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Soccer players awarded one or more red cards exhibit lower 2D

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sexual differentiation of the brain (Auyeung, Lombardo, & Baron-Cohen, 2013; Lombardo et al., 2012; Morris et al., 2004). A second surge of testosterone occurs shortly after birth, reaching a peak at 3–4 months postpartum. This second hormonal surge plays a role in early penile size and growth rate, both in non-primate apes and humans (Boas et al., 2006; Brown, Nevison, Fraser, & Dixson, 1999). Recent evidence suggests that the postnatal testosterone surge might be implied in the development of male-typical behavior (Alexander, 2014). During puberty, testosterone has further virilizing effects, controlling the development of secondary sexual characteristics, stimulating spermatogenesis, and promoting muscle and bone growth (Guatelli-Steinberg & Boyce, 2012).

There is growing evidence that prenatal testosterone is also responsible for the sexual dimorphism observed in the second-to-fourth (2D:4D) digit ratios. Males typically exhibit longer fourth digits relative to second digits (low 2D:4D) than females (high 2D:4D) (for a review, see Manning, Kilduff, Cook, Crewther, & Fink, 2014). While there is evidence of differences in 2D:4D ratios across different ethnicities, sex differences in second-to-fourth finger ratios seem to be robust (e.g., Butovskaya, Burkova, Karelin, & Fink, 2015; 2000; Oladipo, Fawehinmi, Edibamode, Osunwoke, & Kenneth, 2009, but see Apicella, Tobolsky, Marlowe, & Miller, 2015). It has been shown that high levels of amniotic fluid testosterone relative to estradiol in the second trimester of pregnancy are associated with low 2D:4D ratios, at two years of age (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). Consistently, females exposed to high levels of adrenal androgens during embryonic development as a result of congenital adrenal hyperplasia (CAH) exhibit significantly lower right-hand 2D:4D ratios than females without CAH, while males with this condition exhibit lower left-hand 2D:4D ratios than their unaffected relatives (Brown, Hines, Fane, & Breedlove, 2002; Ökten, Kalyoncu, & Yaris, 2002). Buck and co-workers failed to find any significant differences in 2D:4D ratios between CAH females and controls females using direct measurements from left hand radiographs (Buck, Williams, Hughes, & Acerini, 2003). However, in a previous study, Manning and co-workers showed that 2D:4D ratios showed a typical dimorphism when derived from scanned digits and no sex differences could be observed when the ratios were determined from hand radiographs (Manning, Trivers, Thornhill, & Singh, 2000; but see Xi, Li, Fan, & Zhao, 2014).

Another line of evidence for the role of prenatal androgens influencing second-to-fourth finger ratios comes from the study of digit ratios in opposite-sex twin

pairs. van Anders and co-workers observed significantly lower left hand 2D:4D ratios (more masculinized) in females from female/male twin pairs than in females from same sex pairs (van Anders, Vernon, & Wilbur, 2006). In a similar study, Voracek and Dressler (2007) also reported lower 2D:4D ratios for both hands in females from opposite-sex twin pairs; however, in a larger study such an effect could not be found (Medland, Loehlin, & Martin, 2008).

Molecular mechanisms underlying the sex-related differential growth of the second and fourth fingers have been elucidated by experimental manipulation of sex steroids levels in different animal models (Manning et al., 2014; Romano, Rubolini, Martinelli, Bonisoli Alquati, & Saino, 2005; Zheng & Cohn, 2011). For instance, mice exhibit sexual differences in 2D:4D ratios, both in fore and hind limbs, and these differences can be explained by the differential activation of the Androgen Receptor (AR) or the Estrogen Receptor (ER- α) present at higher densities in the cartilage condensations of the developing fourth toe than in the cartilage condensations of the second toe (Zheng & Cohn, 2011).

Recently, there has been some questioning of the role of prenatal testosterone in the establishment of the sexually dimorphic digit patterns. Initially, Manning and co-workers reported a correlation between CAG repeats copy number in the coding sequence of the AR—which are known to affect its transactivation function (Chamberlain, Driver, & Miesfeld, 1994)—and the 2D:4D ratios (Manning, Bundred, Newton, & Flanagan, 2003). Further studies have failed to replicate these results, or find a relationship between GGN repeats—also known to modulate AR transactivation function (Brockschmidt, Nöthen, & Hillmer, 2007)—and 2D:4D ratios (for meta-analyses, see Hönekopp, 2013; Voracek, 2014). It should be noted, though, that modulation of testosterone action does not rely exclusively on CAG and/or GGN repeats copy number (see Discussion in Hönekopp, 2013). In fact, AR function is modulated through several complex mechanisms, such as: interaction with co-factors, intracellular trafficking of the hormone/receptor complex, and interaction with general transcription factors (Heinlein & Chang, 2002; Matsumoto et al., 2013).

Hickey et al. (2010) failed to find a correlation between androgens levels in maternal and umbilical cord blood and offspring 2D:4D ratios. However, assessment of sex steroid levels in maternal serum might not be the method of choice for measuring embryonic hormone levels (Van De Beek, Thijssen, Cohen-Kettenis, Van Goozen, & Buitelaar, 2004), and umbilical cord hormone levels refer rather to the perinatal period and not to earlier critical developmental stages, when brain

sex differentiation is believed to occur. Ventura et al. (2013) reported that while 2D:4D sex differences are already present at the time of birth, this dimorphism is only significant for the left hand in newborns, and suggest that the perinatal surge of testosterone and additional developmental processes might be determinant for the 2D:4D ratios dimorphism observed in later life (see also Galis, Ten Broek, Van Dongen, & Wijnaendts, 2010). Additionally, these authors found a significant correlation between mid-gestation testosterone levels in amniotic fluid and 2D:4D ratios in female newborns, but not in male newborns (Ventura et al., 2013). It should be considered, though, that amniotic fluid samples were taken at quite different time points across the sample (15.7–23.6 weeks of pregnancy), and the testosterone levels considered in this study might not reflect the actual differences in steroid concentrations at a critical time point. In the same vein, Hollier et al. (2015) failed to find a correlation between umbilical cord levels of androgens, estrogens or androgens/estrogens and 2D:4D ratios after controlling for co-variables.

The study of the relationship between prenatal androgens and second-to-fourth digit ratios, particularly in humans, has produced some mixed results, but there is a large body of evidence that support an association between prenatal androgens and 2D:4D ratios in later life. Further research will be needed to fully understand the exact mechanisms by which prenatal, and probably perinatal testosterone, influence second and fourth finger development.

Given the organizing role of fetal androgens on brain structures, and that second-to-fourth digit ratios seem to reflect the relative levels of fetal testosterone in relation to fetal estradiol, numerous studies have analyzed the relationship between 2D:4D ratios and sexually dimorphic characters and behavior (e.g., Voracek, 2011). In this study we focus on the relationship between second-to-fourth digit ratios and two different behavioral characteristics that have been previously related to testosterone—aggressive behavior and sporting performance.

Aggression cannot be considered as a unitary construct (Archer & Coyne, 2005; Buss & Shackelford, 1997; Geen, 2001). Several forms of aggressive behavior have been identified, including indirect, verbal and physical aggression (e.g., Archer, 2004). Sex differences have been observed in some forms of aggressive behavior. For instance, meta-analytic studies have revealed sex differences in physical aggression—towards men—throughout the lifespan, while sex differences in indirect forms of aggression—towards women—seem to be less pronounced, restricted to specific life stages, and dependent on method of

measurement (Archer, 2004; Card, Stucky, Sawalani, & Little, 2008; Eagly & Steffen, 1986).

Most studies that have explored the relationship between 2D:4D ratios and aggressive behavior were based on self-reported assessments of aggressive behavior. Following a meta-analysis of data from 18 studies, Hönekopp and Watson (2011) concluded that there is no significant relationship between aggression and 2D:4D for females, while there is a very weak negative correlation between both variables for males. Among the few studies conducted in real world conditions, one study showed that imprisoned male offenders exhibit significantly lower 2D:4D ratios than control subjects (Hanoch, Gummerum, & Rolison, 2012). Particularly relevant for the present research, aggressive behavior in sports has also been related to 2D:4D ratios. Among elite fencers, those who favor the more aggressive forms of fencing (i.e., sabre fencing) have lower 2D:4D ratios than those who prefer other forms of this sport (Voracek, Reimer, & Dressler, 2010). Similarly, patients with boxers' fractures—fractures of the fourth or fifth metacarpal necks—secondary to an aggressive act, have lower 2D:4D ratios than controls (Joyce et al., 2013). In a recent study, Perciavalle et al. (2013) reported a negative correlation between the number of yellow and/or red cards per match and 2D:4D ratios in professional soccer players.

In recent years an additional anthropometric index, the facial width-to-height ratio (fWHR)—linked to puberty testosterone levels—has also been related to aggressive behavior both in the laboratory and in hockey players (Carré & McCormick, 2008). Facial width-to-height ratio was also found to be positively correlated with fouls committed and goals scored, particularly in midfielders and forwards (Welker, Goetz, Galicia, Liphardt, & Carré, 2014), and to predict fighting ability in professional mixed-martial arts fighters (Zilioli et al., 2015). Prenatal testosterone has also been linked to physical prowess in a wide variety of sports (for a meta-analysis, see Hönekopp & Schuster, 2010). It has been proposed that prenatal levels of androgens influence the development of gender differences (Hines, 2011), some of which might be advantageous for athletic performance, including endurance (Manning, Morris, & Caswell, 2007), maximal oxygen uptake (Hill, Simpson, Millet, Manning, & Kilduff, 2012), certain aspects of visuospatial cognitions (Falter, Arroyo, & Davis, 2006), and perceived dominance (Neave, Laing, Fink, & Manning, 2003).

The present study aims to further the study of the relationship between 2D:4D ratios—a proxy for prenatal testosterone levels—and both physical aggression and sporting performance. Rather than relying on self-report or inferential measurements of aggression and sporting performance, the current study is based on actual

aggressive behavior (i.e., foul play penalized by yellow or red cards), and a global assessment of sporting success.

METHODS

Participants

A total of 94 male junior soccer players, ages 13 to 17 ($M = 15.33$, $SD = 1.15$), belonging to different age categories from a professional Uruguayan soccer club, participated freely in this study. All participants were Caucasian, had a mean height of 174.80 cm ($SD = 7.59$), a mean body mass of 67.65 kg ($SD = 7.64$), and a mean body mass index (BMI) of 22.11 kg/m² ($SD = 1.56$). According to the team's authorities, none of the players suffered from any significant medical or psychological condition, or was using drugs at the time the study was performed.

Two participants were identified as outliers and were excluded from the correlation analyses between 2D:4D ratios and yellow cards per match, and 2D:4D ratios and weighted cards per match, because their cards per match scores were larger than three interquartile ranges from percentile 75. One participant was identified as an outlier and was excluded from the correlation analysis between 2D:4D ratios and red cards per match, because his red cards per match scores were larger than three interquartile ranges from percentile 75.

2D:4D Ratios

The ventral surfaces of the right and left hands of the soccer players were scanned using a CanoScan LiDE 100 Canon Scanner, at a 150 dpi resolution. Fingers were measured independently by two researchers. Length measures were taken from the midpoint of the proximal metacarpo-phalangeal crease to the tip of the finger using the measure tool of the GNU Image Manipulation Program (GIMP) 2.8. When the difference between the two measurements was greater than three standard deviations the measurement of that particular finger was repeated.

2D:4D ratios were calculated for both hands as the length of the second digit divided by the length of the fourth digit. The scanned images of one or both hands of two players were out of focus and could not be used in this study. The 2D:4D ratios of two additional players were not considered in this study since they reported lesions in the second and/or fourth fingers.

Following Weinberg, Scott, Neiswanger and Marazita (2005) the technical error of measurement (TEM) and the relative technical error of measurement (rTEM) were calculated for all measurements and ratios. TEM values of .48 mm and .61 mm, and rTEM's of .62% and .82% were obtained for the right and left hands measurements respectively. Additionally, the intraclass correlation

coefficients (ICC)—two-way, mixed measure, absolute agreement—were also calculated. For all measurements and coefficients ICCs ranged between .95 and .99.

Red Cards, Yellow Cards, and Minutes Played

Yellow, red, and weighted cards were used as a proxy for aggressive behavior. In order to account for the different number of minutes played by each single player, yellow, red, and weighted cards per time unit were computed. For ease of comparison, these scores are given as number of yellow, red and weighted cards per match (1 match = 80 minutes, the canonical duration of a match for junior soccer players). Based on previous studies (Caruso & Di Domizio, 2013; Gandelman, 2009), the weighted sum cards per match was also computed (1 yellow card = 1 weighted card, 1 red card = 2 weighted cards). For example, if a player played 800 minutes in the season analyzed, and was awarded 2 yellow cards and 1 red card, he would have a score of .4 weighted cards per match. In support of the proposed weighting of the cards, it should be noted that a red card is also awarded as a result of the accumulation of two yellow cards in a single match (FIFA, 2014). The players in our sample were awarded a median of .115 yellow cards per match ($IQR = .000-.241$), a median of .000 red cards per match ($IQR = .000-.033$), and a median of .145 weighted cards per match ($IQR = .000-.269$).

In the context of the current study, we used the number of minutes played by each player, in official matches, as an overall measure of sporting performance. This is based on the assumption that if a player performs well in a match, he will be included in the team in subsequent matches and will continue playing for as long as the coach considers he is performing well. Otherwise, he would be replaced by a substitute player. Teams from each age category played a total of 30 matches in the time period analyzed (15 matches in the Campeonato de Clausura 2012, and 15 matches for the Campeonato de Apertura 2013). For junior categories, matches consist of two 40 minutes-halves. According to the development of the match, the referee can add extra time to be played. In the period of time considered in this study, the number of minutes played by individual players ranged from 8 to 2600, with a mean of 1075.04 minutes ($SD = 786.11$).

Data on the number of awarded red and yellow cards, and number of minutes played by each individual player were obtained from the soccer club authorities.

Statistical Analyses

Continuous data were summarized using means and standard deviations (SD) or medians and interquartile

ranges (IQR) depending on data distribution. Digit ratio differences between players who received no cards, players awarded only yellow cards and players bestowed red cards were tested by one-way ANOVA. Since the assumption of homogeneity of variance was violated for these data, Welch F ratios were calculated. Post-hoc comparisons between these three groups were performed by the Games-Howell test given that equal variances assumption was violated, and groups were unequal in size. Since the sample size n is sufficiently large, Pearson correlations were used for exploring the association between 2D:4D ratios and the following variables: yellow cards per match, red cards per match and weighted cards per match. For all analyses, the level of statistical significance was set at $P \leq .05$.

RESULTS

2D:4D and Aggressive Behavior

We first analyzed aggressive behavior measured as type of cards (i.e., none, yellow or red) awarded to the players during the complete season. Given that yellow and red cards might reflect qualitatively different behaviors (FIFA, 2014; Miguel, Saiegh, & Satyanath, 2011), we considered the groups formed by i) players who received no cards ($n = 27$), ii) players awarded one or more yellow cards, but no red cards ($n = 39$), and iii) players awarded one or more red cards ($n = 24$).

A one-way between subjects ANOVA was performed to assess the existence of differences in 2D:4D ratios between these groups. The analysis revealed significant differences between the groups both for the right hand ($F_{(2,54.074)} = 8.321$, $P = .001$) and the left hand ($F_{(2,54.318)} = 6.136$, $P = .004$). Games-Howell post hoc tests revealed significant differences in 2D:4D ratios between players awarded no cards and those awarded one or more red cards ($P = .001$ for the right hand ratios, $P = .003$ for the left hand ratios). The least aggressive players—those awarded no cards—exhibit the largest 2D:4D digit ratios (i.e., have a more feminine finger pattern), while the most aggressive individuals—those awarded one or more red cards—show the lowest 2D:4D finger ratios (i.e., have a more masculinized hand). As expected those players with intermediate levels of aggressive behavior—reflected by the award of one or more yellow cards, but no red cards—exhibit intermediate finger ratios (see Fig. 1).

In order to further explore the relationship of 2D:4D ratios and aggressive behavior, we calculated Pearson correlation coefficients between second-to-fourth ratios and yellow cards per match, red cards per match and the weighted sum of cards per match. A significant (two-tailed) negative correlation was found between 2D:4D ratios and yellow cards per match for the right hand

Fig. 1. Right- and left-hand 2D:4D ratios in soccer players exhibiting different levels of aggressive behavior. Error bars represent the 95% confidence interval (CI) of the means. R2D4D: right hand 2D:4D ratios, L2D4D: left hand 2D:4D ratios.

($r_{85} = -.275$, $P = .011$), but not for the left hand ($r_{85} = -.180$, $P = .098$). Significant (two-tailed) correlations were also found between 2D:4D ratios and red cards per match, both for the right hand ($r_{86} = -.215$, $P = .047$) and left hand ($r_{86} = -.237$, $P = .028$); and between 2D:4D ratios and weighted cards per match, for the right hand ($r_{85} = -.305$, $P = .005$) and also for the left hand ($r_{85} = -.221$, $P = .042$). That is, with decreasing right hand 2D:4D ratios (i.e., more masculinized hands), the yellow cards per match, red cards per match, and weighted sum of cards per match (i.e., aggressive behavior) increased. The same association is observed between left hand 2D:4D ratios and red cards per match, and 2D:4D ratios and weighted cards per match (see Fig. 2).

2D:4D and Sporting Performance

In order to investigate whether second-to-fourth digit ratios are related to sporting performance—measured as number of minutes played—Pearson correlations were calculated. Results showed that neither the right hand 2D:4D ratio ($r_{87} = -.137$, $P = .204$, two-tailed), nor the left hand 2D:4D ratios ($r_{87} = -.062$, $P = .567$, two-tailed) were significantly related to sporting performance.

DISCUSSION

In this study, we investigated whether the second-to-fourth digit ratios—a putative marker of exposure to different levels of intrauterine androgens—are related to

Fig. 2. Relationship between right- and left-hand 2D:4D ratios and yellow, red and weighted cards per match. R2D4D: right hand second-to-fourth digit ratios; L2D4D: left hand second-to-fourth digit ratios.

differences in aggressive behavior and physical performance in later life, particularly in a sample of male junior soccer players. Our results show a significant difference in the 2D:4D ratios of soccer players who have been awarded one or more red cards (low 2D:4D ratios) and those awarded no cards at all (high 2D:4D ratios), suggesting that soccer players who have been exposed to highest levels of prenatal androgens are most prone to engage in aggressive behavior on the field, those exposed to lowest levels are least prone to aggressive behavior, while those exposed to intermediate levels are intermediate in their aggressive behavior (Fig. 1). The differences in 2D:4D ratios seem to reflect differences in the nature of the punished behavior. A stepwise progression in aggression levels can be observed between soccer players who received no cards, to those who received at least a one yellow card, and then to those who received one or more red cards—while yellow cards are usually awarded by breaking the rules or unsporting behavior, red cards usually result from serious physical or verbal aggressions, or from denying an obvious goal scoring opportunity through unlawful behavior (FIFA, 2014). These results are consistent with the role of prenatal androgens in influencing aggressive behavior in later life (Hines, 2011).

It should be noted, though, that red cards can also be awarded by accumulation of yellow cards within a single match (FIFA, 2014). In fact, where data were available, it could be observed that only 35–40% of the red cards were directly awarded (Miguel et al., 2011). This information was not available for our study. It would be interesting to study the relationship between 2D:4D ratios and yellow cards, red cards resulting from the accumulation of yellow cards and direct red cards. We expect the differences between the digit ratios of players awarded no cards and those awarded direct red cards to be even larger than the differences observed in this study.

Since it could be argued that these results might be affected by the number of minutes played by each individual player, we also analyzed the relationship between 2D:4D ratios and the number of yellow, red and weighted sum of cards per match. Pearson correlation analyses revealed moderate significant (two-tailed) negative correlations for all cards and 2D:4D ratios from both hands (the correlation was not significant between yellow cards per match and left hand 2D:4D ratios), that is, players with more masculinized hands were awarded more yellow and/or red cards per match than individuals with more feminized hands. These results are consistent with a role of testosterone in facilitating aggressive behavior in later life by means of affecting early neural development.

Recently, Perciavalle et al. (2013) reported a negative correlation of right hand 2D:4D ratios and the unweighted sum of red and yellow cards per match, in a sample of 18 male professional players from a first level Italian soccer team. Our findings are in line with these results. In a meta-analytic study, Hönekopp and Watson (2011) report a significant weak negative correlation between physical aggression and right hand 2D:4D ratios in males ($r = -.064$, $P = .013$). It should be noted, though, that a large number of the studies considered measured aggressive behavior using the Buss and Perry Aggression Questionnaire (Buss & Perry, 1992) or some alternative aggression questionnaires. One interesting possibility is that actual aggressive behavior, but not aggressive attitudes or feelings, are correlated with 2D:4D ratios. Alternatively, this difference in the magnitude of the correlation could be explained by the age range of the participants—the studies considered in this meta-analysis included a single adolescents sample (Hönekopp & Watson, 2011)—or even by the nature of the sample considered in this study (i.e., soccer players), since sports may allow a socially accepted channel for venting violent behavior.

In an attempt to confirm previously reported data (e.g., Hönekopp & Schuster, 2010; Perciavalle et al., 2013; Tester & Campbell, 2007), we analyzed the relationship between second-to-fourth digit ratios and sporting performance. A Pearson correlation analysis failed to find the previously reported relationship between 2D:4D ratios and sporting performance. In different studies, sporting performance has been measured in a variety of ways. In particular, soccer performance has been equated with playing in the first- or reserve-team (Manning, Bundred, & Taylor, 2003) and the number of goals per game (Perciavalle et al., 2013). Since the chance of a soccer player scoring a goal depends heavily on his playing position, we believe that the number of goals—or number of goals per match—does not provide an objective measure of sporting performance, unless the players' positions are taken into consideration. The number of players in the junior divisions considered in the present study greatly outreach the playing opportunities, and given that players are included in the team based on the coach's judgment of previous performance, we believe that the number of minutes played provides an overall measure of sporting performance throughout the season.

Future research could profitably examine the relationship between prenatally determined (and presumably perinatally modulated) digit ratios and other anthropometric characteristics established during puberty (e.g., facial width-to-height ratios), and study their combined effects on aggressive behavior and sporting performance.

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