The adolescent brain : unraveling the neural mechanisms of cognitive and affective development

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Chapter 8

The link between testosterone and amygdala connectivity in adolescent alcohol use

This chapter is based on:

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Abstract

Alcohol consumption is one of the most problematic and widespread forms of risk taking in adolescence. It has been hypothesized that sex hormones such as testosterone play an important role in risk taking by influencing the development of brain networks involved in emotion and motivation, particularly the amygdala and its functional connections. Connectivity between the amygdala and the orbitofrontal cortex (OFC) may be specifically related to alcohol use, given the association of this tract with top-down control over behavioral approach tendencies.

In line with this, prior studies in adults indicate a link between alcohol use and functional connectivity between the amygdala and the OFC, as well as between testosterone and amygdala-OFC connectivity. We consolidated these research lines by investigating the association between alcohol use, testosterone and resting state functional brain connectivity within one large-scale adolescent sample (N = 173, aged 12-25 years). Mediation analyses demonstrated an indirect effect of testosterone levels on alcohol use through amygdala-OFC intrinsic functional connectivity, but only in boys. That is, increased testosterone in boys was associated with reduced amygdala-OFC connectivity, which in turn was associated with increased alcohol intake. This study is the first to demonstrate the interplay between adolescent alcohol use, sex hormones and brain mechanisms, thus taking an important step to increase our understanding of the mechanisms behind this form of adolescent risk taking.

Introduction

Adolescents are prone to increased risk taking and impulsive behavior (Steinberg, 2008). Although risk taking behavior in adolescence can be adaptive (Crone & Dahl, 2012), it is also associated with negative consequences for health and safety. Alcohol use is one of the most widespread forms of risk taking in adolescence (Hibell et al., 2012). Adolescent alcohol use is associated with impaired cognitive functioning and school performance (Zeigler et al., 2005) and alcohol-related problems in adulthood (Grant et al., 2006). Understanding the mechanisms behind alcohol use in adolescence is an important step towards preventing alcohol-related problems.

Risk taking behavior, including alcohol use, has been related to the dramatic rise in sex hormones during puberty (Forbes & Dahl, 2010). Indirect evidence for a link between increased sex hormone production during puberty and increased alcohol use comes from studies showing that adolescents with advanced pubertal maturation show relatively higher levels of alcohol intake (Biehl, Natsuaki, & Ge, 2007; Bratberg, Nilsen, Holmen, & Vatten, 2005; Westling, Andrews, Hampson, & Peterson, 2008). Moreover, a direct association was found between higher production of the sex hormone testosterone and an earlier onset of alcohol consumption in adolescent boys (de Water et al., 2013). Consequently, a prominent hypothesis predicts that sex hormones affect risk taking by influencing the development of limbic brain areas involved in emotion and motivation (Peper & Dahl, 2013).

The amygdala is one such limbic brain area that plays a key role in adolescent functional brain organization (Scherf, Smyth, & Delgado, 2013) and is associated with both testosterone and alcohol use. For instance, the amygdala is among the brain areas with the highest density of androgen receptors as shown in animal studies (Simerly, Chang, Muramatsu, & Swanson, 1990). Moreover, the amygdala response to emotional faces can be modulated by testosterone (Derntl et al., 2009; Hermans, Ramsey, & van Honk, 2008; Manuck et al., 2010; Stanton, Wirth, Waugh, & Schultheiss, 2009). With regard to alcohol, the amygdala is one of the key regions of interest in animal studies on alcohol use (McBride, 2002) and human research shows that alcohol ingestion leads to reduced amygdala activity for fearful/angry faces (Gilman, Ramchandani, Crouss, & Hommer, 2012; Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008; Sripada, Angstadt, McNamara, King, & Phan, 2011) and a lower amygdala response to fearful faces is linked to increased risk for future alcohol abuse (Glahn, Lovallo, & Fox, 2007).

Since the amygdala is highly interconnected with other brain regions (Cole, Pathak, & Schneider, 2010), it is important to also take into account the functional connections of the amygdala. The connection with the orbitofrontal cortex (OFC) is of particular interest, as the OFC is directly connected to the amygdala through the uncinate fasciculus (Von Der Heide, Skipper, Klobusicky, & Olson, 2013), an association tract that develops well into adolescence (Lebel & Beaulieu, 2011). In adults, it has been demonstrated that functional connectivity between the amygdala and the OFC during emotional face processing was reduced after alcohol ingestion

(Gorka, Fitzgerald, King, & Phan, 2013). Interestingly, testosterone administration (Bos, Hermans, Ramsey, & van Honk, 2012; van Wingen, Mattern, Verkes, Buitelaar, & Fernandez, 2010) and high endogenous testosterone (Spielberg et al., 2014) showed similar reducing effects on amygdala-OFC functional connectivity.

We therefore argue that it is vital to study the interplay between testosterone and amygdala-OFC connectivity in adolescents to explain individual differences in alcohol consumption. We tested this in a large cross-sectional adolescent sample using a resting state paradigm, which is a valuable tool to investigate functional networks in the developing brain (Uddin, Supekar, Ryali, & Menon, 2011) and has several advantages over task-based brain activity, including high test-retest reliability (Zuo & Xing, 2014) and broader generalizability. We hypothesized that higher levels of testosterone would be associated with increased alcohol consumption, mediated through lower amygdala-OFC connectivity.

Methods

Participants

The included sample consisted of 173 healthy participants (86 girls, 87 boys), between 12.05 and 25.95 years old (M = 15.85, SD = 3.10), for whom data on alcohol consumption, brain imaging and hormonal samples were available. Because this study was part of a larger project also involving younger participants, we collected complete resting state scans, high-resolution functional scans and T1 scans for 295 participants between 8 and 25 years old. Only participants who were twelve years and older (N = 209) were asked to fill out the alcohol questionnaire and younger participants were therefore excluded from further analyses. Other reasons for exclusion were: not completing the alcohol questionnaire (N = 11), missing testosterone levels (N = 16), excessive movement in the MRI scanner (> 3 mm; N = 3) and excessive micromovements (> 20% of volumes with > .05 mm movement; N = 5). Note that several participants were excluded for multiple reasons, e.g. both excessive movement and missing testosterone data.

Participants were recruited through local schools and advertisements. IQ was estimated with two subtests of the WAIS-III or WISC-III (Similarities and Block Design). All estimated IQ scores were within the normal range (M = 109.39, SD = 9.67, range: 80-135) and there was no correlation with age (r = .05, p = .53). Adults (18 years and older) received payment (60 euros) for participation, children received presents and their parents received 30 euros for travel reimbursement. The study was approved by the Institutional Review Board at the Leiden University Medical Center. The participants (or in case of minors, participant's parents) signed a written informed consent. All anatomical MRI scans were reviewed and cleared by a radiologist. None of the participants reported neurological or psychiatric disorders or current use of psychotropic medication. See Table 1 for the demographics for male and female participants in the sample.

Table 1: Demographic variables for boys and girls separately.

	Males (N = 87)		Females (N = 86)	
	Mean	**SD**	**Mean**	**SD**
Age	16.10	3.39	15.60	2.77
IQ	109.63	9.85	109.07	9.66
Lifetime alcohol use	30.82	40.30	28.69	36.23
Recent alcohol use	7.97	14.70	5.68	10.71
Testosterone (pmol/l)	258.37	164.79	26.66	38.71
Right amy-OFC connectivity	0.50	0.79	0.69	0.71
Left amy-OFC connectivity	0.66	0.78	0.77	0.77

Testosterone levels

Testosterone levels were extracted from saliva samples (de Water et al., 2013; Peper, Mandl, et al., 2013). Participants collected saliva by passive drool at home on the day of the MRI scan. In order to minimize effects of diurnal fluctuations of hormonal levels, saliva samples were collected immediately after waking up in all participants. Girls using contraceptives (N = 17) collected saliva on the last day of the stop period (day 7) and post-menarchal girls not using contraceptives (N = 46) collected saliva on the 7th day of the menstrual cycle. Testosterone levels from saliva were determined by isotope dilution - online solid phase extraction liquid chromatography – tandem mass spectrometry (ID-XLC-MS/MS; Intra-essay coefficient of variation (CV) was 11% and 4%, at 10 and 140 pmol/L, respectively and inter-assay CV was 8% and 5%, at 31 and 195 pmol/L, respectively. Testosterone levels were not normally distributed, so a log-transformation of the scores was used for further calculations.

Alcohol Questionnaire

Participants filled in an on-line questionnaire at home on recent and lifetime alcohol use (Ames et al., 2007; de Water et al., 2013; Thush et al., 2008). Self-report measures of alcohol use have been shown to be reliable if confidentiality is ensured (Brener et al., 2002; Sobell & Sobell, 1990). The instructions explicitly stated that participant's answers were confidential and would not be disclosed to anyone. Participants were instructed to fill out the questionnaire at a time point as close as possible to the MRI scan.

Lifetime alcohol use was reported as the lifetime amount of glasses consumed on an 11-point scale (0, 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, and > 90). Bottles and cans of alcohol had to be counted as 1.5 glasses, because these contain more of the alcoholic beverage than a standard glass in the Netherlands (Thush et al., 2008). Recent alcohol use was reported as the number of glasses of alcohol participants had consumed over the past 30 days on a 10-point scale (0, 1–2, 3–4, 5–6, 7–10, 11–15, 16–20, 21–30, 31–50, and > 50). To create a scale variable,

the ordinal data on quantity of alcohol use were converted by calculating the mean of the answer (for > 50 and > 90, 51 and 91 were used, respectively). On average, participants had consumed 29.76 glasses of alcohol in their lives (*SD* = 38.24) and 6.83 glasses in the last month (*SD* = 12.89). There were no gender differences or gender x age interactions in alcohol consumption. See Figure 1 for the amount of glasses consumed per age group and per gender. Adult participants between 18-25 years were grouped together due to a lower sample size in that age group (sample sizes: 12y: *N* = 27, 13y: *N* = 33, 14y: *N* = 25, 15y: *N* = 20, 16y: *N* = 16, 17y: *N* = 22, 18-25y: *N* = 30).

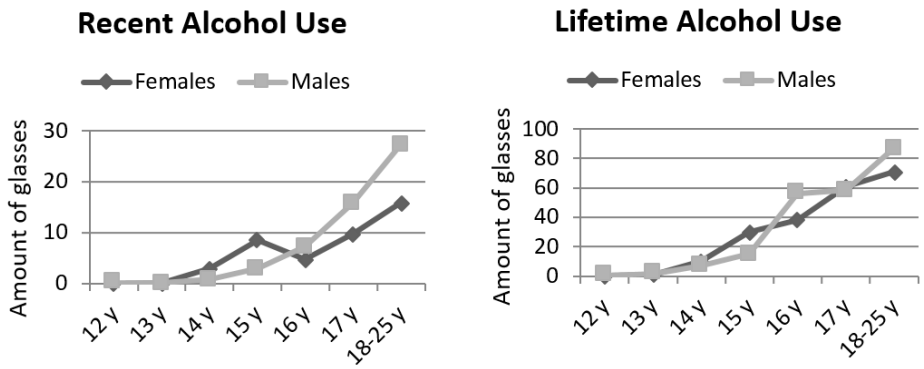

Figure 1: Glasses of alcohol consumed over the past month (recent) and over lifetime, per age and per gender.

MRI data Acquisition

MRI scans were acquired with a standard whole-head coil on a Philips 3.0 Tesla MRI scanner. Functional resting state scans were acquired with T2*-weighted echo-planar imaging (EPI). The first two volumes were discarded to allow for equilibration of T1 saturation effects. The following scan parameters were used: 140 volumes; 38 slices; sequential acquisition; TR = 2200 ms, TE = 30 ms; flip angle = 80°; FOV = 220 x 220 x 114.67 mm; slice thickness = 2.75 mm. A high-resolution anatomical scan (T1-weighted; 140 slices; TR = 9.76 ms; TE = 4.59 ms; flip angle = 8°; FOV = 224 × 177.33 × 168 mm; in-plane resolution = 0.875 x 0.875 mm; slice thickness = 2 mm) and a high-resolution T2*-weighted gradient echo EPI scan (84 slices; TR = 2200 ms; TE = 30 ms; flip angle = 80°; FOV = 220 x 220 x 168 mm; in-plane resolution = 1.96 x 1.96; slice thickness = 2 mm) were acquired after the resting state scan. Participants were instructed to close their eyes during the resting state, and a video was presented during the structural and high-resolution T2*-weighted scan. Before the MRI scan, participants were accustomed to the MRI environment and sounds with a mock scanner.

FMRI data preprocessing

All resting state scans were submitted to visual quality control to check for artifacts. Next, FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following preprocessing steps were used: motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002); non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high-pass temporal filtering of 100 s (Gaussian-weighted least-squares straight line fitting, with sigma = 50 s). The resting state scan was registered with FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001) to the high resolution T2*-weighted scan, which in turn was registered to the T1-weighted scan, and the T1-weighted scan was registered to the 2 mm MNI-152 standard space image.

FMRI data analysis

A seed-based correlation approach (Fox & Raichle, 2007) was used to find brain regions with functional connectivity to the amygdala. Amygdala masks were obtained by using atlas-based masks of left and right amygdala (Automatic Anatomical Labeling atlas; see Figure 2).

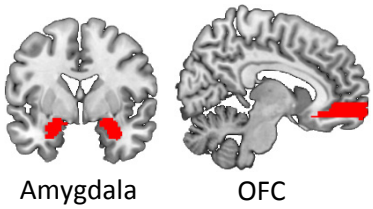

Figure 2: Amygdala and OFC masks based on anatomical masks (Automatic Anatomical Labeling atlas)

Amygdala masks in MNI space were transformed to native space (each individual's resting state scan) with a binary threshold of 0.5. Next, the mean time courses were extracted from each individual's left and right amygdala, i.e. all voxels located within the amygdala mask. These mean time courses were entered as a regressors in a general linear model (separately for left and right amygdala), with nuisance regressors for the white matter signal and CSF signal (obtained from a bilateral 4 mm sphere in white matter (left: x = 54, y = 44, z = 44; right x = 35, y = 44, z = 44) and CS*F* (left: x = 59, y = 55, z = 50; right: x = 30, y = 55, z = 50), global signal, and six motion parameters (rigid body: three translations and three rotations). For participants with excessive micromovements (> .05 mm) between volumes, we included additional regressors (binary for all volumes with movement > .05) to remove the specific volumes where micromovements occurred

from the analysis. Participants for whom more than 20% of volumes were affected by micromovements (> .05 mm) were excluded from further analyses. Next, we performed a first-level analysis with FEAT for each participant. The individual parameter estimate maps and within-subject variance maps were resliced into MNI space and used for higher-level analyses. Higher-level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 (Beckmann, Jenkinson, & Smith, 2003; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004; Woolrich, 2008). Z statistic images were thresholded with an initial cluster-forming threshold of Z > 2.3 and a (corrected) cluster threshold of $p < .05$ (Worsley, 2001).

ROI analyses

Region-of-interest analyses on the a-priori selected OFC were performed on an OFC anatomical mask (based on Autonomic Anatomical Labeling: Medial Orbital Frontal Gyrus; see Figure 2) in which left and right OFC were combined. OFC masks in MNI space were, similar to the amygdala masks, transformed to native space with a binary threshold of 0.5. Next, we extracted Z-scores for amygdala connectivity from the OFC. The results were further analyzed with SPSS 19 for the indirect effect of testosterone on alcohol use via amygdala-OFC connectivity, with age as a covariate of no interest. All analyses were performed for girls and boys separately. This is preferred in puberty related research given the possible differential effects of testosterone in boys and girls (Bramen et al., 2011) and the difference in timing at which puberty emerges, approximately 1.5 years earlier for girls than boys (Shirtcliff, Dahl, & Pollak, 2009). Moreover, there was a substantial gender difference in testosterone levels in our sample (t (128.67) = 14.59, $p < .001$).

To investigate the interplay between amygdala-OFC connectivity, alcohol use, and testosterone, we performed mediation analyses (Preacher & Hayes, 2008). We investigated the association between testosterone and alcohol use and examined whether amygdala-OFC connectivity mediated this association. This approach is similar to previous work demonstrating that the association between testosterone and adolescent risk taking is mediated by OFC morphology (Peper, Koolschijn, & Crone, 2013). Throughout all analyses, we corrected for age. We used the bootstrapping method of Preacher and Hayes (Preacher & Hayes, 2004, 2008) to test the indirect effect of testosterone on alcohol use (through amygdala-OFC connectivity) for significance. With this method it is possible to test for indirect effects even in the absence of direct effects (Hayes, 2009). A bootstrapped mediation analysis uses re-sampling of raw data to estimate the confidence intervals (CI) to formally test the indirect effects of which the mediation model consists.

Whole-brain analyses

In addition to the ROI analyses, we performed whole-brain analyses (using higher-level FEAT in FSL) to examine whether effects were specific for the coupling between the amygdala and the OFC or whether other regions were implicated as well. We examined the relation between amygdala connectivity and testosterone, as well as the relation between amygdala connectivity and

alcohol use (lifetime and recent use). Age was included as covariate of no-interest. Z statistic images were thresholded with an initial cluster-forming threshold of Z > 2.3 and a (corrected) cluster threshold of $p < .05$ (Worsley, 2001). Left and right amygdala were analyzed separately.

Results

Mediation effect in boys

To test the hypothesis that testosterone influences alcohol use through an effect on amygdala-OFC connectivity, we used mediation analyses with alcohol use as outcome variable and amygdala-OFC connectivity as mediator variable (corrected for age). For boys, the results showed that testosterone influenced alcohol use through amygdala-OFC connectivity, for recent alcohol use (right and left amygdala-OFC connectivity: 95% CI = 0.16 – 5.50 and 0.12 - 4.69, respectively) and lifetime alcohol use (right amygdala-OFC connectivity only (95% CI = 0.34 – 10.61) (Figure 3). That is, higher testosterone levels were associated with less functional connectivity between the amygdala and OFC (path a), and less amygdala-OFC connectivity was in turn associated with more alcohol use (path b). There was no direct relation between testosterone and alcohol consumption (path c and c') (see Figure 3 for the statistical values), but a significant direct effect is not a prerequisite for a significant mediation effect (Hayes, 2009; MacKinnon, Krull, & Lockwood, 2000; MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002; Rucker, Preacher, Tormala, & Petty, 2011; Shrout & Bolger, 2002; Zhao, Lynch, & Chen, 2010).

No mediation effects in girls

No significant effects for any of the mediation paths were found for girls, even when girls using contraceptives ($N = 17$) were excluded from the analyses. Note that there was no correlation between age and amygdala-OFC connectivity for either girls or boys. Therefore, the follow-up analyses described in the next paragraphs were performed for boys only.

Follow-up analyses in smaller age ranges

Because of the relatively large age range in our sample, we also tested for mediation effects in separate age groups in boys. We created three age groups of equal size ($N = 29$, young adolescents 12.3-14.0 y, mid-adolescents 14.0-16.7 y, late adolescents/young adults 16.7-25.9 y). The mediation effect was only significant for the mid-adolescent group for right amygdala-OFC connectivity for recent use (95% CI = 0.19 – 5.69) and lifetime use of alcohol (95% CI = 0.23 – 10.56). The mediation in this mid-adolescent group showed comparable effects to the results in the group as a whole. For these analyses in smaller age ranges, similar results were found for mediation analyses performed with and without age correction. These analyses might indicate that the effects of testos-

terone on alcohol use via amygdala-OFC connectivity are mostly driven by the mid-adolescent age group.

A possible explanation for the lack of a direct effect of testosterone on alcohol use is that alcohol use is strongly related to age (recent: $r = .67$, $p < .001$; lifetime: $r = .68$, $p < .001$) and age in turn is strongly related to testosterone ($r = .50$, $p < .001$). Possibly, effects of testosterone are less pronounced due to the correction for age. When analyzing the relationship between testosterone and alcohol use in the smaller age groups (without age correction), this relation was significant for lifetime use in the young adolescent group ($r = .37$, $p = .05$, but this disappears with age correction: $r = .31$, $p = .11$) and for lifetime use in the late adolescent/young adult group ($r = .46$, $p = .01$, with age correction: $r = .44$, $p = .02$).

Age vs. testosterone effects on amygdala-OFC connectivity

We found no correlation between amygdala-OFC connectivity and age in either boys or girls. This correlation with age was not significant independent of whether we did, or did not control for testosterone levels. There was a significant correlation between amygdala-OFC connectivity and testosterone in boys, which remained significant when controlling for age (right: $r = -.26$, $p = .02$, left: $r = -.23$, $p = .03$). These results suggest that connectivity between the amygdala and the OFC is associated more with testosterone rather than age per se.

Whole-brain analyses

To examine the specificity of the mediation effects in boys to the OFC, we investigated effects of alcohol use and testosterone on amygdala connectivity with the rest of the brain. We performed an analysis with testosterone as a regressor, including age as a regressor of no interest. The results indicated reduced functional connectivity between the right amygdala and the orbitofrontal cortex and other medial frontal areas with relatively higher levels of testosterone (see Figure 4, Table 2). The pattern in the left amygdala was in the same direction, albeit less pronounced. Besides the medial frontal cortex, we did not find any other regions that showed an effect of testosterone on amygdala connectivity.

For the analyses with alcohol use as a regressor, we found reduced functional connectivity in boys between the right amygdala and the orbitofrontal cortex and other medial frontal areas for both recent and lifetime alcohol use (see Figure 4, Table 3). Similar but less pronounced effects were found for the left amygdala. There were no other regions that showed an effect of alcohol use on amygdala connectivity. These results provide further evidence for the hypothesis that testosterone and alcohol use are related to similar brain mechanisms and indicate that the effects are specific to amygdala-OFC coupling.

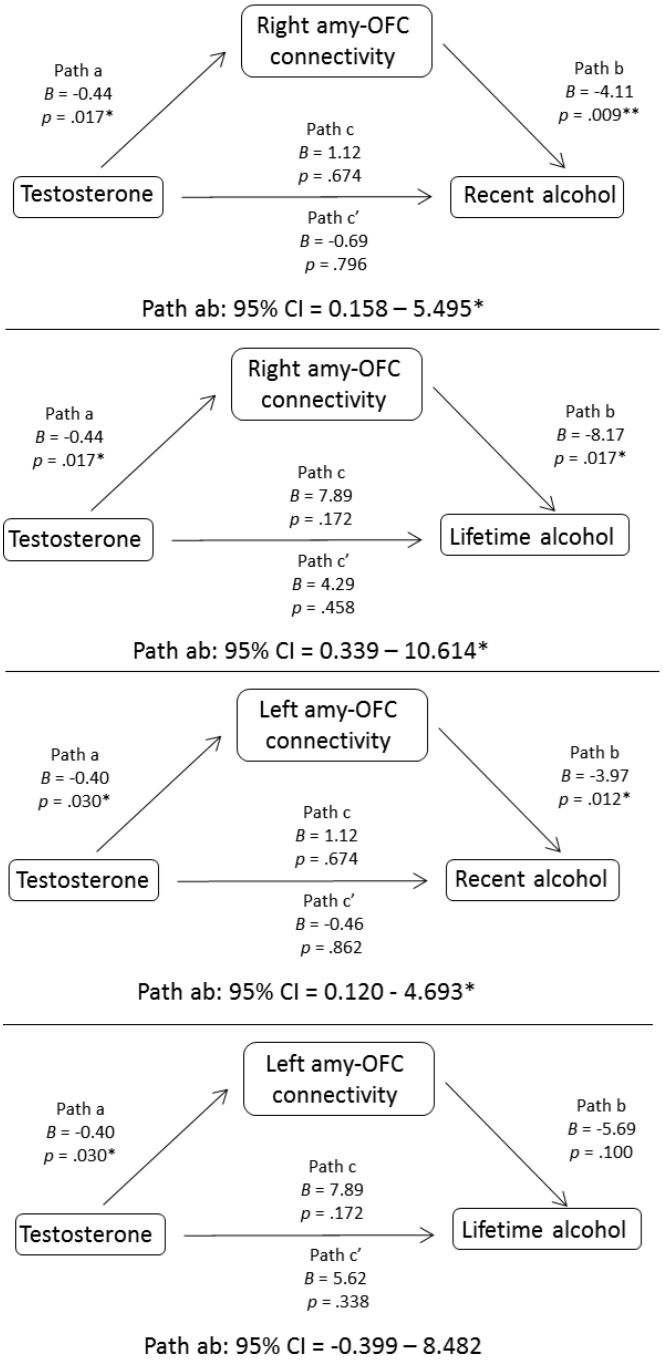

Figure 3: Mediation analyses for the relation between testosterone, amygdala-OFC connectivity and alcohol consumption for left and right amygdala-OFC connectivity and lifetime and recent alcohol use in boys.

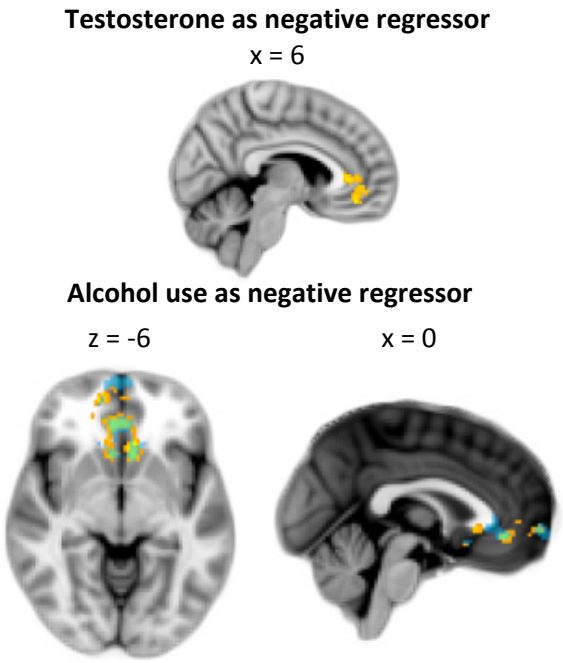

Figure 4: a) Right amygdala connectivity in boys with testosterone as a negative regressor and age as regressor of no interest. b) Right amygdala connectivity in boys with alcohol use as a negative regressor and age as regressor of no interest (yellow=recent alcohol use, blue=lifetime alcohol use, green = overlap).

Table 2: MNI-coordinates for local maxima for right amygdala connectivity in boys with testosterone as a negative regressor and age as regressor of no interest.

Brain Region	Z	x	y	z	voxels
R cingulate gyrus	3.73	10	38	4	397
R frontal medial cortex	3.43	12	34	-10	s.c.
R frontal pole	3.26	20	42	-16	s.c.
R frontal medial cortex	3.2	6	40	-14	s.c.
R frontal pole	3.16	28	52	-12	s.c.
R frontal pole	3.12	22	32	-6	s.c.

Note: s.c. = same cluster

Table 3: Local maxima for connectivity with the right amygdala with alcohol use as a negative regressor and age as regressor of no interest.

Brain Region	Z	x	y	z	voxels
Recent alcohol use					
L subcallosal cortex	4.16	-6	24	-6	786
R subcallosal cortex	3.98	8	20	-10	s.c.
R frontal pole	3.74	4	58	-2	s.c.
R frontal medial cortex	3.68	14	52	-4	s.c.
R cingulate gyrus	3.66	6	38	-4	s.c.
Paracingulate gyrus	3.4	0	38	-8	s.c.
Lifetime alcohol use					
L frontal orbital cortex	4.57	-16	14	-12	853
R frontal orbital cortex	3.89	24	18	-12	s.c.
L paracingulate gyrus	3.72	-6	32	6	s.c.
L subcallosal cortex	3.63	-8	20	-10	s.c.
R cingulate gyrus	3.55	2	38	-6	s.c.
R frontal orbital cortex	3.45	20	22	-10	s.c.

Discussion

We investigated the association between testosterone, amygdala-OFC functional connectivity and alcohol use in a sample of 173 typically developing adolescents. In agreement with our hypothesis, increased testosterone levels were related to increased alcohol consumption, through a mediation effect on reduced intrinsic amygdala-OFC connectivity, but only in boys. In other words, higher testosterone levels in boys were associated with reduced connectivity between the amygdala and the OFC, and reduced amygdala-OFC connectivity in turn was related to increased alcohol use.

Amygdala-OFC connectivity and risk taking behavior

The results of this study fit well with prior research reporting reduced amygdala-OFC connectivity with increased testosterone using task-based functional connectivity in adults (Bos, Panksepp, Bluthe, & van Honk, 2012; van Wingen et al., 2010) and adolescents (Spielberg et al., 2014), as well as a prior study that found reduced task-based amygdala-OFC connectivity after alcohol ingestion in adult heavy social drinkers (Gorka et al., 2013). These results suggest that testosterone and alcohol use are linked to similar brain mechanisms. Because our sample included

a relatively large age range, we also performed these analyses in smaller age ranges. The findings indicated that the effects were most pronounced in mid-adolescence (14-16.7 years). In addition, we tested how amygdala-OFC connectivity changed with age. We did not find a correlation between amygdala-OFC connectivity and age, but there was a negative correlation in boys between amygdala-OFC connectivity and testosterone, controlling for age (but see also Gabard-Durnam et al., 2014).

The amygdala and the OFC are hypothesized to work together during the processing of both negative and appetitive emotional stimuli (Baxter & Murray, 2002). That is, it has been argued that the amygdala detects the valence of affective stimuli, while the OFC guides decisions and goal-directed behavior in reaction to affective and rewarding stimuli (Bechara, Damasio, & Damasio, 2000; Kringelbach, 2005). Prior studies showed that increased task-related connectivity between the amygdala and the OFC is associated with better emotion regulation and self-control (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Lee, Heller, van Reekum, Nelson, & Davidson, 2012) which is in turn associated with less risk taking behavior.

However, further research is warranted to understand the link between task-related versus intrinsic brain activity on behavior. Raichle (2010) argues that brain function should be seen as mostly intrinsic, rather than reflexive (task-related) in nature. In terms of energy allocation, this fits with the finding that task-related brain activity -compared to intrinsic activation- only uses 5% additional energy (Raichle, 2010). It is possible that decreased intrinsic connectivity between the amygdala and the OFC biases a person towards acting in a less top-down controlled and more risky way.

On the other hand, a recent study comparing a group of 18 risk taking individuals with 18 non risk taking individuals (DeWitt, Aslan, & Filbey, 2014) did not find evidence for reduced intrinsic amygdala-OFC connectivity in a group of risk taking individuals, but instead found increased connectivity between the amygdala and left cingulate gyrus, left precuneus, right middle frontal gyrus and right inferior parietal lobule. Several factors might explain these opposing findings: i) DeWitt et al. (2014) compared a group of risk taking individuals with a group of non risk taking individuals (based on an assessment of many different forms of risk taking), rather than only alcohol use as a real-life form of risk taking measured on a continuous scale. ii) DeWitt et al.'s sample was relatively small compared to the current study, in an age range of 12 to 17 with both boys and girls. Future studies should unravel the heightened versus reduced amygdala connectivity in relation to increased risk taking within separate networks of intrinsic brain activity.

Note that with the functional connectivity analyses used in this study, it remains unclear whether there is less 'top-down' connectivity from the OFC to the amygdala or more 'bottom-up' connectivity from the amygdala to the OFC (i.e., the direction of connectivity). We examined functional connectivity using the amygdala as a seed region because of its high density of androgen receptors (Simerly et al., 1990), the correlation between amygdala activation and

testosterone (Derntl et al., 2009; Hermans et al., 2008; Manuck et al., 2010; Stanton et al., 2009), as well as the relation between amygdala activation and alcohol (Gilman et al., 2012, 2008; McBride, 2002; Sripada et al., 2011). Future research should further explore effects of alcohol and testosterone on functional brain connectivity, by –for instance- focusing on the OFC as a seed region. As a result, a broader network of brain regions might be revealed in relation to testosterone and alcohol use.

In sum, our findings suggest that a decreased coupling between the amygdala and the OFC which relates to increased testosterone, may be instrumental in explaining risk taking behavior in boys, such as adolescent alcohol use through the inability to assert top-down control over behavioral approach tendencies.

Indirect effect of testosterone on alcohol use

Although we found evidence for an indirect relationship between testosterone and alcohol consumption (through amygdala-OFC connectivity) in boys, a direct effect of testosterone on alcohol use was not significant. From a statistical point of view, a significant direct effect is not necessary for a significant mediation effect (MacKinnon et al., 2000; MacKinnon et al., 2002; Shrout and Bolger, 2002; Hayes, 2009; Rucker et al., 2011; Zhao et al., 2010). For instance, it is possible that multiple indirect effects are influencing alcohol use, which can explain why a direct effect was not found (Hayes, 2009). The lack of a direct effect was, however, contrary to our expectations as prior studies indicated an effect of advanced pubertal maturation on alcohol intake (Biehl et al., 2007; Bratberg et al., 2005; Westling et al., 2008).

A possible explanation can be found in the fact that testosterone can either have slow effects through genetic mechanims, such as on the structural organization and functional coupling of brain pathways, or fast nongenomic effects in the order of minutes to seconds, such as activitation of brain areas after the administration of testosterone (Bos, Panksepp, Bluthe, & van Honk, 2012). Possibly, endogeneous and gradual increases of testosterone during adolescence might not have influenced a person's immediate decision about consuming alcohol, but might have indirectly influenced brain mechanisms involved in less behavioral control and increased approach-related behavior. Indeed, prior studies in adolescents showed that adolescent testosterone relates to gray matter and white matter tracts within limbic brain areas (Herting, Maxwell, Irvine, & Nagel, 2012; Paus et al., 2010; Peper, Koolschijn, et al., 2013).

No relation between testosterone, amygdala-OFC connectivity and alcohol use in girls

It is interesting that we only found a relation between testosterone, brain connectivity and alcohol intake in boys, but not in girls. Prior research also showed different effects of testosterone in boys than in girls for risk taking behavior (Peper, Koolschijn, et al., 2013) and brain development (Bramen et al., 2011; Nguyen et al., 2013). Note that we did not find any gender difference in alcohol use in our sample, partly similar to prior studies which reported no gender difference in

alcohol intake until the age of about 17, after which men tend to drink more than women (Witt, 2007).

However, Van Wingen et al (2010) and Bos et al. (2012) did show an effect of testosterone administration on task-based amygdala-OFC connectivity in (adult) women. The relatively low endogenous levels of testosterone in adolescent girls compared to boys were possibly not able to reliably affect functional connectivity during a resting state scan.. In addition, although we corrected for menstrual cycle in girls at the time of saliva collection (all post-menarchal girls collected saliva at day 7 within their cycle), it was not feasible to also plan the MRI scan on the 7th day. Consequently, testosterone levels at the time of scanning in girls could have been different from the testosterone levels in the samples we collected.

Limitations and directions for future research

It is important to note that the assessment of alcohol intake relied on self-report. Self-report may lead to both over- and underestimations of actual alcohol intake. Especially for lifetime alcohol use, participants may have difficulty estimating how much alcohol they consumed in their lives. On the other hand, lifetime and recent alcohol use were highly correlated, and prior studies have shown that self-report measures of alcohol use are reliable if confidentiality is ensured (Brener et al., 2002; Sobell & Sobell, 1990), which was the case in our study. Although we excluded participants who were diagnosed with a psychiatric disorder, we did not include a measure for alcohol abuse. In future studies, it is important to additionally investigate age of onset, criteria for alcohol abuse and binge drinking behavior, as binge drinkers are especially at risk for negative consequences related to alcohol use (Wechsler & Nelson, 2001).

Another possible direction for future research would be a large-scale longitudinal study. The current study was cross-sectional, therefore we could only evaluate whether there was an association between testosterone and alcohol use, but it was not possible to assess whether future alcohol use can be predicted based on current testosterone levels. Future longitudinal studies should unravel factors predicting alcohol use. An important advantage of longitudinal studies is that they might eventually lead to early interventions for adolescents at high-risk for excessive alcohol use.

Conclusion

In conclusion, this is the first large-scale study in adolescence on the interplay between pubertal hormones, intrinsic brain connectivity and alcohol use as a measure of risk taking behavior. We found evidence for an indirect effect of testosterone on alcohol consumption, through reduced amygdala-OFC connectivity in boys. This provides important insights into the mechanisms behind alcohol consumption, and may contribute to the development of prevention work aimed at reducing the chance of the transition from normative into abnormal forms of risk taking.