Author version of: Peters, S., Peper, J. S., van Duijvenvoorde, A. C. K., Braams, B. R., & Crone, E. A. (2017). Amygdala-orbitofrontal connectivity predicts alcohol use two years later: A longitudinal neuroimaging study on alcohol use in adolescence. *Developmental Science, 20*(4), e12448. doi: 10.1111/desc.12448

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Highlights

- Large adolescent sample (8-27 years) measured at two time points
- Amygdala-OFC connectivity predicted alcohol use two years later
- Alcohol use did not predict amygdala-OFC connectivity two years later
- Reduced subcortical-cortical connectivity may bias towards risk-taking

Abstract

This study tested the relation between cortical-subcortical functional connectivity and alcohol consumption in adolescents using an accelerated longitudinal design, as well as normative developmental patterns for these measures. Participants between ages 8 and 27 completed resting state neuroimaging scans at two time points separated by two years (*N*=274 at T1, *N*=231 at T2). In addition, participants between ages 12 and 27 reported on recent and lifetime alcohol use (*N*=193 at T1, *N*=244 at T2). Resting state connectivity analyses focused on amygdala-orbitofrontal connectivity given prior research linking reduced coupling between these regions to alcohol use. Mixed-model analyses revealed that age had a cubic relationship with alcohol use, with little to no use in childhood, steep increases in adolescence and leveling off in adulthood. No age effects were found for amygdala-OFC connectivity. Prediction analyses showed that left amygdala-orbitofrontal connectivity at the first time point predicted recent and lifetime alcohol use two years later. There was no evidence for the reversed relation, suggesting that brain connectivity measures precede explorative risk-taking behavior in adolescence, possibly because decreased subcortical-frontal connectivity biases towards more explorative or risky behavior.

Introduction

Adolescence is a developmental period that is associated with increased risk-taking behavior (Steinberg, 2008). One of the most prevalent forms of risk-taking in adolescence is alcohol consumption (Hibell et al., 2012). There is considerable evidence that alcohol use increases sharply in adolescence and has negative consequences for cognitive functioning and school performance (Zeigler et al., 2005). Despite the presumed relations between alcohol use and brain development (Peeters et al., 2015), surprisingly little is known about how longitudinal changes in alcohol use in normally developing adolescents are related to changes in brain function over time. The current study addressed this question with an assessment at two time points for alcohol use and brain connectivity in an accelerated longitudinal design with participants between 8-27 years old. Specifically, we tested the direction of the relation, by studying whether alcohol use could be predicted from brain connectivity, or whether alcohol use predicted later brain connectivity. In addition, normative developmental patterns for alcohol use and brain connectivity were tested for linear, quadratic or cubic trajectories.

A well-suited approach to address this question is by using resting-state analyses to measure changes in brain connectivity over time. This technique involves measuring connectivity between brain regions at rest, i.e. during the absence of a specific task. Resting-state analyses are especially suitable for testing longitudinal questions in children and adolescents as performance differences and practice effects are often observed at different time points, which may confound the results as they influence brain activity. With resting-state analyses, there is no overt behavioral index which has the benefit that performance differences can be excluded as a confounding factor (Dosenbach et al., 2010). Here we focused on connectivity between subcortical and cortical systems. It has been hypothesized that during adolescence there is an imbalance between the relative maturity of subcortical brain regions (including the amygdala and ventral striatum), and prefrontal cortex regions that

exert control over subcortical brain regions, possibly explaining the increased incidence of risk-taking in adolescence (Ernst, Pine, & Hardin, 2006; Somerville & Casey, 2010). With regard to resting-state connectivity, prior authors have argued that during adolescent development, short-range connections (e.g. subcortical-subcortical) become weaker, whereas long-range connections become stronger (e.g. subcortical-cortical) (Dosenbach et al., 2010). This would fit with the hypothesis that, with development, there is increased top-down control over emotional impulses.

We recently demonstrated that decreased connectivity between the amygdala and orbitofrontal cortex (OFC) was related to increased alcohol use in adolescents (Peters, Jolles, Van Duijvenvoorde, Crone, & Peper, 2015). This effect was modulated by testosterone levels: higher testosterone production was related to lower brain connectivity and increased alcohol use. These data support the hypothesis that the amygdala-OFC brain network is shaped by pubertal hormones and is related to risk-taking behavior as measured by consumption of alcohol. A focus on the amygdala when studying alcohol use also fits with task-based fMRI studies in adults, which show a crucial role for the amygdala in alcohol use. For instance, an attenuated amygdala response to emotional faces has been demonstrated after alcohol ingestion (Gilman, Ramchandani, Crouss, & Hommer, 2012; Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008; Sripada, Angstadt, McNamara, King, & Phan, 2011) and reduced coupling between the amygdala and the OFC during an emotional face processing task after alcohol ingestion (Gorka, Fitzgerald, King, & Phan, 2013). Animal studies have also shown an important role for the amygdala in the context of alcohol use in multiple ways, such as by mediating the locomotor stimulating effects of alcohol, and the finding that receptors in the amygdala appear to contribute to regulation of alcohol use (for a review see McBride, 2002).

However, relatively little is known about the direction of the longitudinal relationship between brain connectivity and alcohol consumption. That is, it is unclear whether alcohol use

affects *subsequent* brain development, or whether aberrant brain connectivity *precedes* an individual's propensity to alcohol use. Support for the hypothesis that alcohol influences subsequent brain development in adolescence comes from numerous animal studies and neuroimaging studies in human participants, which showed that substance use is linked to abnormalities in white matter, grey matter volume and abnormal activation during cognitive tasks (for a review see Squeglia, Jacobus, & Tapert, 2009). On the other hand, it is also possible that aberrant connectivity between subcortical and cortical areas biases adolescents towards risk-taking behavior. It is important to investigate this question from a developmental perspective. One of the main reasons why it is crucial to study the link between brain connectivity and alcohol use in adolescence, is that many researchers have argued that this is an especially vulnerable period for brain development. That is, the brain is still undergoing major developmental changes, for instance in connectivity between regions (Uddin, Supekar, & Menon, 2010; van Duijvenvoorde, Achterberg, Braams, Peters, & Crone, 2016). Because major connectivity tracts are not fully established yet in adolescence, it is hypothesized that these relatively fragile paths are more vulnerable and more easily disrupted than in adults, who have more established and stronger connections between regions which are not as easily affected by external agents (Guerri & Pascual, 2010; Zeigler et al., 2005). However, as many prior studies were cross-sectional, it is currently not clear whether alcohol use affects subsequent brain connectivity, or whether brain connectivity influences future alcohol use.

In this study, we investigated the directionality of the relationship between alcohol use and amygdala-OFC-connectivity with a longitudinal approach. We examined a large sample of adolescent participants between 8-27 years old who underwent resting state MRI scanning, and who filled out questionnaires on recent and lifetime alcohol use at two time points with a two-year interval. This large-scale longitudinal sample allowed us to elucidate whether

changes in functional connectivity between amygdala and OFC precedes or follows from alcohol use at the first time point.

Methods

Participants

This study was part of a larger project on cognitive and affective development (e.g., Braams, van Duijvenvoorde, Peper, & Crone, 2015; Peper, Koolschijn, & Crone, 2013; Peters, Braams, Raijmakers, Koolschijn, & Crone, 2014). Results from cross-sectional data on alcohol use and resting state connectivity at the first time point (T1) are published in Peters et al. (2015). Participants (8-25 years old at T1 were recruited through local schools and advertisements (N=299). Demographics for participants who had complete data of sufficient quality for at least one of the measures (alcohol use or brain connectivity) were as follows: N=292, 153 females, 139 males, 97,1 % Caucasian. No SES information was obtained. Ages were between 8.01 and 25.95 at T1 (M=14.06, SD=3.61). IQ was estimated with two subtests of the WAIS-III or WISC-III (Similarities and Block Design). IQ ranged between 80 and 143 (M=109.72, SD=10.52). The follow-up measurement (time point 2 (T2)) was approximately two years later (Mean time between T1 and T2: 2.01 years, SD=0.20) (N=254). Ages were between 10.02 and 26.62 at T2 (M=15.90, SD=3.50). IQ was estimated again using the WAIS-III and WISC-III subtests Picture Completion and Vocabulary, and at T2 ranged between 80 and 147.50 (M=108.28, SD=10.34).

At both time points, adults (18 years and older) received payment (60 euros) for participation, and children received presents and their parents received 30 euros (for 12-17 year old children) or 25 euros (for 8-11 year old children) for travel reimbursement. The study was approved by the Institutional Review Board at the 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 at T1.

Complete MRI data at T1 was collected for 295 participants (4 of the 299 participants did not complete the MRI scan), but there was data of sufficient quality for 274 participants. Reasons for exclusion were: >2 mm movement on the fMRI scan (n=11), >10 % of volumes affected by micromovements (see criteria in the fMRI analysis section) (n=14), a psychiatric diagnosis disclosed after participation (n=1), and insufficient quality data (n=2). At T2, 13 of the 299 initial participants could not or did not want to participate a second time. At T2, a further 32 participants could not participate in the MRI session due to braces, resulting in complete MRI data at T2 for 254 participants. There was sufficient quality data for 231 participants for resting state fMRI (exclusions: movement >2 mm: n=5; >10 % of volumes affected by micromovements: n=9).

The alcohol questionnaire was only administered to participants who were 12 years or older. This resulted in 193 participants at T1 and 244 participants at T2. All analyses were conducted in a pairwise manner, i.e. using all available data for each particular analysis. See Table 1 for an overview of the number of participants in each analysis.

Alcohol Questionnaire

Participants filled out an on-line questionnaire at home on recent and lifetime alcohol use developed by (Ames et al., 2007). Prior studies have shown that self-reported alcohol use is reliable when confidentiality is ensured (Sobell & Sobell, 1990; Winters, Stinchfield, Henly, & Schwartz, 1990) and has predictive validity for actual alcohol use (Graham et al., 1984). This questionnaire has been used often in earlier studies (Braams, Peper, Heide, Peters, & Crone, 2015; de Water, Braams, Crone, & Peper, 2013; Grenard et al., 2008; Peters et al., 2015; Thush et al., 2007, 2008). 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 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). In the question, participants were instructed to count bottles and cans as 1.5 glasses, because these contain more of the 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, thus the scales were recoded as the average of the two numbers, i.e. for 31-50, 40.5 was used (for >50 and >90, 51 and 91 were used, respectively). On average, participants had consumed 28.65 glasses of alcohol in their lives (*SD*=37.68) and 6.35 glasses in the last month (*SD*=12.36), at T1, and had consumed 36.00 glasses in their lives at T2 (*SD*=39.21) and 9.25 in the past month (*SD*=14.48). Alcohol use correlated with age at both T1 (lifetime: r=.779, p<.001; recent: r=.606, p<.001) and T2 (lifetime: r=.775, p<.001; recent: r=.644, p<.001), but not with change in alcohol use. Lifetime use may be relatively more difficult to estimate than recent alcohol use. However, the following findings strengthen our confidence in the lifetime alcohol scale: 1) none of the participants reported a lower lifetime alcohol use at T2 than at T1, 2) in our data set, reliability over two time points for lifetime alcohol use was high (α=.883), even though the time between the measurement points was quite long (average = 2.01 years), 3) there was a strong correlation with age, as one would expect, and 4) because we used a sample of children and adolescents, many participants did not reach the maximum amount of the scale (See Figure 2), making the total amount easier to estimate.

MRI data Acquisition

Scans were acquired with a Philips 3T MRI scanner. The same scanner and settings were used at T1 and T2. Functional 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=220x220x114.67 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=0.875x0.875 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=220x220x168 mm; in-plane resolution=1.96x1.96; slice thickness=2 mm) were acquired after the resting state scan. Participants were instructed to close their eyes during the resting state scan. Before the MRI scan, participants were accustomed to the MRI environment and sounds with a mock scanner.

FMRI data preprocessing

FMRI preprocessing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (www.fmrib.ox.ac.uk/fsl). These 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=50.0s). The resting state scan was registered with FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001) to the high resolution T2*-weighted scan, which was registered to the T1-weighted scan, and the T1-weighted scan was registered to the 2 mm MNI-152 standard image.

FMRI data analysis

In keeping with the prior cross-sectional study (Peters et al., 2015), left and right amygdala were selected for a seed-based correlation approach (Fox & Raichle, 2007) to test for functional connectivity with the OFC. Amygdala masks were obtained using atlas-based masks of the amygdala (Automatic Anatomical Labeling; see Figure 1). Amygdala masks in MNI-space were transformed to native space (each individual's resting state scan) with a binary threshold of 0.5. Next, mean time courses were extracted from each individual's amygdala, i.e. all voxels located within the amygdala mask. These mean time courses were entered as regressors in a GLM (separately for left and right amygdala), with nuisance regressors for white matter 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 CSF (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 specific volumes where micromovements occurred from the analysis (also referred to as 'scrubbing'). Participants where more than 10% of volumes were affected by micromovements (>.05 mm) were excluded from further analyses (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Satterthwaite et al., 2013).

Statistical analyses

We issued a region-of-interest (ROI) approach to investigate specifically amygdala-OFC-connectivity using an OFC anatomical mask (based on AAL: Medial Orbital Frontal Gyrus) with left and right OFC combined. OFC masks were transformed to native space with a binary threshold of 0.5. Next, we extracted Z-scores for amygdala connectivity with the OFC. To confirm that the amygdala and OFC were functionally connected, whole-brain

analyses were performed for visual inspection. Left and right amygdala showed positive functional connectivity with the OFC at both T1 and T2 (Figure 1). The ROI results were further analyzed with SPSS 19 and R 3.1.1.

Age effects: mixed model analyses

As a first goal, we assessed how all measures changed as a function of age. To model developmental trajectories (linear, quadratic or cubic shapes) for alcohol use and brain connectivity, we used mixed model analyses (Braams, van Duijvenvoorde, et al., 2015; Ordaz, Foran, Velanova, & Luna, 2013). We tested a linear effect of age (i.e., monotonic development), a quadratic (i.e., adolescent-specific effect) and a cubic effect (i.e., adolescent-emergent pattern). These analyses are a more advanced version of multiple regression, but taking the longitudinal nature of the data into account. That is, both absolute (i.e., the intercept) and change values for each individual were analyzed, and it was not necessary to calculate change scores. The analyses were performed with the NLME package in R (Pinheiro, Bates, DebRoy, & Sarkar, 2007). Models were compared using the Akaike Information Criterion (AIC) with lower values indicating a better model fit. We additionally tested with log-likelihood-tests whether changes in AIC model fit were significant. These model-building steps were used: First, we tested for each variable (left and right amygdala-OFC-connectivity, recent and lifetime alcohol use; at two time points) which pattern best described the developmental trajectory. The base model consisted of a fixed and a random intercept, describing variation in starting points (intercepts) of individuals. Next, we tested with polynomials (Braams, van Duijvenvoorde, et al., 2015) whether a model with age as a linear effect resulted in a better fit compared to the base model without age. Then, a model including a linear and quadratic term for age was compared to the linear model, and finally, we tested if a combined linear, quadratic and cubic model predicted the data better than a combined linear and quadratic model. For the best age model, we tested whether age as an

effect with a random slope resulted in a better fit, which would indicate that the age effect differs for each individual. We did not find evidence for significant random slopes and did not report this further in the results section.

Prediction analyses for alcohol use and brain connectivity

Intra-class correlation analyses were performed to examine whether there was consistency between T1 and T2 for alcohol use and amygdala-OFC-connectivity. We used a two-way mixed model with absolute agreement and reported the average measure. Next, prediction analyses were performed to examine the direction of the relationship between alcohol use and amygdala-OFC-connectivity. We performed multiple hierarchical regressions with alcohol use (recent and lifetime in separate analyses) at T2 as dependent variable, age at T1 and sex as first predictor and amygdala-OFC-connectivity at T1 (left and right amygdala-OFC-connectivity in separate analyses) as second step. In addition, we tested for the reverse direction, with amygdala-OFC-connectivity at T2 as dependent variable, age at T1 as first predictor and alcohol at T1 as second predictor. These analyses were also performed with baseline alcohol use/amygdala-OFC-connectivity at T1 entered as additional (control) step.

Results

The results section is organized along the following lines: First, consistency for all measures between T1 and T2 was calculated. Next, prediction analyses were performed to examine the directionality of the relationship between alcohol use and amygdala-OFC-connectivity. As a last step, we assessed developmental trajectories for alcohol use and amygdala-OFC-connectivity.

Consistency between T1 and T2

ICC analyses showed that for alcohol use, both recent (ICC=.79, p<.001, 95% CI=.62 - .87) and lifetime alcohol use (ICC=.83, p<.001, 95% CI=.50 - .92) were highly consistent between T1 and T2. In addition, amygdala-OFC-connectivity was modestly consistent over time, for both left (ICC=.20, p=.024, 95% CI=-.02 - .37) and right amygdala (ICC=.20, p=.015, 95% CI=-.16 - .38). There were no sex differences in recent and lifetime alcohol use, nor in left or right amygdala-OFC connectivity, at T1 or T2. Sex effects were therefore not investigated any further.

Age effects on alcohol use and amygdala-OFC-connectivity

We also investigated how alcohol use and amygdala-OFC-connectivity changed as a function of age. Mixed models were used to test the longitudinal pattern of development (linear, quadratic or cubic). These analyses revealed that both lifetime and recent alcohol use were best described by cubic patterns for age (i.e., rising quickly in mid adolescence and leveling off in early adulthood, Figure 2; Table 2). For recent alcohol use, a combined linear and cubic pattern best described the data, whereas for lifetime alcohol use, the best fitting function was a combination of a linear, quadratic and cubic function. For amygdala-OFC-connectivity, mixed linear modeling revealed that a model without age was the best fit to the data, suggesting no significant age-related change over time (Table 2).

Prediction analyses for amygdala-OFC-connectivity and alcohol use: direction of the effect

The next set of analyses addressed the question whether current alcohol use can be predicted from amygdala-OFC-connectivity at an earlier time point, or whether current amygdala-OFC-connectivity can be predicted from earlier alcohol usage. In the analyses reported below, we corrected for age differences and sex differences in alcohol use.

First, we investigated whether amygdala-OFC-connectivity at T1 predicted alcohol use at T2. A hierarchical regression with alcohol use at T2 as dependent variable, age and sex as first predictor and amygdala-OFC-connectivity at T1 as second predictor, showed a significant effect of left amygdala-OFC-connectivity on alcohol use two years later, for both lifetime (β=-.13, p=.001) (Table 3) and recent alcohol use (β=-.10, p=.040) (Table 3). That is, less positive connectivity at T1 was associated with increased alcohol use at T2. The relation between left amygdala-OFC-connectivity at T1 and lifetime alcohol use at T2 remained significant when adding lifetime alcohol use at T1 as a second predictor above age and sex (β=-.10, p=.019) (Table 3). Note that for these analyses including a baseline measure, there were less data points (for some participants who were 12 years and older we collected data on alcohol use on T2 but not on T1). These analyses showed that less positive connectivity between the amygdala and the OFC predicts alcohol use two years later, and that amygdala-OFC-connectivity explains lifetime alcohol use two years later even when controlling for baseline alcohol use at T1. The results did not change when adding also quadratic and cubic effects for age, besides linear effects, in step 1.

To test for the reversed direction, we investigated whether alcohol use at T1 predicted amygdala-OFC-connectivity at T2, but no significant results were found. Together, these analyses suggest that brain connectivity precedes alcohol use, but we found no evidence for the reverse direction, i.e. alcohol use preceding brain connectivity.

Discussion

In this study, our goal was to investigate the longitudinal relationship between alcohol use and amygdala-OFC-connectivity. In particular, our aims were 1) to describe developmental trajectories of alcohol use and amygdala-OFC connectivity in a large sample of typically developing adolescents, and 2) to investigate whether amygdala-OFC-connectivity could be predicted from earlier alcohol use, or instead, whether alcohol use could

be predicted from amygdala-OFC-connectivity two years earlier. The results indicated that alcohol use demonstrated a cubic relationship with age, with little to no alcohol use in childhood, steep increases in adolescence and leveling off in adulthood. No age effects were found for amygdala-OFC connectivity. The prediction analyses indicated that amygdala-OFC-connectivity at the first time point predicted alcohol use two years later, but there was no evidence for the reverse direction. The results are described in more detail in the following sections.

Stability and change of alcohol use and amygdala-OFC-connectivity over a two-year period

We first assessed the level of stability and age-related changes in alcohol use and amygdala-OFC-connectivity within a two-year period. All measures showed significant relations between T1 and T2, confirming that they are valid indices of individual variation. Alcohol use showed relatively high stability over time. The correlation of amygdala-OFC-connectivity over two time points was modest but significant. It should be noted that a limitation of this study was the relatively short assessment time for resting state analyses. That is, prior studies have argued that resting state connectivity is a reliable measure of brain function, but this appears to be mostly the case for scans of relatively long duration (i.e. >9-12 minutes), compared to our acquisition time (6 minutes) (Birn et al., 2013). Nonetheless, the study resulted in consistent patterns over time.

Next to this substantial level of individual stability, we investigated whether alcohol use and amygdala-OFC-connectivity showed age-related changes during adolescence. Consistent with prior studies, we observed a strong increase in alcohol use with increasing age (Hibell et al., 2012). With mixed model analyses for longitudinal data, we assessed the shape of developmental trajectories for alcohol use (linear, quadratic or cubic patterns). These analyses indicated that the developmental trajectory for alcohol use was best described by a

cubic effect of age. That is, alcohol use was relatively stable in children, then showed a steep increase in adolescence, and leveled off again towards young adulthood. These cubic age-effects were both found for lifetime consumption and recent alcohol use (over the past month). It should be noted that the index of lifetime alcohol use reached a ceiling effect (i.e., the maximum amount of glasses that could be chosen in the questionnaire was '91 or more glasses') which makes the last phase less reliable, but the same pattern was found for recent alcohol use (see also Chassin, Pitts, & Prost, 2002; White, Xie, Thompson, Loeber, & Stouthamer-Loeber, 2001).

With regard to developmental patterns in amygdala-OFC-connectivity, we found no linear, quadratic or cubic effect of age using longitudinal mixed models on amygdala-OFC-connectivity. These results do not concur with an earlier cross-sectional study in a smaller-scale task-based study (Gee et al., 2013), who reported a shift from positive to negative connectivity with increasing age, and a prior cross-sectional resting state study (Gabard-Durnam et al., 2014) which reported an age-related increase in connectivity, suggesting that cross-sectional and longitudinal studies, as well as task-based vs. resting state studies may reveal different findings when studying connectivity during adolescent development. Future studies should investigate age-related changes in amygdala-prefrontal connectivity in more detail, with more optimized acquisition times (Birn et al., 2013). The current results suggest that amygdala-OFC-connectivity may be a developmental marker that is predictive for future explorative or risk-taking behavior. Note that in this study we found no sex differences in alcohol use or amygdala-OFC connectivity. A prior developmental resting-state study did find evidence for sex differences in amygdala connectivity, but these authors investigated connectivity from sub-regions of the amygdala which may be more informative with regard to sex differences (Alarcón, Cservenka, Rudolph, Fair, & Nagel, 2015) and is therefore an

important direction for future research into the link between amygdala-connectivity and risk-taking behavior.

Longitudinal relationship between amygdala-OFC-connectivity and alcohol use

Next, we investigated the longitudinal relationship between brain connectivity and alcohol use. In our prior study based on cross-sectional comparisons we reported a correlation between reduced amygdala-OFC-connectivity and increased alcohol use (Peters et al., 2015). Our main goal in the current study was to investigate the directionality of the relationship between amygdala-OFC-connectivity and alcohol consumption using longitudinal data on two time points. We tested whether reduced amygdala-OFC-connectivity preceded alcohol use (suggesting vulnerability to alcohol use due to reduced coupling of prefrontal and subcortical brain systems), or whether increased alcohol use preceded reduced amygdala-OFC-connectivity (suggesting a 'damaging' effect of alcohol use on amygdala-OFC-connectivity). The results indicated that amygdala-OFC-connectivity preceded alcohol use two years later, but we found no evidence for the reverse direction. This effect was found for both lifetime and recent alcohol consumption, and was specific for left amygdala-OFC-connectivity. Importantly, the prediction of lifetime alcohol use from left-amygdala OFC connectivity remained significant when controlling for alcohol use at the first time point, suggesting that brain connectivity explains unique variance in future alcohol use over and beyond behavioral assessments.

These findings are in line with the hypothesis that subcortical-prefrontal connectivity is important for top-down control over behavioral approach tendencies. For instance, prior studies showed that increased connectivity between the amygdala and the OFC was associated with improved emotion regulation and behavioral control (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Lee, Heller, van Reekum, Nelson, & Davidson, 2012). This suggests that increased connectivity is protective against risk-taking, which fits with the current findings

that decreased amygdala-OFC-connectivity predicts increased alcohol use. However, a study by DeWitt et al. (2014) showed that individuals with higher risk-taking tendencies showed *more* connectivity between amygdala and frontal regions. In future studies, it is important to resolve these contradictory findings and assess whether other forms of risk-taking behavior or impulsivity can also be linked to amygdala-frontal connectivity. It should also be investigated whether these findings have relevance for interventions targeting teenage alcohol use. Possibly, aberrant amygdala-OFC connectivity may eventually be useful as a biomarker enabling early detection for children and adolescents at risk for alcohol abuse. Another important direction for further research would be to also investigate connectivity between the ventral striatum and frontal areas and its relation to risk-taking behavior in adolescence. Besides the amygdala, ventral striatum activation has been associated with alcohol use (Braams, Peper, et al., 2015). Resting state connectivity between the ventral striatum and medial prefrontal cortex changes during adolescent development (Fareri et al., 2015) and has recently been shown to be reduced in youth at risk for alcohol dependence (Cservenka, Casimo, Fair, & Nagel, 2014).

When we studied the reverse direction, i.e. alcohol use preceding reduced connectivity between the amygdala and the OFC, we found no significant effects. Although prior studies reported that alcohol consumption can affect brain structure and function (Squeglia et al., 2009), this is the first longitudinal study specifically investigating amygdala-OFC-connectivity during resting state. Our findings suggest that, with regard to the specific connectivity between the amygdala and the OFC, increased alcohol use does not affect coupling between these regions. However we want to be careful to emphasize that we did not find evidence for a damaging effect of alcohol use for this specific connectivity path, but other connectivity paths may result in different effects. These should be investigated in more detail in future studies.

Limitations and future directions

There are several limitations to this study that should be taken into account. First, note that in our sample, alcohol use resulted in a non-normal distribution due to the fact that younger participants often reported no alcohol use, whereas older participants sometimes reached the maximum amount specified in the questionnaire. In future studies, alcohol use could be tested within a sample of same-aged participants to avoid this issue. On the other hand, such an approach has the problem that only a small range of adolescent alcohol use is captured. Second, although our large-scale longitudinal data could be used to find support for the direction of the relation between alcohol use and amygdala-OFC-connectivity, such studies in human participants still cannot provide true causal evidence. Individuals who consume relatively large amounts of alcohol may differ from peers who consume less alcohol in other aspects which could not be controlled for in this study. Third, the alcohol measures in this study were based on self-report, which may lead to overestimations or underestimations of actual alcohol consumption. However, prior studies showed that self-report measures of alcohol can be reliable if confidentiality of answers is ensured (Brener et al., 2002; Sobell & Sobell, 1990). It should also be taken into account that in this study, our main aim was to assess predictive relations rather than change-change relations over time. That is, our goal here was mainly to investigate whether, if brain connectivity measures are known, this information can be used to predict alcohol use two years later. When we additionally added a baseline measure for alcohol use at the first time point to our analyses, there was still a significant prediction of lifetime alcohol use two years later from left-amygdala-OFC connectivity, but not for recent alcohol use. Including a baseline measure has the added benefit of ensuring that brain connectivity explains extra variance in addition to alcohol use at T1, which may also be correlated with brain connectivity at T1. As is often the case in developmental research, younger participants were more likely to be excluded due to

excessive global motion or micromovements. This should be taken into account when interpreting our findings. However, our stringent approach to correcting for motion is based on the most recent insights into resting-state connectivity research (Power et al., 2012; Satterthwaite et al., 2013).

Conclusion

In conclusion, this large-scale longitudinal study provided evidence that future alcohol use can be predicted from amygdala-OFC-connectivity. These results have important implications for understanding the onset and progression of alcohol use in particular, and more generally, the link between subcortical-frontal connectivity and risk-taking behavior in adolescence. Possibly, relatively reduced subcortical-cortical connectivity in early to mid-adolescence creates a vulnerable window for starting alcohol use (Ernst et al., 2006; Somerville & Casey, 2010). Eventually, these results may inform early interventions aimed at adolescents with relatively more sensitivity to exploration and risk-taking.

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Tables

Table 1: overview of the number of participants for each variable. MRI data were collected for all participants who took part in the study. *Alcohol self-report data were only collected in case participants were 12 years or older.

	N		Age Range	
	T1	T2	T1	T2
Participation	299	286	8-25	10-27
MRI scan of sufficient quality	274	231	8-25	10-27
Alcohol data*	193	244	12-25	12-27

Table 2: AIC and loglikelihood *p*-values for a base model (without age), linear, quadratic and cubic age pattern. The best-fitting model is highlighted in bold font.

	Base	Linear		Quadratic		Cubic	
	AIC	AIC	*p*	AIC	*p*	AIC	*p*
Lifetime alcohol	4321	3972	<.001	3956	<.001	**3920**	<.001
Recent alcohol	3435	3252	<.001	3254	.917	**3235**	<.001
Left amygdala-OFC	**1348**	1348	.273	1351	.870	1353	.727
Right amygdala-OFC	**1384**	1386	.615	1386	.173	1388	.396

Table 3: Regression parameters for significant relations between amygdala-OFC connectivity and alcohol use.

Steps	Predictor	*β*	*p*	*F*	R^2
Dependent: Lifetime alcohol use T2					
1	Overall model			183.207***	.784
	Age T1	.789	<.001		
	Sex	-.093	.025		
2	Overall model			130.976***	.795
	Age T1	.776	<.001		
	Sex	-.098	.016		
	Left amy-OFC connectivity T1	-.133	.001		
Dependent: Lifetime alcohol use T2 (lifetime alcohol use T1 as additional regressor)					
1	Overall model			142.406***	.615
	Age T1	.789	<.001		
	Sex	-.093	.048		
2	Overall model			136.135***	.698
	Age T1	.427	<.001		
	Sex	-.063	.134		
	Left amy-OFC connectivity T1	.460	<.001		
3	Overall model			106.192***	.707
	Age T1	.433	<.001		
	Sex	-.068	.101		
	Lifetime alcohol use T1	.441	<.001		
	Left amy-OFC connectivity T1	-.098	.019		
Dependent: Recent alcohol use T2					
1	Overall model			82.378***	.418
	Age T1	.649	<.001		
	Sex	-.025	.565		
2	Overall model			57.122***	.429
	Age T1	.639	<.001		
	Sex	-.029	.565		
	Left amy-OFC connectivity T1	-.104	.040		

* p<.05 ** p<.01 *** p<.001

Figures

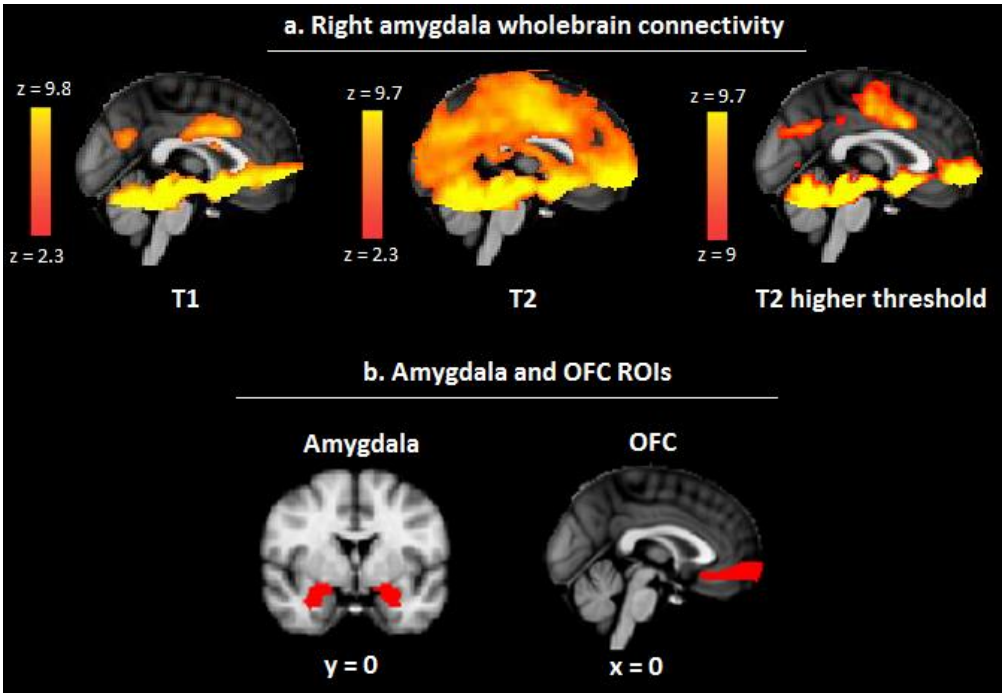

Figure 1a: Positive whole-brain connectivity with the right amygdala as seed (cluster-thresholded at 2.3, $p<.05$). The threshold at T2 was manually set to intensity 9 out of 9.7 for visual inspection. Figure 1b: Amygdala and orbitofrontal cortex anatomical ROIs.

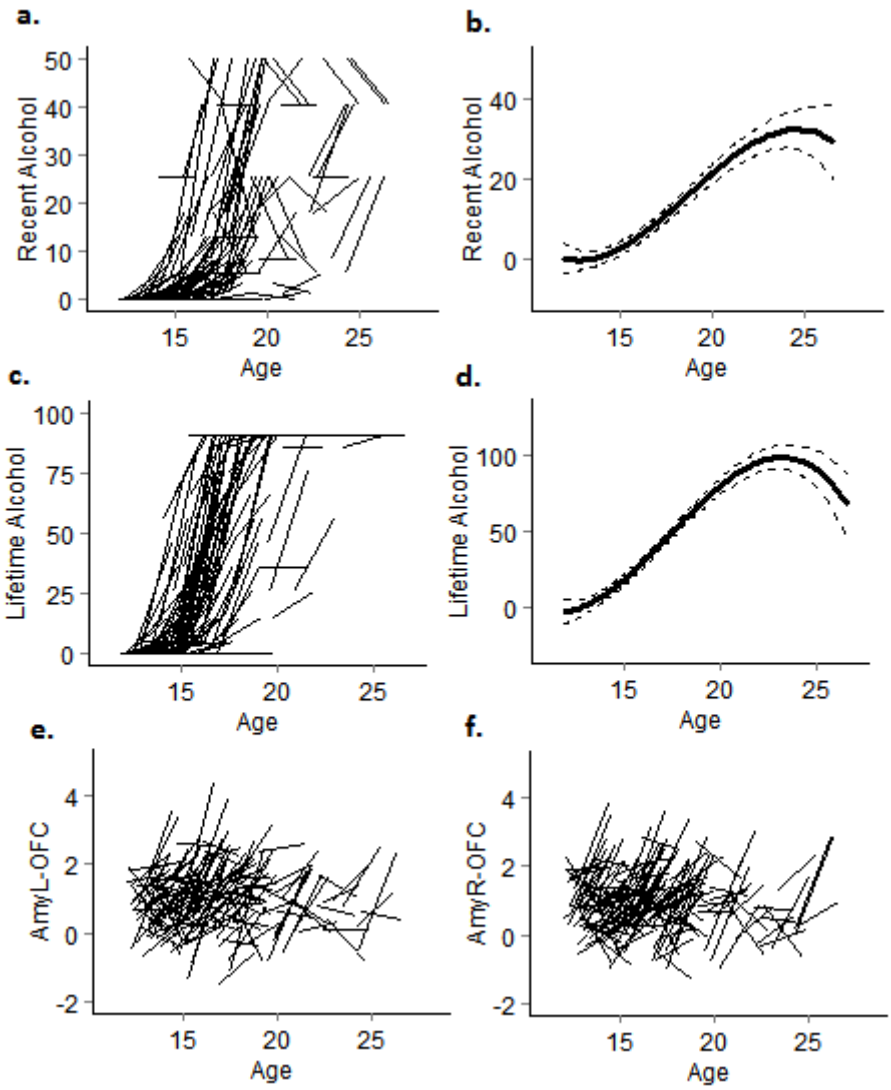

Figure 2: Predicted values (2b, 2d) and raw data (2a, 2c) for the cubic relationship between recent and lifetime alcohol use and age. Figure 2e and 2f depict raw data for left and right amygdala-OFC-connectivity, which revealed no age effect.