

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

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# Chapter 9

# Amygdala-orbitofrontal connectivity predicts alcohol use two years later



#### This chapter is based on:

Peters, S., Peper, J.S., Van Duijvenvoorde, A.C.K., Braams, B.R. & Crone, E.A. Amygdalaorbitofrontal connectivity predicts alcohol use two years later: A longitudinal neuroimaging study on alcohol use in adolescence (in revision, 2015).

## Abstract

This study tested the relation between cortical-subcortical functional connectivity and alcohol consumption in adolescents using an accelerated longitudinal design. Participants (N = 299 at T1 and N = 254 at T2) between ages 8 and 27 completed resting state neuroimaging scans at two time points separated by two years. In addition, participants between ages 12 and 27 reported on recent and lifetime alcohol use. Resting state connectivity analyses focused on amygdala-orbitofrontal connectivity given prior research linking reduced coupling between these regions to alcohol use. The results indicated that amygdala-orbitofrontal connectivity at the first time point predicted 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 function in an accelerated longitudinal design with participants between 8-27 years old.

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, which makes it suitable for testing longitudinal questions as 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 et al., 2006; Somerville & Casey, 2010). 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. These results are also consistent 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 et al., 2012, 2008; Sripada et al., 2011) and reduced coupling between the amygdala and the OFC during an emotional face processing task after alcohol ingestion (Gorka et al., 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, gray 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. In this study, we investigated the directionality of the relationship between alcohol use and amygdala-OFC connectivity, by using 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 precede or follow 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, et al., 2013; Peters et al., 2014). Participants (8-25 years old at the first time point (T1)) were recruited through local schools and advertisements (N = 299). Ages were between 8.01 and 25.95 at T1 (M = 14.06, SD = 3.61). Participants were recruited from different schools in the Netherlands to ensure that the sample reflected the general population. 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 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 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.

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

#### Alcohol Questionnaire

Participants filled out an on-line questionnaire at home on recent and lifetime alcohol use (Ames et al., 2007; de Water et al., 2013; Peters et al., 2015; Thush et al., 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). Bottles and cans were counted 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 (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).

#### **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 =  $80^\circ$ ; FOV =  $220\times220\times114.67$  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 =  $8^\circ$ ; FOV =  $224\times177.33\times168$  mm; in-plane resolution =  $0.875\times0.875$  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 =  $80^\circ$ ; FOV =  $220\times220\times168$  mm; in-plane resolution =  $1.96\times1.96$ ; 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 (Gaussianweighted 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 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. Participants where more than 10 % of volumes were affected by micromovements (> .05 mm) were excluded from further analyses.

#### Statistical analyses

We issued a region-of-interest (ROI) approach to specifically investigate 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.



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 7 out of 9.62 for visual inspection. Figure 1b: Amygdala and orbitofrontal cortex anatomical ROIs

#### Prediction analyses for alcohol use and brain connectivity

Correlation analyses were performed to examine whether there was consistency between T1 and T2 for alcohol use and amygdala-OFC connectivity. Next, prediction analyses were performed to examine the direction of the relationship between alcohol use and amygdala-OFC connectivity. We performed regressions with alcohol use (recent and lifetime in separate analyses) at T2 as dependent variable, age at T1 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. These analyses were also performed with baseline alcohol use/amygdala-OFC connectivity at T1 entered as additional (control) step.

#### Age effects: mixed model analyses

As a final 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 et al., 2015; Ordaz et al., 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 take 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 et al., 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 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.

## 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

Correlation analyses showed that for alcohol use, both recent (r = .71, p < .001) and lifetime alcohol use (r = .79, p < .001) were highly correlated between T1 and T2. In addition, amygdala-OFC connectivity was modestly correlated over time, for both left (r = .14, p = .042) and right amygdala (r = .15, p = .023).

#### 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 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 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 ( $\beta$  = -.13, *p* = .002) and recent alcohol use ( $\beta$  = -.10, *p* = .042). 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 ( $\beta$  = -.10, *p* = .024). 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.

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.



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.

#### Age effects on alcohol use and amygdala-OFC connectivity

We additionally 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).

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.

	<u>Base</u>	<u>Linear</u>		<u>Quadratic</u>		<u>Cubic</u>	
	AIC	AIC	р	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

# Discussion

In this study, our goal was to investigate the longitudinal relationship between alcohol use and amygdala-OFC connectivity. In particular, our aim was 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 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.

#### Longitudinal relationship between amygdala-OFC 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 leftamygdala 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 idea 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 et al., 2007; Lee et al., 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.

No evidence was found for the reverse direction, i.e. alcohol use preceding reduced connectivity between the amygdala and the OFC. 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. This highlights the importance of longitudinal designs to determine the direction of a cross-sectional association between brain connectivity and behavior.

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

In addition to these main analyses, we 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 correlations 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 young adolescents, then showed a steep increase in midadolescence, and leveled off again towards young adulthood. These cubic age-effects were found for both 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 (and 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 taskbased 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.

#### Limitations and future directions

There are several limitations to this study that should be taken into account. First, 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. Second, 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).

#### 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-cortical 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.