◆ Human Brain Mapping 36:1043–1052 (2015) ◆

Short Fused? Associations Between White Matter Connections, Sex Steroids, and Aggression Across Adolescence

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Abstract: Functional neuroimaging studies in adults show that aggression involves reduced brain communication between subcortical and cortical areas dedicated to motivation and control, respectively. Prior research indicates that sex steroid hormone production during adolescence negatively influences the rapid development of white matter connectivity between subcortical and cortical areas during adolescence and may potentiate aggression. Here, we tested this hypothesis in 258 participants between 8 and 25 years of age by using Diffusion Weighted Imaging to examine the microstructure of white matter connections within the fronto-temporal-subcortical network. Trait aggression was measured using the Buss Perry Aggression Questionnaire and testosterone and estradiol levels were measured in saliva. Results indicated that higher levels of testosterone were associated with less white matter integrity within the fronto-temporal-subcortical network (i.e., higher mean diffusivity [MD] longitudinal [LD], and radial diffusivity [RD]). Furthermore, lower fractional anisotropy and higher MD, LD, and RD values within this network increased expressive forms of aggression and reduced inhibited forms of aggression (hostility). Our study indicates higher levels of testosterone relating to lower quality of structural cortical-subcortical connectivity, arguably resulting in a shift from inhibited towards expressive forms of aggression. Our data adds evidence to the idea that aggressive tendencies are subcortically driven, but individuals with relatively high testosterone might have lower structural connectivity within cortical control areas, resulting in a stronger tendency to act on these aggressive tendencies. *Hum Brain Mapp 36:1043–1052, 2015.*

Additional Supporting Information may be found in the online version of this article.

Martijn P. van den Heuvel and Dennis J. L. G. Schutter contributed equally to this work.

Contract grant sponsor: Dutch Science Foundation (NWO-Veni); Contract grant number: 451-10-007 (to J.S.P.); Contract grant sponsor: Dutch Science Foundation (NWO-Veni); Contract grant number: 451-12-001 (to M.P.v.d.H.); Contract grant sponsor: Dutch Science Foundation (NWO-Vidi); Contract grant number: 452–07–012 (to D.J.L.G.S.)

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Received for publication 28 April 2014; Revised 22 October 2014; Accepted 23 October 2014.

DOI: 10.1002/hbm.22684

Published online 31 October 2014 in Wiley Online Library (wileyonlinelibrary.com).

Key words: adolescence; aggression; connectivity; diffusion tensor imaging; testosterone; white matter

INTRODUCTION

Extreme forms of aggression pose a threat to both individuals and society. Higher than normal levels of aggression have been linked to psychopathological conditions including conduct disorder, oppositional defiant disorder and antisocial personality disorder [Matthys et al., 2013]. Studying the underlying etiology of individual differences in aggression is, therefore, of critical importance for understanding this often destructive behavior.

Aggression can be defined as overt behavior to deliberately inflict harm to others, objects or one-self [Anderson and Bushman 2002]. Aggression comes in different forms and includes "inhibited" aggression (e.g., hostility), aggressive emotions (e.g., anger), and "disinhibited" aggressive behavior (e.g., physical and verbal aggression [Anderson and Bushman, 2002]. Even though aggressive behavior is complex and involves an intricate interplay between the individual and environment, social scientific research has shown that aggressive behavior is often associated with low impulse control, anger, and decreased capacity of regulating emotions [Anderson and Bushman, 2002; Dodge, 2006].

Neuroimaging studies in adults have shown that aggressive tendencies involve an imbalance between heightened reactivity in motor and subcortical areas dedicated to motivation [Hermans et al., 2008; Pawliczek et al., 2013], paralleled by relative low regulatory capacities of the prefrontal brain areas (for review see [Coccaro et al., 2011]). This imbalance may point to suboptimal brain communication between cortical and subcortical brain areas that could represent a neural predisposition for aggressive behavior (e.g., [Hofman and Schutter, 2012; Schutter and Harmon-Jones, 2013]. Indeed, abnormalities in white matter integrity within subcortico-frontal and fronto-temporal connections were reported in adolescents with conduct disorder [Haney-Caron et al., 2014; Sarkar et al., 2013], as well as in adult psychopathic offenders [Hoppenbrouwers et al., 2013]. Interestingly, the integrity of white matter pathways between cortical and subcortical brain areas increases from childhood into adolescence [Brouwer et al., 2012; Ladouceur et al., 2012; Lebel and Beaulieu, 2011; Simmonds et al., 2013]. The increase in subcortico-cortical white matter quality has been proposed as a possible mechanism by which cognitive control over motivational tendencies grows during this transition period [Peper et al., 2013a]. As a result of the increase in quality of the white matter tracts between subcortical, frontal, and temporal brain regions, it is not unreasonable to assume that aggressive tendencies will decrease with age.

In addition, there is now evidence showing that the sharp rise in the sex steroid hormones testosterone and estradiol may account for changes in brain development and aggression during adolescence (for reviews see [Blakemore et al., 2010; Peper and Dahl, 2013]).

Relatively early pubertal development and enhanced testosterone and estradiol levels, compared to same aged peers, have been associated with decreased behavioral control [de Water et al., 2013; Hemphill et al., 2010] and lowered brain connectivity [Klapwijk et al., 2013; Peper et al., 2013a]. It can therefore be argued that if sex steroids influence white matter connectivity, then increased sex steroid production during puberty may result in decreases in structural connectivity and increase aggressive tendencies.

In this study, we examined the association between sex steroids, aggressive traits, and white matter connections between frontal, temporal, and subcortical brain areas in a large normative group of participants between 8 and 25 years. We hypothesized that with age increasing white matter integrity of frontal-temporal-subcortical connections is associated with decreases in aggressive tendencies. We further anticipated that higher endogenous levels of sex steroid hormones suppresses this effect, in such a way that higher levels of sex steroids decrease white matter integrity in the frontal-temporal-subcortical network and contributes to aggression.

MATERIALS AND METHODS

Participants

Two hundred and fifty eight (126 [48%] boys) healthy volunteers between 8 and 25 years of age (Mean 14.2; SD 3.8) participated in the study, as part of the "Braintime" study [Braams et al., in press; Peper et al., 2013b; Peters et al., 2014] (Table I). Children were recruited from Elementary Schools and High Schools in The Netherlands and young adults were recruited from Leiden University and surrounding community. All participants had normal intelligence (Mean 109.8; SD 10.7) estimated by block design and similarities of the WISC-III for children up to 16 years of age and of the WAIS-IV from 16 years and older [Wechsler, 1997]. Participants were free from any history of psychiatric, endocrinological or neurological illnesses as verified by interview. Informed consent was obtained from all adult volunteers and from the parents of children under the age of 18. In addition, informed consent was obtained from volunteers under the age of 18 as well. The internal ethical review board from the Leiden University approved the study.

Aggressive Behavior

The Buss Perry Aggression Questionnaire (BPA-Q) was administered [Buss and Perry, 1992], consisting of 29

TABLE I. Sample characteristics

	Boys (N = 126)	Girls (N = 132)
Age	14.5 (3.8)	13.9 (3.8)
T (pmol/L)*	215.2 (192.8)	19.0 (12.8)
E2 (pmol/L)	0.91 (0.44)	0.89 (0.44)
Total aggression	2.96 (0.69)	2.96 (0.63)
Physical aggression**	2.92 (1.00)	2.58 (0.81)
Verbal aggression	4.05 (0.71)	4.06 (0.72)
Anger**	2.47 (0.95)	2.79 (1.00)
Hostility	2.77 (1.03)	2.87 (0.99)

*$P < 0.001$, **$P < 0.01$

Mean (*sd*) ages, hormonal levels and aggression of boys and girls. T = testosterone, E2 = estradiol.

Aggression scores represent the means (*sd*) on the total Buss-Perry Aggression questionnaire and its subscales, ranging from 1 (extremely uncharacteristic of me) to 7 (extremely characteristic of me).

items. Four subscales are distinguished: physical aggression (e.g., "I get into fights very often"; 9 items), verbal aggression (e.g., "most of the time I disagree with people"; 5 items), anger (e.g., "I have difficulties controlling my temper"; 7 items), and hostility (e.g., "I believe that people are making fun of me behind my back"; 8 items). The questionnaire consists of 29 items and participants rate themselves on a 7-point scale from "extremely uncharacteristic of me" (1) to "extremely characteristic of me" (7). The cumulative score on total aggression as well as on the subscales was divided by the number of items, to generate mean scores between 1 and 7.

The BPA-Q had a high reliability with a Cronbach's α of 0.84 in the whole sample. In children under 12, Cronbach's α was 0.79, between 12 and 17 years 0.85 and >18 years 0.87

Neuroimaging

Diffusion weighted imaging

Scans were acquired on a 3-Tesla Philips Achieva MRI system. Two transverse Diffusion Weighted Imaging (DWI) scans were obtained with the following parameter settings: 30 diffusion-weighted volumes with different noncollinear diffusion directions [Jones et al., 1999] with b-factor 1,000 s/mm^2 and 5 diffusion-unweighted volumes (b-factor 0 s/mm^2); parallel imaging SENSE factor = 3; flip angle = 90 degrees; 75 slices of 2 mm; no slice gap; reconstruction matrix 128 × 128; Field of view (FOV) = 240 × 240 mm; TE = 69 ms; TR = 7,315 ms; total scan duration = 271 s per DWI set. The second DWI set had identical parameter settings as used for the first set except that it was acquired with a reversed *k*-space readout direction enabling the removal of susceptibility artifacts during postprocessing [Andersson et al., 2003]. During scanning, the FOV was angulated according to the anterior commissure-posterior commissure line, and diffusion gradients were adjusted accordingly during data processing. Subsequently, diffusion scans were realigned to the averaged b0 scan and corrected for motion, eddy current, and susceptibility distortions [Andersson and Skare, 2002; Andersson et al., 2003]. A tensor was fitted to the diffusion profile in each voxel using a robust tensor fitting method [Chang et al., 2005, 2012], and the main diffusion direction was determined as the principal eigenvector of the eigenvalue decomposition of this fitted tensor. Based on the eigenvalue decomposition, within each voxel the fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and longitudinal diffusivity (LD) were computed, as metrics of white matter integrity. Specifically, FA and RD values are thought to be measures of myelination and axonal diameter, respectively, whereas MD and LD values are putative measures of bulk water around axons and axonal coherence, respectively [cf. Alexander et al., 2007; Yoshida et al., 2013].

White matter pathways were reconstructed using deterministic streamline tractography, based on the Fiber Assignment by Continuous Tracking algorithm [Mori et al., 1999]. Within each voxel of the cerebral white matter, eight streamlines were started, following the computed diffusion directions from voxel to voxel until one of the stopping criteria was reached (being FA < 0.1, sharp turn of 45 degrees or more, or exceeding brain tissue). This procedure resulted in a collection of reconstructable white matter tracts, from which fiber tracts of interest could be selected.

Tracts of interest

Of each participant a T1 weighed image was acquired for anatomical reference and for the selection of cortical and subcortical regions of interest. T1 scanning parameters were as follows: 3D FFE using parallel imaging; TR/TE 10 ms/4.6 ms; FOV 240 × 240 mm, 200 slices, 0.75 mm isotropic voxel size. Tissue classification, including automatic segmentation of gray/white/csf tissue was performed using the Freesurfer suite (V5, http://surfer.nmr.mgh.harvard.edu/) [Fischl et al., 2004]. Freesurfer was also used for automatic segmentation of the cortex into 68 distinct regions (34 per hemisphere) and the subcortical gray matter into 14 (7 per hemisphere) distinct regions.

Intrahemispheric white matter tracts between frontal and temporal lobes and subcortical brain areas were selected (Fig. 1), and the following pathways (with mean [SD] streamlines) were reconstructed: (1) subcortico-subcortical (2,125 ± 295 streamlines), (2) subcortico-frontal (2,276 ± 470 streamlines), (3) subcortico-temporal (865 ± 243 streamlines), (4) fronto-frontal (1,646 ± 335 streamlines), (5) fronto-temporal (619 ± 203 streamlines), and (6) temporo-temporal (1,182 ± 291 streamlines) connections. Connections within the occipital lobe (794 ± 407 streamlines) were analysed as "control" tracts.

To access the level of white matter complexity of reconstructed white matter connections, FA, MD, RD, and

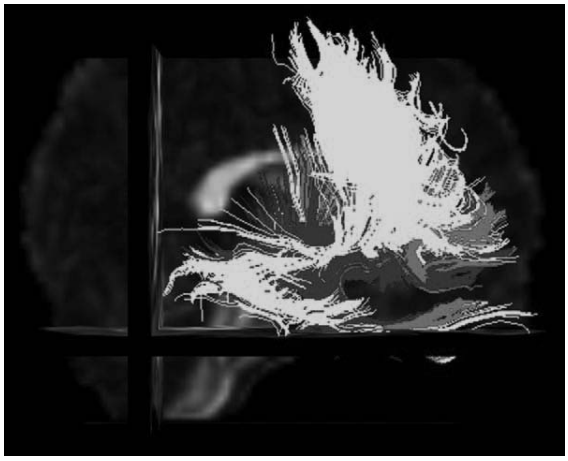

Figure 1.

Example of white matter tracts within a fronto-temporal-subcortical network of a representative subject in our sample. The following pathways (with mean [SD] streamlines) were reconstructed: (1) subcortico-subcortical (2125 ± 295 streamlines), (2) subcortico-frontal (2276 ± 470 streamlines), (3) subcortico-temporal (865 ± 243 streamlines), (4) fronto-frontal (1646 ± 335 streamlines), (5) fronto-temporal (619 ± 203 streamlines), and (6) temporo-temporal (1182 ± 291 streamlines) connections. Connections within the occipital lobe (794 ± 407 streamlines) were analysed as "control" tracts. Shown in the figure are white matter tracts between frontal and subcortical brain areas (red), between frontal and temporal brain areas (green), and between subcortical and temporal brain regions (blue). FA and MD values along the tracts were used for primary analyses-and RD and LD values were used for post hoc analyses in case MD effects were found. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

LD values along aggregated white matter pathways were averaged and used for further analyses. Tract analysis was initially carried out within each hemisphere separately, as findings suggest differential contributions of the hemispheres in aggression [Schutter and Harmon-Jones, 2013]. In the current sample however, we could not detect any substantial differences between white matter properties in the left and right hemisphere with respect to the relation with hormonal levels and/or aggression. We, therefore, combined the results of the left and right hemisphere.

Testosterone and Estradiol

Testosterone and estradiol were determined on the day of MR-scanning in morning saliva samples that were collected at home, directly after waking up. Participants were instructed not to eat or brush their teeth before collecting saliva. To control for hormonal fluctuations across the menstrual cycle, postmenarcheal girls collected saliva samples on the same day within the early follicular phase of the menstrual cycle (Day 7), when hormone levels (e.g., progesterone) are relatively low [Mihm et al., 2011]). Similarly, girls using oral contraceptives ($n = 16$) collected a saliva sample on the last day within their stopping period (Day 7). Girls using contraceptives without a stopping period, such as hormonal intrauterine devices, were excluded from participating in this study.

Saliva samples were assayed for testosterone and estradiol levels at the Department of Clinical Chemistry of the Free University Medical Centre. The lower limit of detection was 4 pmol/L for testosterone, and 0.1 pg/mL for estradiol. Salivary testosterone was determined by isotope dilution—online solid phase extraction liquid chromatography—tandem mass spectrometry (ID-XLC-MS/MS) [De Ronde et al., 2011; Peper et al., 2013b]. Intraassay coefficient of variation (CV) was 11% and 4%, at 10 and 140 pmoL/L, respectively, and interassay CV was 8% and 5%, at 31 and 195 pmol/L, respectively. Salivary estradiol was determined using an enzyme linked immunosorbant assay (DRG Instruments, Marburg, Germany). Interassay CV was 8% and 15% at 10 and 40 pg/L, respectively [Peper et al., 2013b]. Age positively correlated with testosterone in boys ($r = 0.72$, $P < 0.0001$) and girls ($r = 0.55$, $P < 0.0001$) and with estradiol in girls ($r = 0.20$, $P = 0.01$) and boys ($r = 0.29$, $P = 0.001$).

Statistical Analyses

Using parametric correlation analyses, we first examined the effects of age on FA and MD of the six white matter pathways of interest: (1) subcortico-subcortical, (2) subcortico-frontal, (3) subcortico-temporal, (4) fronto-frontal, (5) fronto-temporal, and (6) temporo-temporal connections. Conducting partial correlations, corrected for age, we subsequently tested the contributions of testosterone and estradiol to these six connections. In case of effects on FA or MD, we describe associations with RD and/or LD values as well.

Similar to analyses on white matter, we examined the contributions of age, testosterone and estradiol on overall aggression. In case the anticipated relation with total aggression was found, post hoc analyses were carried out for the subscales physical aggression, verbal aggression, anger, and hostility. Due to substantial sex differences in hormonal profiles, analyses involving sex hormones were carried out in boys and girls separately.

Finally, interrelations between white matter connections and aggression were investigated using partial correlations, controlling for age. As we investigated six white matter tracts we report for the white matter analyses whether effects remained significant at a Bonferroni-corrected P-value of 0.008 (0.05/6).

RESULTS

White Matter Connections: Effects of Age and Sex Steroids

Significant age-related increases in FA were found in the majority of white matter connections under study

TABLE II. Correlations between white matter connections, age, and testosterone, in (A) boys (*N* = 126) and (B) girls (*N* = 132)

	Age		T (age-corrected)	
Connections	FA	MD	FA	MD
(A) Boys				
Sub–Sub	**0.37** (<0.001)	**−0.36** (<0.001)	—	0.18 (0.05)
Sub-Front	**0.45** (<0.001)	**−0.38** (<0.001)		
Sub-Temp	**0.40** (0.001)	**−0.44** (<0.001)	—	—
Front–Front	**0.57** (<0.001)	**−0.40** (<0.001)	—	0.21 (0.01)
Front-Temp	**0.41** (<0.001)	**−0.45** (<0.001)		
Temp–Temp	—	**−0.43** (<0.001)		—
Occ–Occ	—	0.37 (<0.001)		
(B) Girls				
Sub–Sub	**0.45** (<0.001)	**−0.38** (<0.001)	—	0.19 (0.04)
Sub-Front	**0.39** (<0.001)	**−0.37** (<0.001)	−0.20 (0.03)	0.20 (0.03)
Sub-Temp	**0.29** (<0.001)	**−0.37** (<0.001)	—	**0.24** (0.008)
Front–Front	**0.38** (<0.001)	**−0.38** (<0.001)	—	0.19 (0.04)
Front-Temp	**0.36** (<0.001)	**−0.41** (<0.001)		0.23 (0.01)
Temp–Temp	—	**−0.34** (<0.001)	—	0.22 (0.02)
Occ–Occ	—	**0.43** (<0.001)		

Front = frontal, Occ = occipital, Sub = subcortical, Temp = temporal. T = testosterone.
Correlation coefficients printed in **bold** are significant at a Bonferroni correction for multiple comparisons ($P \leq 0.008$). Other printed correlations are significant at an uncorrected level of $P < 0.05$.

(Table II) with r's between 0.29 and 57; $P's < 0.0001$). Age-related increases in FA could not be demonstrated in temporo-temporal connections and in the control connections within the occipital lobe ($P's > 0.05$).

In contrast, age-related decreases of MD were found in all white matter connections within the subcortico-fronto-temporal network (r's between −0.45 and −0.34; $P's < 0.0001$; Table II). These age-related decreases in MD were both driven by a decrease in LD as well as in RD values (r's between −0.46 and −0.32; $P's < 0.0001$) (Supporting Information Table I). Occipital connections showed an age-related increase in MD ($r = 0.43$; $P < 0.0001$).

These results confirm commonly reported development effects on white matter microstructure during adolescence [Brouwer et al., 2012; Ladouceur et al., 2012; Lebel and Beaulieu, 2011; Simmonds et al., 2013].

In boys, corrected for age, a higher level of testosterone was related to more MD in subcortico-subcortical connections ($r = 0.18$, $P = 0.05$) and in fronto-frontal connections ($r = 0.21$, $P = 0.02$) (Table II). Testosterone-related increases in MD could be equally explained by an increase in LD as well as in RD (Supporting Information Table I). The correlations between testosterone in boys and DTI-measures did not survive a Bonferroni-correction for multiple comparisons.

In girls, corrected for age, in all connections under study, higher testosterone levels in girls were related to more MD (r's ranging from 0.19 to 0.24, with P's between 0.04 and 0.008) (Fig. 2, Table II) and both to increases in RD and LD (r's between 0.19 and 0.24, $P's < 0.05$; Supporting Information Table I). Specifically, at a corrected level, higher testosterone in girls was related to higher MD ($r = 0.24$, $P = 0.008$) and LD ($r = 0.24$, $P = 0.008$) within subcortico-temporal connections.

Uncorrected for multiple comparisons, a higher level of testosterone was related to less FA in subcortico-frontal connections ($r = -0.20$, $P = 0.03$; Table II).

Neither in girls nor boys, estradiol levels significantly correlated with white matter properties (e.g., FA, MD, RD all $P's > 0.40$).

Aggression: Effects of Age and Sex Steroids

In boys, total aggression did not change with age (Table III). However, estradiol levels in boys were positively related to total aggression ($r = 0.30$, $P = 0.002$).

Post hoc analyses showed that the estradiol-related increase of aggression was driven by the subscales physical aggression ($r = 0.26$, $P = 0.006$), verbal aggression ($r = 0.19$, $P = 0.04$), and hostility ($r = 0.21$, $P = 0.03$). Higher levels of testosterone in boys were also related to more physical aggression ($r = 0.24$, $P = 0.01$). In girls, total aggression significantly decreased with age ($r = -0.21$, $P = 0.02$).

Post hoc analyses mainly showed an age-related decrease in physical aggression ($r = -0.28$, $P = 0.001$). Unlike in boys, neither testosterone nor estradiol explained variance in total-, physical-, verbal aggression, and anger in girls, above and beyond age effects (Table III). However, higher levels of testosterone were related to lower levels of hostility ($r = -0.20$, $P = 0.03$).

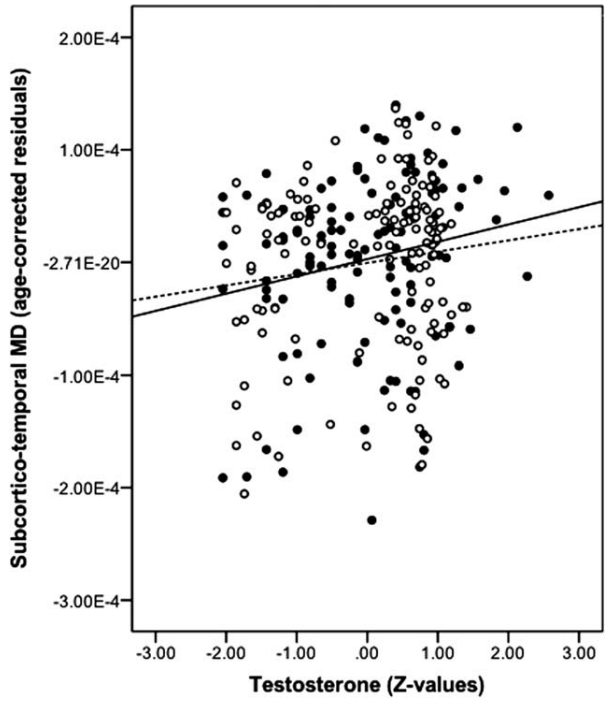

Figure 2.
Testosterone levels and MD values within subcortico-temporal connections in boys and girls. For visualization purposes, testosterone levels are Z-transformed within each sex. MD values of subcortico-temporal connections are corrected for age. The open dots with the dashed regression line represent the boys ($r = 0.16$, NS) and the closed dots with the solid line represent the girls ($r = 0.24$, $P = 0.008$).

In sum, irrespective of general age-related development, a higher level of estradiol (and to a lesser extent testosterone) in boys relates to relatively higher levels of aggression. To a lesser extent, increased levels of testosterone in girls relate to lower levels of "inhibited" aggression (i.e., hostility). In girls, changes in aggression appear to be predominantly related to age.

Associations Between Aggression and White Matter

FA and MD values of white matter connections were related to individual differences in aggression, controlling for age, revealing that individual differences in total aggression could not simply be explained by individual differences in white matter integrity. However, when the subscales were analysed separately, higher verbal aggression was, at an uncorrected level, associated with lower FA within subcortico-frontal ($r = -0.15$, $P = 0.01$), fronto-frontal ($r = -0.16$; $P = 0.01$), and fronto-temporal ($r = -0.13$, $P = .03$) connections (Table IV). Moreover, higher verbal aggression was associated with higher MD within temporo-temporal connections ($r = 0.17$, $P = 0.006$). To a lesser extent at an uncorrected level, higher verbal aggression was related to higher MD within subcortico-subcortical connections ($r = 0.16$, $P = 0.01$), subcortico-temporal connections ($r = 0.13$, $P = 0.04$) and fronto-temporal connections ($r = 0.15$, $P = 0.02$) (Fig. 3). Increased MD within these white matter connections in relation to increased verbal aggression was both driven by increased LD and RD (r's between 0.13 and 0.17, with P's between 0.005 and 0.05; Supporting Information Table II), reflecting lower white matter integrity.

In contrast to verbal aggression, higher hostility correlated with decreased MD in subcortico-temporal connections

TABLE III. The association between aggression, age and sex steroids in (A) Boys ($N = 126$) and (B) girls ($N = 132$)

Aggression	Age	T	E2
(A) Boys			
Total	—	—	0.30 (0.002)
Physical	—	0.24 (0.01)	0.26 (0.006)
Verbal	—	—	0.19 (0.04)
Anger	—	—	—
Hostility	—	—	0.21 (0.03)
(B) Girls			
Total	−0.21 (0.02)	—	—
Physical	−0.28 (0.001)	—	—
Verbal	—	—	—
Anger	—	—	—
Hostility	—	−0.20 (0.03)	—

T = Testosterone, E2 = estradiol.
Correlation coefficients pertaining to sex steroid analyses are partial correlations, corrected for age.
Correlation coefficients printed in **bold** are significant at a Bonferroni correction for multiple comparisons ($P \leq 0.008$). Other printed correlations are significant at an uncorrected level of $P < 0.05$

TABLE IV. Correlation between aggression and white matter connections (controlled for age) ($N = 250$)

	Verbal Aggression		Hostility	
	FA	MD	FA	MD
Sub–Sub	—	0.16 (0.01)	—	−0.14 (0.03)
Sub-Front	−0.15 (0.01)	—	—	−0.13 (0.04)
Sub-Temp	—	0.13 (0.04)	0.13 (0.04)	**−0.19** (0.003)
Front–Front	−0.16 (0.01)	—	—	—
Front-Temp	−0.13 (0.03)	0.15 (0.02)	—	−0.14(0.02)
Temp–Temp	—	**0.17** (0.006)	—	**−0.17** (0.006)
Occ–Occ	—	—	—	—

Front = frontal, Occ = occipital, Sub = subcortical, Temp = Temporal.
Correlations in **bold** survive correction for multiple comparisons ($P \leq 0.008$).
Total aggression, physical aggression, and anger were not related to white matter microstructure.

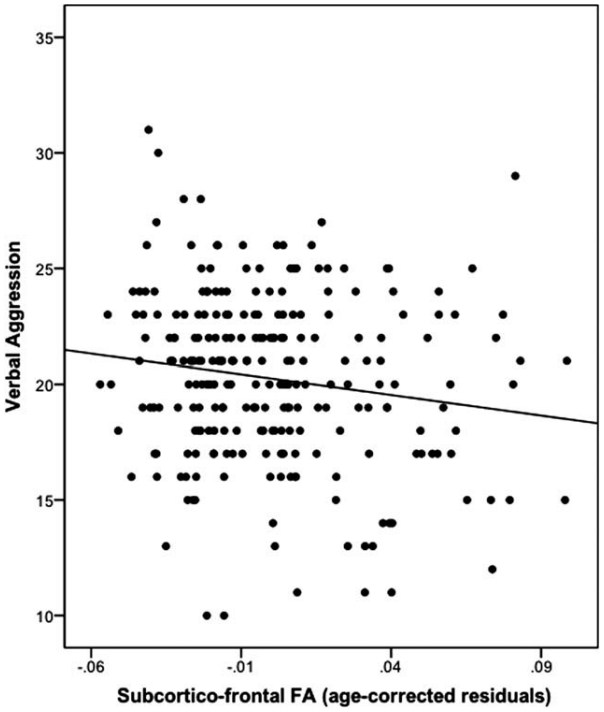

Figure 3.
Verbal aggression and FA values within subcortico-frontal connections. FA values of subcortico-frontal connections are corrected for age. ($r = 0.15$, $P = 0.02$, uncorrected).

($r = -0.19$; $P = 0.003$ and in temporo-temporal connections ($r = -0.17$; $P = 0.006$; Table IV).

To a lesser extent, at an uncorrected level, higher hostility was associated with higher FA within subcortico-temporal connections ($r = 0.13$; $P = 0.04$) and to lower MD within subcortico-subcortical connections ($r = -0.14$, $P = 0.03$), subcortico-frontal connections ($r = -0.13$, $P = 0.04$) fronto-temporal connections ($r = -0.14$, $P = 0.02$). Again, decreased MD within these white matter connections in relation to increased hostility was both driven by decreased LD and RD (r's between -0.19 and -0.13, with P's between 0.002 and 0.03; Supporting Information Table II).

Subscales physical aggression and anger were not found to be significantly associated with FA or MD values of the studied connections (all P-values > 0.12). Also, white matter properties of the "control" tracts within the occipital lobe could not be associated with aggression.

DISCUSSION

In a community-based sample of 258 participants between 8 and 25 years, we examined the associations between sex steroid hormone effects on aggression and white matter connections within a fronto-temporal-subcortical network. We hypothesized that with age, white matter integrity within subcortico-frontal-temporal connections would increase paralleled by a decrease in aggressive traits. Moreover, we expected that a higher sex steroid level suppresses this effect, in such a way that higher levels of sex steroids would relate to lower white matter integrity and increase aggression.

In line with our expectations, we replicated earlier findings of increased white matter integrity with chronological age [Asato et al., 2010; Lebel and Beaulieu 2011; Lebel et al., 2012; Simmonds et al., 2013]. Moreover, above and beyond age-related effects on white matter, our results indicate that a higher testosterone level relates to lower white matter integrity within a subcortico-frontal-temporal network (i.e., lower FA in girls and higher MD in boys and girls). These results fit with earlier neuroimaging studies in adults, reporting less functional and structural connectivity between frontal and subcortical brain areas with higher levels of testosterone [Peper et al., 2013a; van Wingen et al., 2010; Volman et al., 2011]. Indeed, our data suggest that adolescent increases in testosterone in girls and—to a lesser extent—in boys is associated with decreased structural connectivity between subcortical and frontal and temporal brain areas. It should nevertheless be noted, that the associations between testosterone and DTI-metrics did not survive correction for multiple comparisons. Therefore, the interpretation of these data should be with caution and warrants replication. Estradiol levels were not related to white matter integrity.

We also hypothesized that increased sex steroid hormone production—as opposed to general age effects—would relate to increased aggression. Indeed, age-related decreases in aggression were demonstrated, but in girls only. In boys, aggressive traits did not change with age but were associated both with testosterone and to a larger extent to estradiol levels. That is, higher levels of testosterone in boys were related to higher disinhibited (physical) aggression. The association between testosterone and physical aggression in the current sample of normally developing boys is comparable to earlier studies carried out in clinical [Barzman et al., 2013; Popma et al., 2007; Scerbo and Kolko 1994; van Bokhoven et al., 2006] and healthy [Vermeersch et al., 2008a] adolescent samples. The association between estradiol and aggressive traits is less well established. Recently however, rapid effects of estradiol on aggressive behavior in rodents have been described [Laredo et al., 2014]. This study provides the first evidence for possible contributions of endogenous estradiol levels and aggressive traits in boys. However, our data do not suggest that this association is established through estradiol acting on the white matter connections we examined.

Surprisingly, in girls, higher testosterone was related to lower hostility. It should be noted that hostility is characterized by a cynical attitude, feelings of resentment, and mistrust of others [Miller et al., 1996] and has been associated with higher levels of social anxiety and depression [Asberg, 2013; Weiss et al., 2005]. Interestingly, our finding

of higher endogenous testosterone relating to lower hostility adds evidence to the idea that testosterone has anxiolytic properties [van Honk et al., 2005]. Opposed to boys, estradiol was not associated with aggression in girls. To our knowledge, only one study has investigated the relation between female estradiol levels and aggressive behavior and found that a positive association, but only in the luteal phase of the menstrual cycle (i.e., around Day 14) [Vermeersch et al., 2008b]. In our study, postmenarcheal females were examined during the early follicular phase (i.e., Day 7), which could provide an explanation for the absence of an association between estradiol and aggressive behavior.

Interestingly, in adults [Pawliczek et al., 2013] and in extreme [Coccaro et al., 2011] forms of aggression have been associated with enhanced subcortical brain activity and reduced regulatory control by (pre)frontal and temporal brain areas. When directly relating white matter connectivity with aggression, we now for the first time provide evidence that in healthy children and adolescents the integrity of white matter connections between these brain areas is associated with aggressive tendencies. In particular, decreased connectivity relates to higher explicit' verbal aggression, while increased connectivity relates to higher "implicit" aggression (i.e., hostile thoughts). Thus, it can be speculated that although aggressive thoughts and feelings are subcortically generated [Davidson et al., 2000], the tendency to act on these thoughts and feelings (i.e., aggressive behavior) or to control these aggressive tendencies is partially regulated by white matter tracts between subcortico-frontal and temporal connections. Although we found that verbal aggression was associated with lower structural connectivity, this association was neither age nor hormonally related, and may reflect a more stable trait across adolescence. In contrast, higher levels of hostility were linked to increased structural white matter connectivity and lower levels of testosterone, suggesting that implicit forms aggression are (partly) regulated at a hormonal and neuronal level. We could, however, not distinguish between LD and RD effects, as both measures seemed to contribute equally to the effects in MD. We, therefore, argue that the combination of all axonal properties (i.e., axonal diameter, myelination and axonal coherence) most likely seems to play a role in the decreased "integrity" of subcortico-frontal-temporal white matter, subsequently relating to aggressive traits. Although prior research has provided evidence for hemispheric asymmetries in aggressive tendencies [Hofman and Schutter, 2012], the present findings did not show intracortical differences in white matter connectivity between the left and right hemisphere. A possible explanation is that aggression may be better explained by interhemispheric connectivity rather than local intrahemispheric differences in white matter connectivity [Schutter and Harmon-Jones, 2013].

Finally, it must be pointed out that the selected white matter pathways are not predefined tracts by white matter atlases. We preferred to select all anatomical connections between our regions of interest not to exclude relatively small white matter bundles that are not predefined by white matter atlases. It is highly likely however, that subcortico-frontal tracts partly overlap with the uncinate fasciculus which is in agreement with the notion that the integrity of the UF is implicated in conduct disorder [Sarkar et al., 2013] and antisocial personality disorder [Haney-Caron et al., 2014].

CONCLUSION

Our study suggests that the "integrity" of white matter connections within fronto-temporal-subcortical brain areas increases with age, but higher levels of testosterone might-partially-suppress this process by decreasing the integrity of connections. Lower white matter integrity within this network is subsequently associated with more disinhibited aggressive behavior, but with less inhibited aggression. Thus, distinct subtypes of aggression seem to be driven by different neural correlates. Our data add evidence to the general idea that aggressive tendencies are subcortically driven, but individuals with relatively high testosterone have lower structural connectivity within cortical control areas, resulting in a stronger tendency to act on these aggressive tendencies, as opposed to individuals who seem to be less short fused and more capable of regulating aggressive impulses.

ACKNOWLEDGMENTS

The authors do not have conflict of interest to declare. We thank Michelle Achterberg for graphical support and Rene Mandl for helpful discussions on MR acquisition and processing.

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