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Prospective Changes in Neural Alcohol Cue Reactivity in At-Risk Adolescents

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Abstract

Introduction—Adolescence represents an ideal time for elucidating the etiology of cue reactivity profiles. This study examined the influence of three risk factors consistently associated with heavy adolescent drinking on alcohol cue reactivity.

Methods—Youth were first assessed while still naïve to alcohol (12–14 years old) and followed after transitioning into alcohol use (17–21 years old). The effects of family history of substance use disorder, sex, and history of early of dating (i.e., before 14 years of age) on BOLD response contrast to alcohol picture cues were examined in a linear mixed model, controlling for age and alcohol use patterns at follow-up.

Result—Activation to alcohol picture cues differed as a function of risk factor and time. At baseline, family history positive youth showed greater activation to alcohol cues than family history negative peers in the right middle occipital and anterior cingulate gyri. Youth with a history of early-dating showed greater activation to alcohol cues, compared to non-early daters, in the left anterior cingulate/white matter region. Girls showed greater activation to alcohol than boys at baseline in left middle frontal gyrus. At follow-up, after drinking started, patterns reversed for each risk factor.

Discussion—These results indicate that even prior to initiating alcohol use, adolescents showed differences in activation to alcohol cues based on their family history, dating history, and sex.

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Compliance with Ethical Standards

All authors (TTN, KEC, LMS, KB, SE, RM, ARA, SFT, CP) of this study declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Keywords

alcohol cue reactivity; neuroimaging; alcohol use; family history; gender; early dating

Introduction

Significant attention has been devoted to understanding the phenomenology of alcohol cue reactivity (i.e., psychophysiological responses), largely predicated on associative learning principles. Repeated pairing of substance-related cues with substance use produces conditioned reinforcement such that the cues become conditioned stimuli capable of eliciting craving and modulation of affective states, thereby helping to maintain substance use behaviors (Curtin et al. 2005; O'Brien et al. 1990). Importantly, distinct profiles can be observed in functional magnetic resonance imaging (fMRI) measures of cue reactivity among drinkers with and without an alcohol use disorder ((e.g., greater activation of the posterior cingulate, precuneus and superior temporal gyrus during alcohol cue vs. control cue presentation; Schacht et al. 2013). These findings suggest the potential utility of fMRI markers for elucidating risk profiles for alcohol use disorder (AUD) trajectories.

Alcohol cue reactivity shows substantial inter-individual variability (Ivory et al. 2014; Rees and Heather 1995; Schacht et al. 2013), suggesting possible etiological contributions from factors beyond learned cue-response pairings. Such factors include previous level of exposure to alcohol (White and Staiger 1991), severity of alcohol use problems (Claus et al. 2011; Courtney et al. 2014; Filbey et al. 2008), subjective responses to alcohol (Courtney et al. 2014), and reward expectancies (Franken 2002; Glautier et al. 2000; Ivory et al. 2014). Genetics (Ray et al. 2014) and family history (Dager et al. 2014) may also moderate neuroimaging markers of alcohol cue reactivity. Compared to counterparts with a negative family history (FHN) of AUD, family history positive (FHP) young adults showed a decrease (i.e., habituation) in their activation patterns over repeated alcohol cues in the hippocampus, amygdala, and temporo-parietal areas over repeated exposure to alcohol picture cues, above and beyond lifetime drinking (Erratum 2016; Dager et al. 2013). Similarly, FHP adolescents with and without AUD show widespread enhanced BOLD activation to alcohol picture cues compared to age- and sex-matched FHN adolescents (Tapert et al. 2003). However, the prospective impact of family history of AUD on alcohol cue reactivity within an individual remains unknown.

Sex has also been related to differential alcohol cue reactivity responses. Specifically, girls exhibited greater BOLD activation to alcohol picture cues in the anterior cingulate and left prefrontal cortex; whereas boys showed greater alcohol cue-induced activation in the left orbital gyrus and bilateral paracentral gyri in a small sample of adolescents with AUD (Tapert et al. 2003). In adults, female social drinkers exhibited greater activation in the superior and middle frontal gyrus versus male social drinkers during alcohol (vs. neutral) picture cues. Further, alcohol craving in men was positively associated with activation of the striatum, right dorsolateral and lateral prefrontal cortex (PFC), anterior cingulate cortex (ACC), and middle/inferior temporal lobe during alcohol cues. Women showed no regions of

significant correlation with alcohol cravings, suggesting men may be more vulnerable to development of AUDs (Seo et al. 2011).

Along with other life events (e.g., relocation, distress), adolescent involvement in romantic and sexual relationships has been shown to be strongly predictive of current and future binge drinking behaviors (Engels and Knibbe 2000; Whelan et al. 2014). Among tenth graders, more experience with romantic relations (e.g., had a boy/girlfriend) was related to higher social acceptance and more friendships, romantic relationships, substance use and delinquent behaviors, even after accounting for social acceptance and sexual behaviors (Furman et al. 2009). A recent study that examined the contributions of neuroimaging (structural and functional MRI), neuropsychological, and psychosocial predictors of future alcohol use in young adolescents found that history of early dating was a very important psychosocial predictor of future drinking (Squeglia et al. 2016), and a more accurate predictor (i.e., greater model improvement and larger effect size) than AUD family history. To date, no published studies exist on whether youth who begin dating early show differential activation to alcohol cues. Evidence of such effect would provide greater support of this as an important risk factor for development of heavy drinking for AUD. Importantly, unlike sex and family history, early dating could potentially be targeted in prevention efforts.

To date, most alcohol cue reactivity studies have focused on adult populations with extensive histories of alcohol use. However, studying youth is essential to increase our understanding of the etiology of cue reactivity profiles as a significant number of adults with AUD initiate alcohol use during adolescence (Hingson et al. 2006). Therefore, it is likely that cue-response profiles are established prior to young adulthood. Further, the literature suggests that external factors may contribute to the etiology of cue reactivity profiles as adolescents with AUD exhibit similar fMRI cue reactivity patterns to adults, despite having shorter alcohol use trajectories (Tapert et al. 2003).

The present study examined the influence of select risk factors on the development of neural markers of alcohol cue reactivity in a prospective study of adolescents; first assessed while naïve to alcohol (12–14 years old at baseline) and followed-up after transition to alcohol use (17–21 years old). The following variables were chosen *a priori*, as they have consistently been shown to be risk factors for initiation and escalation of heavy alcohol use in adolescents: family history of substance use disorder (SUD), biological sex, and history of early-dating. According to the incentive-sensitization model of addiction, heavy use over time increases the salience of previously neutral stimuli, which are then paired with the reinforcing effects of substances through associative learning principles (Robinson and Berridge 1993). At baseline, participants in this study did not consumed any alcohol; thus, it was hypothesized that no differences in task-based BOLD reactivity to alcohol cues would be observed at baseline, regardless of family history, sex, and dating status. At follow-up, after all youth have transitioned into moderate or heavy drinking, it was hypothesized that youth with risk factors for AUD development (i.e., positive SUD family history, being male, and having early-dating experience) would show greater activation to alcohol cues compared to their counterparts (i.e., negative SUD family history, being female, and no history of early-dating). Further, it was expected that activation to alcohol picture cues at follow-up,

but not at baseline, would be positively associated with past-year alcohol consumption at follow-up.

Methods

Participants

This study used data from a larger ongoing longitudinal substance use and neuroimaging project (R01 AA13419), a protocol that was approved by the University of California San Diego Human Research Protections Program. Participants between 12 to 14 years old were recruited from San Diego public middle schools. Eligible youth ($N = 295$) were administered a comprehensive interview assessing substance use and demographic information (e.g., age, ethnicity) and completed an MRI scan at baseline (project entry).

In the parent study, potential participants were excluded for psychiatric diagnoses, neurodevelopmental disorders, and use that exceeded minimal exposure to alcohol and other substances (Nguyen-Louie et al. 2015). Participants were followed-up each year by phone to obtain data about alcohol consumption and other drug use habits and psychosocial changes (e.g., grade in school). Individuals who transitioned into drinking were brought back into the laboratory for a repeat MRI scan at follow-up. Not all participants in the parent study received the Alcohol Pictures Cue Reactivity task, as it was added later during the project. The sample for the current study included only those participants in the parent study who were administered this task at baseline and follow-up. To account for possible effects of prior alcohol and other substance use on cue reactivity at baseline, participants in the parent study who reported having any experience with alcohol greater than several sips and any history of other substance use at project intake were excluded from analysis. Likewise, to examine the effects of initiation of alcohol use on alcohol cue reactivity, participants who had not initiated alcohol use at follow-up (i.e., have not had at least one standard drink) were also excluded from analysis. In total, 51 participants met criteria for inclusions in the current set of analyses.

Measures

Structured clinical interview—The Structured Clinical Interview (Brown et al. 1998) was administered to youth at baseline and follow-up to assess general psychosocial functioning such as academic functioning (e.g., grade point average) and peer relations and attitudes towards substance use, and major medical illnesses. Dating status was defined dichotomously with the question “Do you date” at baseline. Answer choices were “no, never dated”, “no, not now”, “yes, only in group activities”, “yes, rarely”, “yes, occasionally”, “yes, regularly”, “yes, steady partner”, or “I am married.” At baseline, youth were coded as either 1) non-early daters (i.e., no history of dating) if they answered “no, never dated”, or 2) early daters (i.e., have a history of any, or are currently, dating) if answered otherwise. All but one participant reported having dated at least once at follow-up.

Substance use—The Customary Drinking and Drug Use Record (Brown et al. 1998) was administered at baseline and follow-up years to assess onset, frequency, quantity, and sequelae of alcohol and other substance use. Participants were asked to abstain from alcohol

and recreational drug use for 72 hours prior to scan sessions. Breathalyzer and urine toxicology screens were administered in the laboratory to confirm recent abstinence.

Family history—The Family History Assessment Module (Rice et al. 1995) was administered to youth and both parents, or one parent and another family member, to assess familial density of alcohol and other substance use disorders (SUD). Family history negative (FHN) was defined as having no first- or second-degree relative with history of SUD, family history positive (FHP) as having one or more first- or second-degree relative with history of SUD.

Socioeconomic status—The Hollingshead Index of Social Position score (Hollingshead 1965), an index of socioeconomic status (SES), was calculated for each subject using parental socioeconomic background information (i.e., educational attainment, occupation, and salary of each parent) to characterize the youth's home environment. Higher values indicate lower SES.

Imaging

Imaging data were collected using a 3.0 Tesla General Electric (3T GE) short bore Excite-2 system with an eight-channel phase-array head coil. Eight high-bandwidth receivers for ultra-short repetition time (TR) reduced signal distortions and dropout. High-resolution T1-weighted sequence using a sagittally acquired spoiled gradient recalled sequence (field of view [FOV] 24cm, $256 \times 256 \times 192$ matrix, $.94 \times .94 \times 1$ mm voxels, 176 slices, repetition time=20ms, echo time=4.8ms; flip angle 12° , 7:26 minutes). Field maps were acquired to assess field inhomogeneities and signal dropout with two different echo times, which was then applied to the BOLD signal to minimize warping and signal dropouts. BOLD signal was measured with T2*-weighted axially acquired echo-planar images (FOV=24cm, 64×64 matrix, $3.75 \times 3.75 \times 3.8$ mm voxels, 32 slices, echo time=30ms, repetition time=2,000ms, flip angle 90° , task time: 8:32). Task stimuli were back-projected from a laptop to a screen at the foot of the scanner using an angled mirror attached to the head coil. Task accuracy and reaction times were logged with a MRI compatible fiber-optic response box (Current Designs, Pittsburgh, PA).

Task

The Alcohol Cue Reactivity Task (Pulido et al. 2010) consisted of four conditions: alcoholic beverages, non-alcoholic beverage (i.e., neutral cues), active control conditions (i.e., pixilated versions of the beverage images), and fixation (rest) periods. Each of the 4 conditions consisted of 22 stimuli each presented four times on a laptop using E-Prime (Pittsburgh, PA). Stimuli in this event-related task were presented sequentially in pseudo-randomized order, each for 750 ms with a 1250 ms inter-stimulus interval, resulting in a task duration of 8 minutes and 32 seconds (Figure 1). Alcohol and non-alcohol beverage stimuli were previously standardized with a non-drinking youth sample to ascertain adequate identification, and comparable valence, arousal, and visual characteristics between the two conditions (Pulido et al. 2010). Standardized instructions and a practice version of the task were administered outside the scanner. In the scanner, participants were instructed to indicate the valence (i.e., dislike, neutral, like) of each stimulus with their fingers using a

button box. Participants' responses and reaction times were logged while completing the task inside the scanner. BOLD response signal change during viewing of the alcohol picture cues minus neutral picture cues served as the outcome measure of interest. A positive BOLD response contrast was interpreted as greater activation to alcoholic beverage cues vs. neutral cues; a negative BOLD response contrast was interpreted as greater activation to neutral cues vs. alcoholic beverage cues.

Image Processing

Imaging data was analyzed using the Analysis of Functional NeuroImages (AFNI) software (Cox 1996). Artifacts and abnormal signals were removed and time series data were temporally aligned and co-registered to a maximally stable base volume with an iterated least squares algorithm (Cox and Jesmanowicz 1999). Each individual's functional and anatomical data sets were co-registered and warped to standardized Talairach space (Talairach and Tournoux 1988) to reduce effects of anatomical variability. Functional data were resampled into isotropic voxels (3 mm^3), and spatial smoothing was applied using a Gaussian filter (full-width half maximum 5 mm). Motion (i.e., three rotational and three linear displacement parameters) was estimated for each participant and used to control for task-related analyses (Bandettini et al. 1993). An automated program detected outliers in the six motion parameters; time-series data with significant outliers were despiked or censored (i.e., removed from further processing). No participant had 15% or more of repetitions censored.

Deconvolution was conducted on time-series data with a reference function that convolved the behavioral stimuli response time with an ideal hemodynamic response model (Cohen 1997), covarying for linear trends and motion correction parameters. Results of the general linear model included, for each voxel, 1) a fit coefficient that represented change in signal between alcohol beverage pictures minus active control, 2) percentage signal change (i.e., contrast between alcohol and neutral picture cue trials) and 3) threshold statistics.

Statistical Analysis

fMRI BOLD—To test the dual hypotheses that 1) no differences in BOLD activation to alcohol vs. neutral picture cues would be detected between risk groups at baseline and 2) high-risk participants would exhibit increased activation to alcohol vs. neutral picture cues after transitioning into alcohol use at follow-up, a single whole-brain group-level linear mixed effects model was conducted in AFNI (3dLME). The model included two covariates (age and total number of drinks consumed in the past year), one random factor (participants), one fixed-effect within-subject predictor (time: baseline and follow-up), three dichotomous fixed-effects between-subjects predictors (family history, sex, early-dating history), and interactions among the four predictors. Mean activations from functional clusters that showed significant interaction effects were extract (3dROIstats) for each time point (i.e., at baseline and follow-up) and group (e.g., FHN and FHP) and graphed using Microsoft Excel. Graphs were visually inspected for qualitative description of signage (i.e., positive or negative activation) directionality (e.g., increased or decreased over time).

To control for multiple comparisons and Type I error, noise smoothness in the x, y, and z dimensions was first estimated ($3dFWHMx$) with a Gaussian and mono-spherical autocorrelation function (acf). Parameters from this model were then entered into Monte Carlo simulations ($3dClustSim$) that accounted for the estimated smoothing to determine cluster size thresholds that met the following criteria: bi-sided thresholding, first-nearest neighbor clustering, uncorrected (per-voxel) $p < .001$, and corrected (whole volume) $\alpha = .05$. Only regions with 11 or more contiguous voxels were retained for interpretation.

Alcohol Consumption—Differences in past-year total drinking and binge-drinking days at follow-up by family history, sex, and early-dating history were tested in Wilcoxon-Mann-Whitney tests with the *proc nparlway wilcoxon* command in the SAS Studio software (SAS Institute Inc., Cary, NC, USA; Version 3.5). Wilcoxon-Mann-Whitney tests were conducted to reflect the non-normal distribution of the two indices of alcohol use of interest. Six models were tested in total; one for each group (i.e., FHP vs. FHN; male vs. female; early-dating history vs. non-early dating history) and alcohol use index (i.e., past-year total drinking days, past-year binge drinking days).

The relationship between recent (i.e., past year) alcohol consumption at follow-up and alcohol-cue reactivity was examined in two linear regressions ($3dMEMA$). To examine the effect of overall drinking quantity on alcohol cue reactivity, the first linear regression model used the total number of standard drinks consumed in the past year as the predictor (i.e., independent) variable of interest. To examine the effect of risky drinking behavior on activation to alcohol picture cues, the number of binge drinking episodes in the past year (defined as 4 or more drinks per drinking occasion for females and 5 or more drinks per drinking occasion for males) served as the predictor of interest in the second linear regression model. In both regression models, whole-brain BOLD fMRI response to alcohol vs. neutral picture cues at follow-up served as the outcome (i.e., dependent) variable of interest. The same procedure for multiple comparisons corrections as above was followed (uncorrected $p < .001$, corrected $\alpha = .05$, 11 contiguous voxels).

Results

Participant Characteristics

At project entry, participants were 51 adolescents between 12.3 and 14.9 years of age ($M=13.7$, $SD=0.7$). At follow-up, youth were 18.1 to 21.3 years, ($M=19.4$, $SD=0.7$), an average of 5.7 years ($SD=0.7$) post-baseline (see Table 1). These individuals were primarily from middle class families, with a median Hollingshead Index of Social Position score of 19.9 ($SD=8.7$); however, the sample represented youth from a range of SES backgrounds, with scores between 11 and 53. At follow-up, youth were classified either as moderate or heavy drinkers (see Figure 2), based on their past-year alcohol use frequency and quantity (Cahalan et al. 1969). Thirty-nine (46.2% female) individuals were classified as heavy drinkers, and 12 (33.3% female) as moderate drinkers. On average, participants drank 310.1 standard drinks ($SD=409.6$) in the past year, with a wide range from 13.9 to 1,843.2 drinks ($median=144.0$ drinks). Follow-up past-year total drinking days and binge-drinking days did not differ by sex, family history, or history of early dating.

BOLD Response Contrast Main Effects

Controlling for age and total drinks in the past year, a main effect of time (baseline vs. follow-up) was observed for alcohol vs. neutral picture cues (Table 2) in the left middle occipital gyrus ($Z=3.7$). Youth showed greater activation to alcohol vs. neutral cues at follow-up than at baseline, regardless of dating status, sex, or SUD family history. No main effect of dating, sex, or family history was found.

BOLD Response Interaction Effects

Early-dating History $\times$ Time—Controlling for age and total drinks in the past year, a significant early-dating history $\times$ time interaction effect was found in a cluster that includes the left anterior cingulate and white matter, likely the corpus callosum ($Z=3.82$). At baseline, early-daters showed greater activation to alcohol vs. neutral picture cues and non-early daters showed greater activation to neutral vs. alcohol cues. At follow-up, early-daters showed greater activation to neutral vs. alcohol picture cues and non-early daters showed greater activation to alcohol vs. neutral picture cues. Between baseline and follow-up, early-daters showed a decrease in activation whereas non-early daters showed an increase in activation contrast over time.

Sex $\times$ Time—Controlling for age and total drinks in the past year, a significant sex $\times$ time interaction effect was found in the left middle frontal gyrus ($Z=-3.50$). At baseline, boys showed greater activation to neutral vs. alcohol picture cues and girls showed greater activation to alcohol vs. neutral cues. At follow-up, boys showed greater activation to alcohol vs. neutral picture cues and girls showed greater activation to neutral vs. alcohol picture cues. Between baseline and follow-up, boys showed an increase whereas girls showed a decrease in activation contrast over time.

Family History $\times$ Time—Controlling for age and total drinks in the past year, significant family history by time interaction effects (Figure 3) were found in the right anterior cingulate gyrus ($Z=3.81$) and right middle occipital gyrus ($Z=3.98$). In the right anterior cingulate at baseline, FHP youth showed greater activation to alcohol vs. neutral picture cues and FHN youth showed greater activation to neutral vs. alcohol cues. At follow-up, FHP youth showed greater activation to neutral vs. alcohol picture cues and FHN youth showed greater activation to alcohol vs. neutral cues. Between baseline and follow-up, FHP youth showed a decrease, while FHN showed an increase, in activation contrast over time.

In the right middle occipital gyrus at baseline, FHP youth showed greater activation to alcohol vs. neutral picture cues and FHN youth showed greater activation to neutral vs. alcohol cues. At follow-up, FHP youth showed greater activation to neutral vs. alcohol picture cues and FHN youth showed greater activation to alcohol vs. neutral cues. Between baseline and follow-up, FHP youth showed a decrease, whereas FHN youth showed an increase in activation contrast over time.

BOLD Relationship with Alcohol Use and Age

Follow-up, past-year total drinks consumed and past-year binge drinking episodes were not significantly associated with follow-up BOLD activation. Follow-up age was not

significantly related to BOLD activation or past-year binge drinking episodes and total drinks.

Discussion

This study prospectively examined the effects of three important AUD risk factors on BOLD activation to alcohol picture cues in adolescents: early-dating history, sex, and SUD family history. Importantly, youth were alcohol- and substance-naïve at baseline, and transitioned to moderate to heavy drinkers by follow-up. To our knowledge, this is the first study to examine the longitudinal changes in BOLD activation to alcohol picture cues pre- and post-alcohol initiation in adolescents at risk for AUD.

Controlling for age and total number of drinks in the past year, we expected the effects of each risk factor on cue reactivity to interact with time (i.e., family history $\times$ time, sex $\times$ time, and early-dating history $\times$ time), such that no significant differences in activation would be observed at baseline, prior to any personal alcohol use experience, regardless of family history, sex, and early-dating status. At follow-up, it was hypothesized that youth with AUD risk factors (i.e., FHP, male, and history of early dating) would show deviations in their BOLD activation to alcohol picture cues as compared to their low-risk peers (i.e., FHN, female, and non-early daters). If substantiated, this would suggest that the experience of alcohol consumption changes how adolescents process alcohol-related cues. As expected, we found no main effect of group for family history, sex, and early-dating history, but a significant main effect of time and risk factor by time interactions. These results suggest that the influence of each risk factor on neural processing of appetitive alcohol cues is highly dependent on personal experience with alcohol.

Regions implicated in the current study (i.e., occipital, frontal, and limbic areas) are consistent with prior studies that examined differential alcohol cue-reactivity in adolescent drinkers (Tapert et al. 2003; Dager et al. 2013) and in other addictive behaviors (Noori et al. 2016). Unexpectedly, visual inspection of interaction effects suggested that at baseline, youth with and without risk factors showed pre-existing differences in activation to alcohol cues based on their family history, early-dating history, and sex even prior to any alcohol use. FHP youth showed greater activation at baseline, but lower activation at follow-up in the right cingulate and middle occipital gyri. This suggests that, in response to alcohol-related cues, FHP youth may allocate attention (Bryden et al. 2011) and experience craving responses (Goudriaan et al. 2010) differently than FHN youth even in the absence of differences in drinking behavior. Mechanistically, this deviation in the emotional and visual processing of alcohol picture cues has been proposed to originate in the anterior cingulate of the mesocorticolimbic system, innervating the visual cortex and resulting in differences in the individual saliency of alcohol-related cues (Dager et al. 2013; Hyman et al. 2006; Due et al. 2002). Similarly, at baseline, youth with an early-dating history showed greater activation to alcohol picture cues compared to their counterparts, but exhibited an overall decrease in activation at follow-up, in the left anterior cingulate/white matter region. Interestingly, girls showed greater activation to alcohol than boys at baseline but lower activation at follow-up in the left middle frontal gyrus. One may speculate that inefficiencies in the inhibition of alcohol-seeking behaviors upon exposure to appetitive cues may play an important role in

the divergence of alcohol use rates by males vs. females (Goldstein and Volkow 2011; Johnston et al. 2017).

The examination of three different risk factors in one model with a longitudinal design allowed, for the first time, for comparisons in the directionality and effect strength that each factor exerts on alcohol-cue reactivity simultaneously. Importantly, family history and sex are commonly conceptualized as proxies for genetic vulnerabilities (Chartier et al. 2010), implying temporal precedence, such that differences serve as risk factors for, or leads to, maladaptive alcohol use patterns. In the current study, we found that individuals with a positive family history of AUD and those who have engaged in early dating showed greater activation at baseline but lower activation at follow-up, compared to their counterparts (i.e., FHN and no early-dating history). The opposite pattern was found for males vs. females (i.e., boys showed lower activation at baseline but greater activation at follow-up compared to girls). These results have two important implications. First, they suggest that youth with these risk factors indeed show differences in alcohol-cue reactivity even before engaging in any personal experience with the substance. Further, at-risk youth continue to show differences in alcohol-cue reactivity even after transition. Additionally, the use of a single statistical model allowed for comparison of Z-scores to better understand effect strength. Results suggest that early dating appears to exhibit similar magnitudes of effect on alcohol cue-reactivity as family history and sex. This is an important consideration, as the former has yet to be examined in detail in the literature.

The effects of early dating on alcohol use development have been relatively unexplored area in the literature. While research has highlighted the importance of violent romantic relationships in the escalation of substance use (Howard and Wang 2003; Swahn et al. 2008), little is known about how involvement in non-violent romantic relationships affects the development and trajectory of alcohol use, especially among adolescents. In line with previous research suggesting that early dating may be an important risk factor for later alcohol use (Squeglia et al. 2016; Whelan et al. 2014), we found that youth with a history of early-dating showed differential activation at baseline and follow-up in the left anterior cingulate/white matter region, an area implicated in addictions (Goudriaan et al. 2010). Alcohol consumption and dating in adolescents are intricately related. Having more romantic dating experiences is correlated with having an increased social network (i.e., friendships and romantic relationships) and greater social acceptance among peers (Furman et al. 2009). Further, adolescent alcohol and other substance use behaviors is positively correlated with peer attitudes and use patterns (Bot et al. 2005; Mundt 2011). In particular, youth are more likely to acquire romantic partners with similar drinking habits; further, the frequency of alcohol use among adolescents and their romantic partners are positively correlated (van der Zwaluw et al. 2009). The effects of early dating on adolescent drinking may operate in both direct (e.g., disinhibited behaviors during alcohol use may lead to greater risky sexual behaviors) and indirect ways (e.g., early dating as an indicator of greater sociability and increased likelihood of exposure to peer alcohol use behaviors) (Dermen et al. 1998). Neurologically, it is possible that the effects of early-dating history on alcohol-cue reactivity operates through a third covariate, mediator, or moderator factor. For example, youth who engage in early-dating may also exhibit increased impulsivity and/or reward sensitivity, which leads to or is associated with, differential alcohol-cue reactivity.

Considering the dearth of studies on adolescent early-dating history and fMRI response, this may be an important area of exploration in future studies.

In two of the three predictors examined, youth with an AUD risk factor (i.e., FHP and early dating) consistently showed greater activation to alcohol cues than neutral trials at baseline. On the other hand, boys, who are at greater risk for AUD development, tended to show less activation to alcohol cues compared to girls at baseline but increased activation at follow-up. Although the reasons and mechanisms remain unclear, one may postulate that social influences play a role in the latter finding. Themes of masculinity are often depicted in alcohol television ads (Morgenstern et al. 2015). Further, adolescent boys, compared to their female counterparts, receive greater exposure to such depictions through sports watching (Stacy et al. 2004). It is possible that boys exhibited a lower BOLD response to alcohol cues at baseline in part due to desensitization to alcohol picture cues after repeated exposure. These findings controlled for the effects of age, despite a small range at baseline and follow-up, and past-year alcohol consumption at follow-up. Thus, the results reported here are less likely attributable to differences in brain maturation. Further, while most previous reports of alcohol cue reactivity have examined drinker group differences (e.g., drinkers vs. controls), all participants in the current study were moderate to heavy drinkers at follow-up and analyses controlled for past year drinking quantity. Therefore, differences in BOLD activation observed in this study are interpreted as due to the influence of AUD risk factors, above and beyond drinking status. Overall, results suggest that youth at risk for heavier drinking show deviations in brain activation patterns to alcohol picture cues even when their drinking level is no different from youth without these risk factors.

Several limitations of this study should be noted. First, although alcohol use quantity was statistically controlled for, information about other aspects of alcohol consumption were not assessed and therefore cannot be modeled. For example, while participants reported no substance use at baseline, detailed assessments of exposure to alcohol-related events and stimuli, such as television commercials and exposure to peer alcohol use on social media were not obtained. Likewise, all participants were moderate to heavy drinkers at follow-up, but each individual's rearing and social environment is different, and thus, may differentially affect each participant's perception of alcohol in diverse ways. Second, the sample of adolescents in this study is comprised of healthy, highly functional individuals primarily from middle-class families. No participant met diagnostic criteria for substance use disorder (American Psychiatric Association 1994), thus, it is unclear if and how the results of the current study may be applied to individuals with more severe alcohol use patterns and/or substance use disorders. Results of this study suggest that history of dating prior to age 14 may play an important role in differential cue-reactivity to alcohol pictures. However, few studies have examined this index as a risk factor or as a possible influence of drinking behavior. Nevertheless, an important limitation of the current study was the relatively simplistic definition of this index, such that a history of early dating included youth who endorsed any involvement in dating activity, even if rarely. It is unclear whether there may be differences in cue-reactivity among youth who are engaged in different levels of early dating, a question that was not examined in the current study due to inadequate power. This important nuance in dating involvement may be of interest in future studies.

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Fig. 1. Alcohol cue reactivity task

(a) Portion of the fMRI alcohol cue reactivity task (Pulido et al. 2010) design showing fixation trials, alcohol picture cue, and neutral picture cue and (b) portion of the alcohol cue reactivity task reference function generated by RSFgen in Analysis of Functional NeuroImages software. The first 2 minutes of the task (total duration is 8min, 32s) is represented on the x-axis. The five stimuli types are represented on the y-axis. The horizontal lines indicate the condition and duration of the trials.

Pulido, C., Brown, S. A., Cummins, K., Paulus, M. P., & Tapert, S. F. (2010). Alcohol Cue Reactivity Task Development. *Addictive Behaviors*, 35(2), 84-90, doi:10.1016/j.addbeh.2009.09.006.

		Quantity							
		Average drinks/occasion (past three months):	>4	3-4	3-4	1-2	1-2	1-2	
		Max drinks/occasion (past year):	>4	>4	3-4	>4	3-4	1-2	
Frequency	Daily		Heavy Drinker						
	>8x/month								
	4-8x/month		Moderate Drinker			Control			
	1-3x/month								
	<1x/month								
	<1x/year								

Fig. 2. Alcohol use classification at follow-up

Alcohol use classification criteria based on Cahalan et al. (1969). Frequency and quantity were modified to better reflect adolescent drinking patterns.

Cahalan D, Cisin I, Crossley H. New Brunswick, NJ: Rutgers Center of Alcohol Studies; 1969. *American drinking practices*. Monograph No. 6.

Fig. 3. Mean percent signal change in alcohol vs. neutral picture cues by family history, sex, and dating status at baseline and follow-up

Top: Percent signal change in blood-oxygen-level dependent (BOLD) activation to alcohol vs. neutral picture cues at baseline (BL; prior to alcohol use initiation) and follow-up (FU; after transitioning into moderate to heavy drinking on average 5.7 years later) in youth with negative substance use disorder family history (FHN) and positive substance use disorder family history (FNP). **Bottom left:** BOLD percent signal change to alcohol vs. neutral picture cues at BL and FU in boys and girls. **Bottom right:** BOLD percent signal change to alcohol vs. neutral picture cues at BL and FU in youth who reported history of, or are

currently in, a romantic relationship at BL, by age 14 (dated before 14 years; early dater), and those who have never dated at baseline (have not dated before 14 years; non-early dater). Error bars indicate standard error of BOLD activation to alcohol vs. neutral picture cues.

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Table 1

Sample demographics at baseline and follow-up ($N=51$)

Baseline (project entry)	<i>M (SD) or %</i>
Age	13.7 (0.7)
% Male	56.9%
% FHP	54.9%
% Early daters	58.8%
Race (% White)	78.4%
Grades completed	7.0 (0.9)
Grade point average	3.6 (0.4)
Conduct disorder positive	4.0%
CBCL Externalizing T-score	41.3 (7.8)
CBCL Internalizing T-score	41.4 (6.8)
Mother's annual salary (in thousands of dollars)	42.2 (35.7); <i>Range</i> : 0.0–140.0
Father's annual salary (in thousands of dollars)	43.6 (12.4); <i>Range</i> : 30.0–77.0
Mother's educational attainment (years)	16.2 (1.8); <i>Range</i> : 12.0–20.0
Father's educational attainment (years)	16.5 (2.1); <i>Range</i> : 12.0–20.0
Follow-up	
Age	19.5 (0.7)
Years after baseline	5.7 (0.7)
Grades completed	12.5 (0.9)
Grade point average	3.4 (0.8)
Hollingshead Index Score	19.9 (8.7)
Conduct disorder positive	4.0%
ASR Externalizing T-score ^a	49.0 (10.2)
ASR Internalizing T-score ^a	43.6 (12.4)
Past year drinking days	57.2 (56.7); <i>Range</i> : 6.9–244.0
Past year binge drinking days	25.1 (35.6); <i>Range</i> : 0.0–200.0
Past year average drinks per 24 hours	4.6 (2.5); <i>Range</i> : 1.0–15.0
Past year peak drinks per 24 hours	9.2 (5.0); <i>Range</i> : 3.0–34.0

At baseline, youth were substance naïve; at follow-up, youth have begun alcohol use.

^aAt follow-up, four adolescents had an Externalizing and Internalizing T-score 65 (one additional participant had only Internalizing T-score 65). No individual had Externalizing or Internalizing T -scores 65 at baseline.

CBCL = Child Behavior Checklist; ASR = Adult Self Report

Regions of significant BOLD response contrast to alcohol cues relative to neutral cues in linear mixed effect models.

c₁ Family history of substance use disorder