research has examined short-term changes in 2D:4D, and a recent study did not find evidence of significant short-term changes in 2D:4D in females, even in periods of large hormonal fluctuations (Barrett et al., 2015).

[2] Because little research has examined the relationship between hormones and aggression in black samples, we also repeated these analyses among black subjects only (79.7% of the total sample; $n = 178$ black females; $n = 181$ black males). Results are presented in Supplementary Tables 2 and 3. Patterns of interaction were generally consistent with those observed amongst the full sample, although some previously significant interactions predicting self-reported externalizing behavior in males no longer remained significant with this reduced sample size ($p > .05$).

[3] In addition to 2D:4D research, there has also been recent interest in right-left 2D:4D (Dr-l), although there is much less literature linking Dr-l to androgen production. However, given that results may be of interest to some researchers, we also repeat these analyses using Dr-l. Results are available in Supplementary Tables 4 and 6. We also performed a repeated measures ANOVA to determine whether Dr-l significantly changed within-individuals over the 0 month, 3 month, and 6 month measurements. Results are shown in Supplementary Table 6 and Supplementary Fig. 3.

Table 1
Descriptive statistics by sex.

	Males mean (SD)	Females mean (SD)	*t*	Cohen's d	African American mean (SD)	Non-African American mean (SD)	*t*	Cohen's d
Cortisol AUC_G	9.94 (7.01) $n = 212$	10.64 (8.36) $n = 205$	−.93	−.09	10.23 (8.00) $n = 332$	10.55 (6.45) $n = 84$	−.34	−.04
Morning testosterone (pg/mL)	53.27 (26.22) $n = 221$	48.13 (21.97) $n = 214$	2.22*	.21	53.02 (24.71) $n = 337$	41.97 (20.58) $n = 87$	4.29**	.47
Left 2d:4d	.949 (.034) $n = 206$	.958 (.034) $n = 201$	−2.62**	−.26	.953 (.035) $n = 347$	.956 (.033) $n = 78$	−.74	−.09
Right 2d:4d	.963 (.034) $n = 207$	.967 (.033) $n = 201$	−1.14	−.12	.964 (.034) $n = 329$	.968 (.033) $n = 78$	−.87	−.12
YSR externalizing	11.01 (8.03) $n = 219$	10.75 (7.78) $n = 216$	.35	.03	11.15 (9.83) $n = 348$	9.83 (7.18) $n = 86$	1.40	.14
YSR aggression	7.31 (5.45) $n = 218$	7.56 (5.41) $n = 216$	−.48	−.05	7.53 (5.50) $n = 347$	7.08 (5.12) $n = 86$	.69	.08
YSR rule-breaking	3.65 (3.21) $n = 220$	3.19 (2.90) $n = 216$	1.60	.15	3.59 (2.74) $n = 349$	2.74 (2.61) $n = 86$	2.31*	.31
CBCL externalizing	10.75 (8.64) $n = 220$	8.88 (9.36) $n = 214$	2.16*	.21	10.22 (9.13) $n = 348$	8.25 (8.58) $n = 85$	1.81	.22
CBCL aggression	7.20 (5.96) $n = 220$	6.12 (6.53) $n = 214$	1.80	.17	6.82 (6.28) $n = 348$	6.05 (6.21) $n = 85$	1.02	.13
CBCL rule-breaking	3.55 (3.16) $n = 220$	2.76 (3.28) $n = 214$	2.55*	.25	3.40 (3.32) $n = 348$	2.20 (2.71) $n = 85$	3.50**	.38
Puberty stage	3.07 (1.01) $n = 211$	3.48 (.93) $n = 213$	−4.30**	−.42	3.42 (.95) $n = 340$	2.69 (.92) $n = 83$	6.34**	.77
Social adversity	4.07 (2.38) $n = 226$	3.99 (2.44) $n = 220$	.40	.03	4.23 (2.35) $n = 358$	3.22 (2.50) $n = 87$	3.55**	.42
Age	11.91 (.62) $n = 226$	11.92 (.57) $n = 220$	−.13	−.02	11.94 (.60) $n = 358$	11.85 (.60) $n = 87$	1.20	.15
BMI	21.00 (5.18) $n = 226$	22.70 (6.19) $n = 217$	−1.92	−.30	22.15 (6.05) $n = 353$	20.63 (4.17) $n = 86$	2.74**	.27

Note: Cortisol AUC_G = cortisol area under the curve with respect to ground. YSR = Youth Self Report. CBCL = Child Behavior Checklist. BMI = body mass index.

* $p < .05$.

** $p < .01$.

Table 2
Bivariate correlations. Correlations for males (n = 195–221) shown above the diagonal. Correlations for females (n = 190–216) shown below the diagonal.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Cortisol AUC$_G$	–	.10 n = 207	−.03 n = 195	.06 n = 196	−.09 n = 206	−.07 n = 205	−.11 n = 207	.03 n = 206	−.01 n = 206	−.07 n = 206	.08 n = 201	−.02 n = 212	.07 n = 212	.03 n = 211	−.08 *n = 210*
2. Morning testosterone (pg/mL)	.11 n = 199	–	−.09 n = 202	−.04 n = 203	−.02 n = 214	−.03 n = 213	.03 n = 215	−.08 n = 215	−.09 n = 215	−.04 n = 215	.32** n = 206	−.04 n = 221	.47** n = 221	−.17** n = 220	.08 n = 218
3. Left 2D:4D	−.15* n = 190	−.07 n = 196	–	.63** n = 206	.06 n = 200	.07 n = 199	.03 n = 201	.04 n = 200	.03 n = 200	.05 n = 200	−.03 n = 196	−.00 n = 206	.05 n = 206	−.05 n = 205	.05 n = 205
4. Right 2d:4d	−.07 n = 190	−.02 n = 196	.61** n = 201	–	.15* n = 201	.17* n = 200	.09 n = 202	−.04 n = 201	−.03 n = 201	−.04 n = 2–1	.00 n = 197	−.02 n = 207	.02 n = 207	.01 n = 206	.06 n = 206
5. YSR externalizing	.01 n = 202	−.01 n = 210	.04 n = 198	.04 n = 198	–	.95** n = 218	.85** n = 219	.35** n = 216	.36** n = 216	.29** n = n = 216	−.01 n = 205	.05 n = 219	−.04 n = 219	.05 n = 218	.04 n = 217
6. YSR aggression	−.01 n = 201	−.01 n = 210	.04 n = 198	.04 n = 198	.97** n = 216	–	.65** n = 218	.31** n = 215	.31** n = 215	.24** n = 215	−.01 n = 204	.00 n = 218	−.07 n = 218	.10 n = 217	.00 n = 216
7. YSR rule-breaking	.06 n = 202	−.00 n = 210	.02 n = 198	.03 n = 198	.88** n = 216	.73** n = 216	–	.30** n = 217	.30** n = 217	.26** n = 217	.06 n = 206	.08 n = 220	.04 n = 220	−.03 n = 219	.04 n = 218
8. CBCL externalizing	−.03 n = 201	−.06 n = 208	.06 n = 198	.01 n = 198	.42** n = 211	.42** n = 211	.36** n = 211	–	.97** n = 200	.90** n = 220	.97 n = 205	.21** n = 220	−.01 n = 220	−.05 n = 219	−.01 n = 217
9. CBCL aggression	−.04 n = 201	−.06 n = 208	.06 n = 198	.01 n = 198	.40** n = 211	.40** n = 211	.32** n = 211	.98** n = 214	–	.78** n = 220	−.11 n = 205	.18* n = 220	−.03 n = 220	.01 n = 219	−.00 n = 217
10. CBCL rule-breaking	−.00 n = 201	−.04 n = 208	.07 n = 198	.00 n = 198	.41** n = 211	.40** n = 211	.37** n = 211	.91** n = 214	.80** n = 214	–	−.03 n = 205	.23** n = 220	.01 n = 220	−.15* n = 219	−.02 n = 217
11. Pubertal timing	.05 n = 200	.23** n = 208	.00 n = 196	.07 n = 196	.14* n = 210	.14* n = 210	.10 n = 210	.01 n = 208	−.03 n = 208	.09 n = 208	–	.00 n = 211	.00 n = 211	−.27** n = 210	.04 n = 210
12. Social adversity	.04 n = 205	.04 n = 214	.01 n = 201	.05 n = 201	.32** n = 216	.33** n = 216	.25** n = 216	.35** n = 214	.34** n = 214	.32** n = 214	.09 n = 213	–	−.08 n = 226	−.22** n = 225	−.05 n = 223
13. Age	−.11 n = 205	.10 n = 214	.26** n = 201	.22** n = 201	.06 n = 216	.02 n = 216	.11 n = 216	.02 n = 214	.01 n = 214	.04 n = 214	.00 n = 213	.02 n = 220	–	−.09 n = 225	.17** n = 223
14. Race	.01 n = 205	−.20** n = 214	.14* n = 201	.09 n = 201	−.20** n = 216	−.17* n = 216	−.20** n = 216	−.13 n = 214	−.11 n = 214	−.15* n = 214	−.33** n = 213	−.12 n = 220	−.03 n = 220	–	−.08 n = 222
15. BMI	−.03 n = 202	.09 n = 211	.02 n = 200	.07 n = 200	.13 n = 213	.11 n = 213	.14* n = 213	.11 n = 211	.09 n = 211	.14 n = 211	.30** n = 210	.12 n = 217	.04 n = 217	−.13 n = 217	–

Note: Cortisol AUC$_G$ = Cortisol area under the curve with respect to ground. YSR = Youth Self Report. CBCL = Child Behavior Checklist. BMI = Body Mass Index.

* p < .05.

** p < .01.

Table 3
Unstandardized regression coefficients for the interaction terms predicting self-reported and parent-reported outcomes in males.

	Self-reported externalizing outcomes			Parent-reported externalizing outcomes		
	YSR externalizing	YSR aggression	YSR rule-breaking	CBCL externalizing	CBCL aggression	CBCL rule-breaking
Left 2D:4D × Cortisol AUC_G	7.92 (3.62)* $n = 178$	5.32 (2.44)* $n = 177$	2.87 (1.40)* $n = 179$	1.69 (3.83) $n = 178$	.76 (2.7) $n = 178$	.93 (1.34) $n = 178$
Right 2D:4D × Cortisol AUC_G	7.60 (3.33)* $n = 179$	4.56 (2.24)* $n = 178$	3.03 (1.29)* $n = 180$	1.82 (3.63) $n = 179$	.94 (2.56) $n = 179$	.88 (1.28) $n = 179$
Left 2D:4D × Testosterone	.04 (.73) $n = 182$	.11 (.49) $n = 181$	−.15 (.29) $n = 183$	−.18 (.76) $n = 182$	−.03 (.53) $n = 182$	−.15 (.27) $n = 182$
Right 2D:4D × Testosterone	−.38 (.74) $n = 183$	−.14 (.50) $n = 182$	−.29 (.29) $n = 184$	.40 (.78) $n = 183$	.31 (.55) $n = 183$	.08 (.27) $n = 183$

Note: Regression models also included main effects of the variables in the interaction term, as well as saliva sample time, race, social adversity, age, pubertal timing, and BMI. Separate regressions were conducted for each interaction term. Cortisol AUC_G = Cortisol area under the curve with respect to ground. YSR = Youth Self Report. CBCL = Child Behavior Checklist. BMI = Body Mass Index.
*$p < .05$. **$p < .01$.

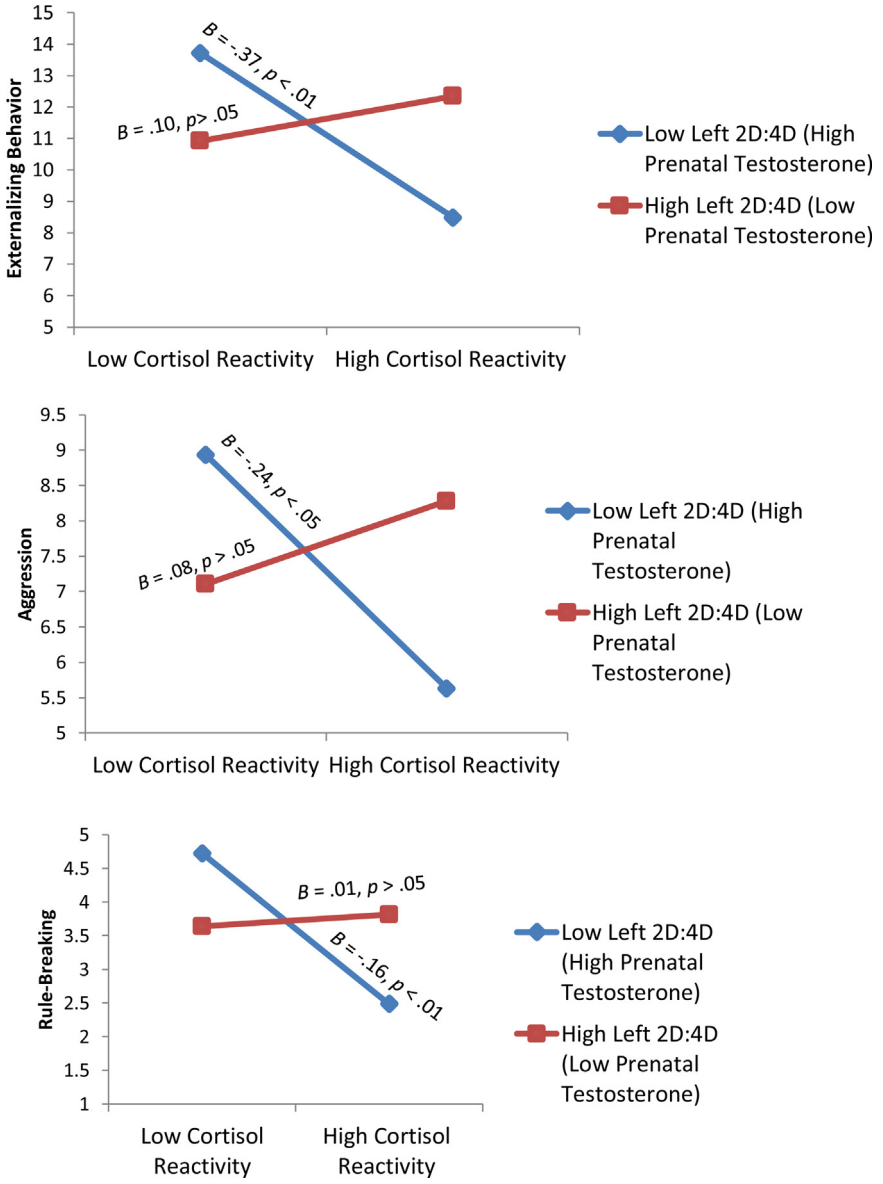

Fig. 1. Simple slopes of self-reported externalizing behavior, aggression, and rule-breaking on cortisol reactivity at high (+1 SD) and Low (−1 SD) levels of left 2D:4D in males.

behavior in a sample of young adolescents. We found that 2D:4D moderated the relationship between cortisol reactivity and self-reported externalizing behavior in males. Specifically, the expected negative relationship between cortisol reactivity and self-reported externalizing behavior was significant only for male subjects with a high 2D:4D. On the other hand, 2D:4D did not moderate the relationship between adolescent testosterone and either parent- or child-reported externalizing behavior. To our knowledge, this is the first study to find that 2D:4D interacts with cortisol reactivity to predict antisocial behavior. Results suggest the need to examine interactions between multiple hormone systems in order to understand externalizing behavior (Terburg et al., 2009). Because bivariate correlations between testosterone and cortisol with externalizing behavior were not significant, these findings also

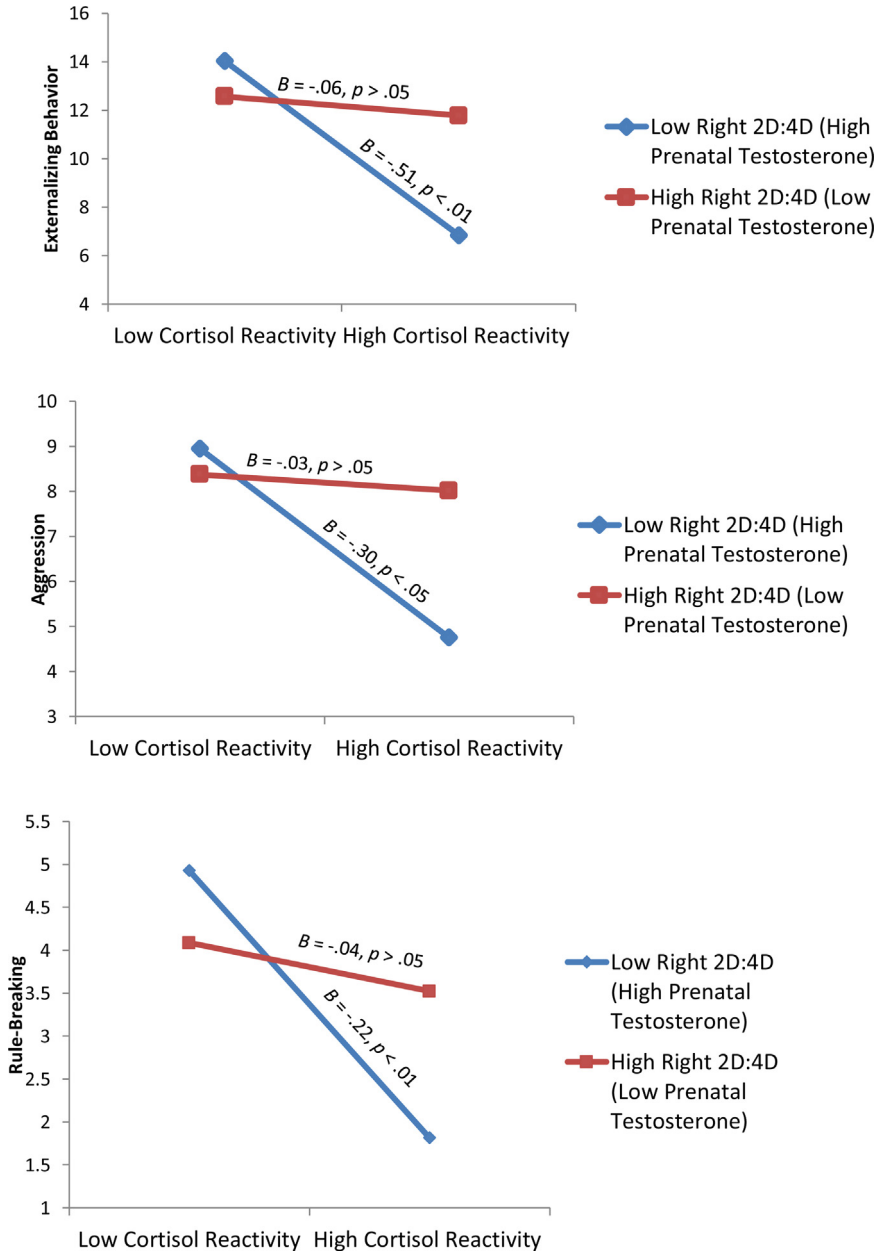

Fig. 2. Simple slopes of self-reported externalizing behavior, aggression, and rule-breaking on cortisol reactivity at high (+1 SD) and low (−1 SD) levels of right 2D:4D in males.

Table 4
Unstandardized regression coefficients for the interaction terms predicting self-reported and parent-reported outcomes in females.

	Self-reported externalizing outcomes			Parent-reported externalizing outcomes		
	YSR externalizing	YSR aggression	YSR rule-breaking	CBCL externalizing	CBCL aggression	CBCL rule-breaking
Left 2D:4D × Cortisol AUC_G	1.71 (2.02) $n = 181$	1.13 (1.40) $n = 181$	.59 (.78) $n = 181$	−2.26 (2.09) $n = 181$	−1.87 (1.46) $n = 181$	−.40 (.73) $n = 181$
Right 2D:4D × Cortisol AUC_G	−.55 (2.05) $n = 181$	.22 (1.42) $n = 181$	−.78 (.78) $n = 181$	−1.10 (2.30) $n = 181$	−.67 (1.61) $n = 181$	−.43 (.80) $n = 181$
Left 2D:4D × Testosterone	−.20 (.69) $n = 188$	−.04 (.48) $n = 188$	−.16 (.27) $n = 188$	−.19 (.84) $n = 188$	−.19 (.59) $n = 188$	−.003 (.29) $n = 188$
Right 2D:4D × Testosterone	−.12 (.86) $n = 188$	.22 (.60) $n = 188$	−.33 (.33) $n = 188$	−.15 (1.02) $n = 188$	−.24 (.71) $n = 188$	.08 (.36) $n = 188$

Note: Regression models also included main effects of the variables in the interaction term, as well as saliva sample time, race, social adversity, age, pubertal timing, and BMI. Separate regressions were conducted for each interaction term. Cortisol AUC_G = Cortisol area under the curve with respect to ground. YSR = Youth Self Report. CBCL = Child Behavior Checklist. BMI = Body Mass Index.
$^{*}p < .05$. $^{**}p < .01$.

suggest that 2D:4D could help to explain heterogeneity in the findings of studies that examine the independent effects of hormones on externalizing behavior.

This study examined a suggested marker of prenatal testosterone exposure as a moderator of the cortisol-externalizing behavior relationship. Although a growing body of prior research has found that adolescent and adult circulating testosterone and cortisol interact to predict violent and antisocial behavior (Dabbs & Jurkovic, 1991; Glenn et al., 2011; Mehta & Josephs, 2010; Popma et al., 2007), this study is the first to find that a marker of prenatal

testosterone also interacts with cortisol to predict these behaviors. Findings are consistent with the pattern of interactions observed in these prior studies; in particular, low cortisol was associated with higher levels of antisocial behavior, but only for subjects with low 2D:4D (high prenatal testosterone). We extended prior findings by documenting this interaction prenatally.

There have been several proposed explanations as to why postnatal testosterone may interact with cortisol to predict externalizing behavior (reviewed in Terburg et al., 2009). For instance, it has been argued that an increased level of testosterone relative to cortisol may reflect an imbalance in reward and punishment sensitivity that increases the likelihood of reward motivated antisocial behavior (Terburg et al., 2009). This model is based on the finding that high testosterone is associated with increased reward sensitivity and decreased punishment sensitivity (van Honk et al., 2004), while low cortisol is associated with reduced punishment sensitivity (van Honk, Schutter, Hermans, & Putman, 2003).

Although existing theories of the cortisol-testosterone imbalance focus on postnatal testosterone, our results also suggest the need to consider the influence of prenatal testosterone in determining the effect of cortisol on behavior. Animal studies that examine corticosterone—the end product of the HPA axis in mice and rats—could help to guide our understanding of the interrelationship between cortisol and prenatal testosterone in humans. An animal study found that mice who were selectively bred for the trait of high voluntary exercise had both a higher 2D:4D (indicating lower prenatal testosterone) and also elevated baseline circulating corticosterone (Yan, Malisch, Hannon, Hurd, & Garland, 2008). These findings were surprising, given that physical fitness—which is considered a male-dominant trait—was associated with a *higher* 2D:4D, leading the authors to conclude that 2D:4D may not be an effective proxy for prenatal androgen exposure. Nonetheless, these findings do suggest that a lower 2D:4D ratio could be associated with increased corticosterone production. Similarly, in experimental research, female rats who were exposed to testosterone prenatally were found to experience masculinization of the HPA axis, as indicated by lower corticosterone secretion in adulthood (Seale et al., 2005a). On the other hand, male rats deprived of prenatal testosterone had increased adult secretion of corticosterone, a more feminine pattern of HPA axis activation (Seale et al., 2005b). This suggests that prenatal testosterone likely plays some role in the masculinization of the HPA axis in rats, although this remains to be seen in human research. Nonetheless, the findings of the current study suggest that low 2D:4D combined with reduced cortisol reactivity could increase the likelihood of antisocial behavior, because these individuals display increased sensitivity to postnatal testosterone in the presence of reduced cortisol.

Contrary to our expectations, in the current study, 2D:4D did not interact with adolescent testosterone level to predict antisocial behavior. This finding was in contrast to a prior study in males, which found that 2D:4D moderated the effect of testosterone on sexually dimorphic socio-cognitive processes (Carré et al., 2015). One possible reason for this null finding relates to the outcome measure used in this study. It is thought that aggression itself is not related to testosterone, but rather that testosterone is associated with increased social dominance, which may or may not take the form of aggressive behavior (Mazur & Booth, 1998; Rowe et al., 2004; Schaal, Tremblay, Soussignan, & Susman, 1996). Thus, the externalizing behavior measures used in this study may not have captured the social dominance construct that is thought to be most directly related to circulating testosterone. Alternatively, the null findings may be related to the developmental period of the subjects in this study. Male subjects were on average between Tanner Stages 3 and 4. This is a period of large hormonal fluctuations, which could have affected results. On the other hand, the findings of this study are suggestive that the organizational influence of prenatal testosterone may have a more important impact than circulating testosterone on the behavior of early adolescents transitioning to puberty.

4.1. Sex differences

Although the interaction between 2D:4D and cortisol reactivity significantly predicted self-reported externalizing behavior in males, this interaction was not significant in females. These results are potentially consistent with a meta-analysis, which found that there was a small, negative relationship between 2D:4D and aggression in males, but not in females (Hönekopp & Watson, 2011). While we were not able to identify the exact source of the null finding in females, there are several potential explanations. For instance, it is possible that amongst adolescents, externalizing and aggressive behavior are less reflective of social dominance in females than in males. Consistent with this, de Bruyn (2012) found that physical aggression was characteristic of social dominance in male, but not in female adolescents. This suggests that testosterone, which is thought to be indicative of social dominance, may be less strongly associated with physical aggression in adolescent females. Alternatively, sex differences in the interaction between cortisol and testosterone may be related to sex differences in androgen production (Montoya et al., 2012). In males, androgens primarily originate from the HPG axis, while in females a larger proportion of androgen production comes from the adrenal cortex, which is part of the HPA axis, where cortisol also originates (Burger, 2002; Montoya et al., 2012). Thus, the relative balance between testosterone and cortisol may be less predictive of behavior in females than in males.

4.2. Limitations, contributions, and future directions

There are several limitations to this study that should be highlighted. One limitation relates to the marker of prenatal testosterone used in the current study, as 2D:4D is an imperfect marker of prenatal testosterone. Given the risks of performing medically unnecessary amniocentesis to assay prenatal hormones and the clear ethical concerns of manipulating in-utero hormone levels, 2D:4D is widely used as a method of retrospectively estimating prenatal androgen exposure (Breedlove, 2010; Manning et al., 1998, 2014). Nonetheless, we should note that the use of 2D:4D as a marker of prenatal androgen exposure remains subject to debate. Some researchers have argued that 2D:4D is only modestly linked to prenatal androgen exposure and therefore may not be a strong proxy for individual differences in androgen exposure (Berenbaum & Beltz, 2011; Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009; Hines, 2010; Voracek, 2014). On balance, recent experimental research in animals has provided both direct and indirect evidence that androgen level affects digit ratio (Auger et al., 2013; Zheng and Cohn, 2011), while other researchers have countered claims used to question the use of 2D:4D as a marker of prenatal androgens (Hönekopp, 2013). In light of ongoing investigations into the validity of 2D:4D as an indicator of individual differences in androgen exposure, we urge our findings to be interpreted with some caution.

We should also mention that our findings only applied to self-reported externalizing behavior, and no significant interactions were found for parent-reported behavior. It is common for parent and child ratings of behavior to be only weakly correlated (Achenbach and Rescorla, 2001), and given differences in the sample of behaviors observed by different informants, it is possible that different raters may capture somewhat different behavioral constructs. Consequently, we would not necessarily expect results to converge across raters. Nonetheless, given this lack of convergence, our findings should be interpreted with some caution.

We should also note that in females, but not in males, left and right 2D:4D were positively and significantly associated with age.

Several prior studies found that 2D:4D increases slightly with age (Coyne, Manning, Ringer, & Bailey, 2007; Gillam, McDonald, Ebling, & Mahew, 2008; Trivers, Manning, & Jacobson, 2006). It is not yet known why 2D:4D changes postnatally. Although 2D:4D has been found to fluctuate with the menstrual cycle in post-pubertal women (Mayhew, Gillam, McDonald, & Ebling, 2007), a recent study found that 2D:4D does not change across the menstrual cycle (Barrett et al., 2015). Because the source of the relationship between 2D:4D and age is not yet fully understood, it is possible that the current study failed to take into account relevant confounding variables, such as the stage of the menstrual cycle in menstruating female subjects. On the other hand, given age effects on 2D:4D, a strength of this study was the relatively small age range studied.

In spite of these limitations, it is believed that the current study has significant strengths. Importantly, this study extends our understanding of the relationship between cortisol and externalizing behavior in males by demonstrating for the first time that the expected negative relationship between cortisol reactivity and externalizing behavior was only present in subjects with low 2D:4D (high prenatal testosterone). This could partly explain why findings on cortisol reactivity and externalizing behavior are inconsistent (Alink et al., 2008). Interestingly, prenatal testosterone interacted with adolescent cortisol reactivity. This finding suggests the importance of early developmental processes in shaping behavior later in life. Although there is evidence that 2D:4D is sensitive to ethnicity (Manning, Stewart, Bundred, & Trivers, 2004), prior hormone research has been conducted in largely in white samples. Therefore, another key contribution of the current study was the investigation of 2D:4D in a sample that was nearly 80% African American. We should note that this study was conducted in a relatively large sample at a critical point in development when both testosterone and behavior are beginning to change dramatically. Therefore, this is a particularly important developmental period to examine the effect of hormones on behavior.

This study provides support for the need to examine interactions between multiple hormones in order to understand behavior. Taken together, the findings of this study suggests a partial hormonal basis to antisocial behavior and point to the critical need to examine biological processes in conjunction with one another in order to understand the etiology of externalizing behavior.

Disclosure statement

In the interest of full disclosure, DAG is founder and Chief Scientific and Strategy Advisor at Salimetrics LLC and Salivabio LLC (Carlsbad, CA) and these relationships are managed by the policies of the committee on conflict of interest at the Johns Hopkins University School of Medicine and the Office of Research Integrity and Adherence at Arizona State University.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopsycho.2015.09.013.

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