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# Sex differences in the development of emotion circuitry in adolescents at risk for substance abuse: a longitudinal fMRI study

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## Abstract

There is substantial evidence for behavioral sex differences in risk trajectories for alcohol and substance use, with internalizing factors such as negative affectivity contributing more to female risk. Because the neural development of emotion circuitry varies between males and females across adolescence, it represents a potential mechanism by which underlying neurobiology contributes to risk for substance use. Longitudinal functional magnetic resonance imaging was conducted in males and females ($n = 18$ each) with a family history of alcohol use disorders starting at ages 8–13 years. Participants performed an affective word task during functional magnetic resonance imaging at 1- to 2-year intervals, covering the age range of 8.5–17.6 years (3–4 scans per participant). Significant age-related sex differences were found in the right amygdala and right precentral gyrus for the negative *vs* neutral word condition. Males showed a significant decrease in both amygdala and precentral gyrus activation with age, whereas the response in females persisted. The subjective experience of internalizing symptomatology significantly increased with age for females but not for males. Taken together, these results reveal sex differences in negative affect processing in at-risk adolescents, and offer longitudinal neural evidence for female substance use risk through internalizing pathways.

**Key words:** fMRI; emotion; amygdala; substance use; sex differences

## Introduction

Adolescence is the developmental period during which many psychopathologies begin to emerge, and they do so in a sex-specific manner. Emotional development in particular is sexually divergent; female adolescents are twice as likely as males to experience depression (Angold *et al.*, 1998; Hankin *et al.*, 1998; Twenge and Nolen-Hoeksema, 2002; Hilt *et al.*, 2011) and anxiety (for review, see Albano and Krain, 2005), which are highly comorbid with one another (Cummings *et al.*, 2014). Both depression and anxiety are characterized by the same underlying core psychopathological process called internalizing, or the tendency to direct feelings or emotional states inward (Achenbach and Edelbrock, 1981; Caspi *et al.*, 2014). Factors or vulnerabilities contributing to internalizing disorders in females, such as negative affectivity, emerge or intensify during adolescence (Hyde *et al.*, 2008; Cummings *et al.*, 2014).

Adolescence is also a time when alcohol and drug use typically begins, making it an important interval in the etiology of

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alcohol (AUD) and other substance use disorders (SUD). Adolescent SUD and internalizing disorders are comorbid in 9–48% of findings from community and clinical samples, but tend to be higher for females than males (O'Neil *et al.*, 2011, for review). Several psychosocial risk factors associated with gender differences in AUD vulnerability, such as coping styles and drinking motives/expectancies, demonstrate how this comorbidity may arise (Nolen-Hoeksema, 2004, 2012). For example, women are more likely than men to drink heavily when experiencing psychological distress, unpleasant emotions, conflict with others, or to alleviate internal stress or tension (Mooney *et al.*, 1987; Lex, 1991; Rubonis *et al.*, 1994; Annis and Graham, 1995; Lau-Barraco *et al.*, 2009; Choi and DiNitto, 2011). Females with AUD report using alcohol as a way to change negative mood compared to female social drinkers (Olenick and Chalmers, 1991), whereas males expect more positive effects from drinking (Mooney *et al.*, 1987; Berger and Adesso, 1991; Satre and Knight, 2001). Therefore, it is not surprising that women more often report having developed depression before they developed an AUD, whereas men more often report developing an AUD prior to depression (Sannibale and Hall, 2001). Together, this evidence suggests a sexually divergent behavioral profile regarding risk for substance use and later AUD and SUD, one that emerges in adolescence and emphasizes internalizing in females.

The link between internalizing behaviors and substance use in adolescence has received less attention than externalizing, despite evidence of a strong comorbidity in youth and adults (Kessler *et al.*, 1996; O'Neil *et al.*, 2010). In particular, the neural mechanisms underlying the contribution of internalizing to *risk* for later substance use and AUD/SUD is not well understood. Several theories suggest that females are more emotionally reactive and report greater emotional intensity to negative events as they appraise them as being more stressful than males do (Rudolph and Hammen, 1999; Ge *et al.*, 2001; Hyde *et al.*, 2008). A number of neuroimaging studies of healthy subjects have identified key brain structures involved in affect processing, specifically the amygdala (Lindquist *et al.*, 2012, for review). The amygdala plays a central role in processing stimuli that are salient and/or emotionally impactful, such as negative emotional stimuli. In individuals with internalizing disorders, such as anxiety (Duval *et al.*, 2015, for review) and depression (Rive *et al.*, 2013, for review), amygdala hyper-activation has been consistently observed during tasks that involve emotion reactivity or the modulation of affect. This stems from decreased inhibitory control of the amygdala and other emotion processing areas by prefrontal regions in response to negative affect (Johnstone *et al.*, 2007; Milad and Rauch, 2007; Motzkin *et al.*, 2015). The prefrontal cortex is one of the last brain regions to fully mature, demonstrating functional and anatomical changes that continue into early adulthood (Giedd *et al.*, 1999; Gogtay *et al.*, 2004). In contrast, subcortical regions such as the amygdala reach maturity much earlier, usually by early adolescence (Giedd *et al.*, 1996; Ostby *et al.*, 2009; Wierenga *et al.*, 2014). This maturational difference creates an imbalance between subcortical and cortical circuitry proposed to underlie a number of stereotypical adolescent behaviors (Casey *et al.*, 2008; Steinberg, 2008; Somerville *et al.*, 2010; Mills *et al.*, 2014), including heightened emotional reactivity (Hare *et al.*, 2008; Perlman and Pelphrey, 2011; McRae *et al.*, 2012; Gee *et al.*, 2013; Spear, 2013). In healthy adolescents, development of the amygdala impacts affective processing in a sex-specific manner (Blanton *et al.*, 2010; Alarcón *et al.*, 2015), and sex differences in the vulnerability to anxiety and depression can also arise from the maturational imbalance between subcortical and cortical regions (Zahn-Waxler *et al.*, 2008; Blanton *et al.*, 2010; Burghy *et al.*, 2012). Together, these factors potentially leave females more vulnerable for substance use and later SUD. Further, the development of emotion circuitry, specifically the amygdala, represents a potential mechanism by which underlying neurobiology contributes to risk for substance use in females.

Here we focus on brain responses to emotional stimuli, specifically the passive viewing of affective words (Heitzeg *et al.*, 2008; Glaser *et al.*, 2014), using functional magnetic resonance imaging (fMRI). Our prior work demonstrates that brain activation during this task in 17- to 21-year-olds differentiates resilient and vulnerable adolescents who are at risk (Heitzeg *et al.*, 2008) and is associated with later alcohol consumption, an effect mediated by negative affectivity (Glaser *et al.*, 2014). In this study, we investigate the developmental trajectory of this circuitry using longitudinal data collected at 1- to 2-year intervals starting at ages 8–13 and covering the range of 8–17 years. Participants were male and female youth with a family history of AUD, which is a significant risk factor for later AUD and SUD. We hypothesize that, across development, at-risk females, but not males, will show increasing activation in neural regions known to be associated with affective processing, such as the amygdala. They will also score higher on subjective measures of internalizing behavior with increasing age, compared to at-risk males.

## Methods

### Participants

Thirty-six right-handed subjects (18 females) aged 8–13 years at baseline participated in the study. Participants were recruited from the Michigan Longitudinal Study, an ongoing, prospective study of families with high levels of parental AUD and a contrast sample of non-alcoholic families (Zucker *et al.*, 1996, 2000). Parental AUD diagnosis was based on DSM-V criteria, and assessed by way of the Diagnostic Interview Schedule–Version 4 (Robins *et al.*, 1981, 2000), supplemented with the Drinking and Drug History (Zucker and Fitzgerald, 1994). All participants in this study came from families where at least one parent had an AUD diagnosis during the child's lifetime. Exclusionary criteria for this study included: neurological, acute, uncorrected, or chronic medical illness; current or recent (within 6 months) treatment with centrally active medications; and history of psychosis or schizophrenia in first-degree relatives. The presence of Axis I psychiatric or developmental disorders that would interfere with the interpretation of the data was also exclusionary; this did not include past history of mood disorder or current unmedicated mood disorder, or current or past history of conduct disorders or attention deficit disorder. Diagnosis was determined using the Diagnostic Interview Schedule–Child (Costello *et al.*, 1984). As part of the Michigan Longitudinal Study, all offspring were assessed annually on substance use and related problems (Zucker *et al.*, 1996; also see Supplementary Data). All participants were told to abstain from alcohol and illicit substances/recreational drugs for 48 h prior to the fMRI scan. For participants aged 15 and older, urine drug screens were conducted immediately prior to the fMRI scan; positive results were exclusionary. In participants aged 14 and younger, we relied on verbal confirmation of drug and alcohol abstinence on the day of the scan. No participants had to be excluded from this study due to a positive drug screen or affirmative self-report of alcohol or drug use. All participants gave

**Table 1.** Participant characteristics

| | All Participants | Female | Male |
|---|---|---|---|
| Total participant, *N* | 36 | 18 | 18 |
| Participants with two scans | 36 | 18 | 18 |
| Participants with three scans | 24 | 12 | 12 |
| Participants with four scans | 16 | 8 | 8 |
| Total scans | 112 | 56 | 56 |
| Age at scan (mean ± s.d.) in years | | | |
| All scans | 12.8 ±2.3 | 12.8 ±2.5 | 12.8 ±2.1 |
| Time 1 | 10.5 ±1.1 | 10.4 ±1.2 | 10.7 ±1.0 |
| Time 2 | 12.7 ±1.3 | 12.7 ±1.5 | 12.7 ±1.1 |
| Time 3 | 14.4 ±1.4 | 14.4 ±1.8 | 14.4 ±1.0 |
| Time 4 | 15.9 ±1.2 | 16.1 ±1.3 | 16.0 ±1.1 |
| Age range | 8.5–17.6 | 8.5–17.6 | 9.1–17.0 |
| IQ (mean ± s.d.) | 104.6 ±14.4 | 105.8 ±16.4 | 103.4 ±12.7 |
| ADHD diagnosis, N[a] | 2 | 1 | 1 |
| Conduct disorder diagnosis, *N* | 0 | 0 | 0 |
| Generalized anxiety diagnosis, N[b] | 2 | 1 | 1 |
| Depression diagnosis, N[b] | 1 | 1 | 0 |
| Alcohol/drug use, *N* | | | |
| Baseline (Time 1) | | | |
| Alcohol use | 2 | 0 | 2 |
| Marijuana use | 3 | 0 | 3 |
| Illicit drug use | 5 | 1 | 4 |
| Total subjects reporting any use at Time 1 | 5 | 1 | 4 |
| Follow-up scans (Times 2, 3 or 4) | | | |
| Alcohol use | 8 | 4 | 4 |
| Marijuana use | 6 | 2 | 4 |
| Illicit drug use | 1 | 0 | 1 |
| Total subjects reporting any use at Times 2, 3 or 4 | 8 | 4 | 4 |

[a]Of the two participants with ADHD diagnoses, neither were on medication for ADHD during study participation.
[b]Diagnosis for generalized anxiety and depression is missing for one subject.

written consent/assent after explanation of the experimental protocol, as approved by the local institutional review board. As participants were under the age of 18, at least one parent gave written informed consent. Table 1 contains subject information.

All participants had at least two fMRI scans ($n = 36$); 24 participants had three fMRI scans ($n = 24$); 16 participants had four fMRI scans ($n = 16$) (Table 1). Thus, each participant had either 2, 3 or 4 observations; each subsequent set of observations was always a subset of the participants from the previous time point. These will be referred to as Time 1 (first fMRI scan), Time 2 (second fMRI scan), Time 3 (third fMRI scan) and Time 4 (fourth fMRI scan) for the remainder of this manuscript; all scans together will be referred to as time points.

## Measures

***Youth Self-Report.*** Emotional and behavioral problems were assessed annually as part of the MLS protocol, using the Youth Self-Report (YSR), designed to be utilized in adolescents (Achenbach, 1991). The YSR assesses problem behaviors along two broadband scales of internalizing and externalizing that include anxiety/depression, withdrawal and somatic complaints (internalizing), as well as aggression and rule-breaking behaviors (externalizing). YSR assessments closest to each participant's fMRI scan were used. Externalizing was included as it has previously been linked to alcohol use problems in males (Kendler *et al.*, 2015).

***fMRI task.*** Emotion arousal while in the scanner was probed using an affective word task (Heitzeg *et al.*, 2008; Hsu *et al.*, 2010; Mickey *et al.*, 2011; Glaser *et al.*, 2014). Previous neuroimaging studies have demonstrated that emotional words are able to elicit comparable neural responses, particularly in the amygdala, to pictures and faces (Kissler *et al.*, 2006; Citron, 2012). Words were selected from the Affective Norms for English Words (Bradley and Lang, 1999) which provides norm ratings for two separate scales, emotional valence and arousal, on a scale of 1 (negative valence, low arousal) to 9 (positive valence, high arousal). The following criteria were used to select 36 words (12 words for each emotional category): negative (valence rating $<3$), neutral ($4.5 <$ valence rating $> 5.5$) and positive (valence rating $>7$). Negative and positive words had an arousal rating $>5$, whereas the arousal rating for neutral words was $<2$.

Words were presented one at a time in a blocked design. Each block consisted of six trials. Each trial started with a 3 s word presentation followed by a crosshair that lasted 1 s. Participants were instructed to press a button while the word was on the screen indicating that they understood the word. Following each task block, participants viewed a blank screen for 18 s. Six task blocks and six rest blocks were included in each run, and each condition was presented twice. The order of presented conditions was counterbalanced using the Latin Squares Design. The entire experiment consisted of three runs in total, which lasted 12 min and 36 s.

Following the scan, participants completed a questionnaire containing 54 words, of which 36 were from the task. Equal numbers of words were included across conditions (negative,

neutral and positive). For each word, participants were asked to identify whether they remembered seeing it in the scanner and rate how pleasant (valence) on a 9-point scale they found the word to be (0 = not pleasant; 9 = pleasant). In order to avoid confounding brain activation, valence ratings were collected after—as opposed to during—the scan. Evidence suggests that evaluation of stimuli with affective qualities alters emotional reactions and brain activation when compared to passive viewing (Taylor *et al.*, 2003).

## MRI data acquisition

Whole-brain blood oxygenated level-dependent (BOLD) images were acquired on a 3.0 Tesla GE Signa scanner (Milwaukee, WI, USA) using a T2*-weighted single-shot combined spiral in-out sequence (Glover and Law, 2001) with the following parameters: repetition time = 2000 ms; time to echo = 30 ms; flip angle = 90°; field of view = 200 mm; 64×64 matrix; in-plane resolution = 3.12×3.12 mm; and slice thickness = 4 mm. The entire volume of 29 axial slices was acquired every 2 s. A high-resolution anatomical T1 scan was obtained for spatial normalization (three-dimensional spoiled gradient-recalled echo, repetition time = 25 ms; minimum time to echo; field of view = 25 cm; 256×256 matrix, slice thickness = 1.4 mm). Participant motion was minimized using foam pads placed around the head along with a forehead strap.

## Data analysis

***Behavioral data.*** For post-scan questionnaire performance, values that fell ±3 s.d. outside of the mean were removed (see Supplementary Data for details). Mean hit rate for each condition (positive, negative, neutral words) was calculated across all time points, and then for each time point individually. This was used to calculate recognition memory performance (below). In SPSS (Version 19.0, IBM Corp., Armonk, NY), linear mixed model analyses were used to investigate sex differences across age for recognition memory performance and memory bias. Recognition memory is the ability to correctly recognize a recently encountered item, and accounts for the number of incorrect words participants say they saw. Recognition memory performance ($p'$) was calculated using the formula $p' = (p - fp)/(1 - fp)$ where ($p$) is the percentage of correct responses to target stimuli and ($fp$) is the percentage of incorrect responses to distractor stimuli (Epstein *et al.*, 2006). Memory bias takes neutral word performance into account, and can compare recognition memory for positive relative to negative words. Negative memory bias was calculated by subtracting recognition memory performance to neutral words from that of negative words (Glaser *et al.*, 2014); a lower score indicates that negative words did not differ much from neutral words. Positive memory bias was calculated by subtracting recognition memory performance to neutral words from that of positive words (Glaser *et al.*, 2014), and a lower score indicates positive words did not differ much from neutral words. Research indicates that emotional memories are stronger for women (Hamann and Canli, 2004), and emotional memory is correlated with amygdala activity in females and males (Stevens and Hamann, 2012). Linear mixed models were run on the following variables to investigate sex differences across age: (i) positive, negative and neutral performance measures for recognition memory performance, and (ii) positive and negative memory bias. Each measure was entered *individually* as a dependent variable, with sex as a factor and age mean-centered as a fixed-effect covariate. Time point (1, 2,. . ., 4) was included as a repeated effect and subject was entered as a random factor. Schwarz's Bayesian information criteria (BIC; Schwarz, 1978) was used to determine the best-fitting covariance structure (autoregressive–first order; all longitudinal models used BIC to determine best fit), and models were fit using maximum likelihood estimation method. Models were also run for each sex separately to determine individual group contributions to significance. Independent samples *t*-tests were run at each time point for each performance variable to determine whether differences existed between males and females. For analysis details regarding valence ratings, see Supplementary Data.

***Youth Self-Report.*** An independent samples *t*-test was used to determine whether group differences existed at baseline (Time 1) and at Time 4. To investigate sex differences across age, each YSR scale was entered individually as a dependent variable into the linear mixed model, with sex as a factor and age mean-centered as a covariate. Restricted maximum likelihood (REML) was the estimation method, and the covariance structure was ante-dependence first order. Models were also run separately for each sex.

***fMRI preprocessing.*** An iterative algorithm was used to reconstruct functional images (Sutton *et al.*, 2003; Noll *et al.*, 2005). Subject head motion was corrected using FSL 5.0.2.2 (Analysis Group, FMRIB, Oxford, UK) (Jenkinson *et al.*, 2002). Analysis of estimated motion parameters confirmed that overall head motion within each run did not exceed 3 mm translation or 3° rotation in any direction (Table S9). All remaining image processing (including slice timing correction) and statistical analysis were completed using statistical parametric mapping (SPM8; Wellcome Institute of Cognitive Neurology, London, UK). Functional images were spatially normalized to a standard stereotaxic space as defined by the Montreal Neurological Institute. A 6 mm full-width half-maximum Gaussian spatial smoothing kernel was applied to improve signal-to-noise ratio and to account for differences in anatomy.

Individual analysis was completed using a general linear model. Four regressors (positive, negative, and neutral words, rest) were convolved with the canonical hemodynamic response function, with event durations of 4 s from stimulus presentation. Motion parameters were modeled as nuisance regressors to remove residual motion artifacts. The main contrasts of interest were: (i) Positive *vs* Neutral words; and (ii) Negative *vs* Neutral words. These two contrasts will be referred to as POS*vs*NEU and NEG*vs*NEU, respectively. These were calculated by linearly combining parameter estimates over all three runs of the task.

***Task effect.*** Task effect was determined for all participants across all scans using a one-sample *t*-test. Areas of activation were deemed significant if they reached a cluster-level false discovery rate (FDR)-corrected threshold of $P < 0.05$ in the NEG*vs*NEU and POS*vs*NEU contrasts.

***Longitudinal analysis.*** Differences in linear developmental trajectories between groups were tested using a second-level multiple regression analysis in Matlab (version R2010a, The MathWorks, Inc., Natick, MA, USA) and SPM. The participant-specific intercept terms and age regressors (linear age, quadratic age, and cubic age) were specified by a script written in Matlab. Age regressors were first constructed across all participants, and then Gram-Schmidt orthogonalization was performed.

Participants were then split into two groups: males and females. The multiple regression model was then built in SPM with these calculated subject-specific intercept terms and the group-specific predictors as a second-level analysis, as in Hardee *et al.* (2014). Contrasts were calculated to examine sex differences as a function of age, with linear age effect differences being the primary focus.

Missing data were handled by utilizing all observations in the calculation of each age effect by group, such that individuals with more time points contributed more data to the estimation of the group-age slope. Notably, the missing data were distributed equally across groups so there was no number of observations by group bias (Laird, 1988). As we did not note that the (unobserved) fMRI response in missing subjects predicted their chance of not having four data points, we are operating with 'Missing Completely at Random' or at most 'Missing at Random' assumptions, and are so justified in using all available data.

Differences between groups for linear age effects were viewed at $P = 0.005$, and regions were deemed significant if whole-brain activation reached a cluster-level FDR-corrected statistical threshold of $P < 0.05$. The amygdala was selected as an *a priori* region of interest (ROI) due to extensive literature demonstrating its role in affect processing; therefore, amygdala activation was deemed significant if it reached a whole-brain statistical threshold of $P < 0.001$, uncorrected. Values from significant clusters were extracted using MarsBaR Region of Interest toolbox (Brett *et al.*, 2002).

Extracted values from the amygdala and precentral gyrus for contrasts of interest were imported into SPSS to quantify interaction effects, *post hoc*. Linear mixed model analyses were conducted for each sex (male, female), using time point as a repeated measure, subject as a random factor and mean-centered age as a fixed-effect covariate. REML estimation was used, and the covariance structure was first-order autoregressive. Next, correlations between the amygdala and YSR internalizing broadband and subscale scores and the amygdala and negative memory bias were examined to determine whether brain activation was linked to internalizing measures and/or played a role in remembering negative words. Each measure (YSR variables, negative memory bias) was investigated independently using separate mixed model analyses with these measures as the dependent variable, extracted amygdala values as a fixed-effect covariate, compound symmetry as a covariance structure, and REML as an estimation method. These analyses were repeated to investigate the effect of sex and brain on YSR measures, this time using each measure (YSR, negative memory bias) as the dependent variable, and compound symmetry as the covariance structure.

# Results

## Participant characteristics

There were no significant differences between groups for IQ, $t(31)=0.47$, $P = 0.64$; overall age, $t(110)=-0.13$, $P = 0.90$; or age at Time 1, $t(34)=-0.81$, $P = 0.42$; Time 2, $t(34)=-0.05$, $P = 0.96$; Time 3, $t(22)=-0.08$, $P = 0.93$; or Time 4, $t(14)=0.70$, $P = 0.50$. Five participants reported substance use (alcohol, marijuana or any illicit drug) at Time 1. In the follow-up scans (Times 2, 3 or 4), an additional eight participants reported substance use at one time point minimally. Table 1 contains subject details (number of scans, age, IQ, diagnoses, substance use information). For detailed information regarding substance use, see Supplementary Data (Tables S1 and S2).

## Behavioral data

*Recognition memory*. There were no significant age-by-gender interaction effects for recognition memory performance; there was a significant main effect of age for negative words [$F(1, 37.39)=5.50$, $P = 0.02$], and a trend toward significance for positive words for age [$F(1, 84.56)=3.72$, $P = 0.06$]; there was no significant effect for age or gender for neutral recognition memory performance. See Table S3 for recognition memory performance means broken down by sex and time point.

*Memory bias*. For negative memory bias, a significant interaction was found [$F(1, 82.43)=4.28$, $P = 0.04$]; males significantly increased with age [$F(1, 39.54)=5.92$, $P = 0.02$] whereas females did not [$F(1, 38.33)=0.12$, $P = 0.74$]. However, there was a significant group difference for negative memory bias at Time 1, $t(32)=2.69$, $P = 0.01$, with females (mean at Time $1 = 0.15 \pm 0.20$) having a greater bias for remembering negative words than males (mean at Time $1 = -0.02 \pm 0.17$) (Table S4). For positive memory bias, there was a main effect of age [$F(1, 83.91)=6.07$, $P = 0.02$], where bias increased with age (see Table S4 for means). Valence rating results can be found in Supplementary Data (Table S5).

## Youth Self-Report

There were no significant differences in YSR scores between groups at baseline (Time 1). By Time 4, however, females had higher internalizing scores, $t(14)=2.14$, $P = 0.05$. Subscale scores, disaggregated components of the internalizing broadband score, showed trend level differences on the anxious, $t(14)=1.94$, $P = 0.07$ and withdrawn subscales, $t(14)=1.90$, $P = 0.08$ (Supplementary Data, Table S6). Linear mixed model analyses revealed significant interactions between sex and age for internalizing; again subscale analyses indicated a significant interaction for anxious and a trend toward significance for withdrawn subscales (Table 2A). *Post hoc* analyses (Figure 1) revealed a significant increase with age in females for internalizing, driven by anxious and withdrawn subscales (Table 2B). Males, on the other hand, showed a significant decrease in internalizing, driven by a decrease in anxious scores with age (Table 2B). There were no significant differences between groups for externalizing scores with age (Table 2A) or at Times 1 or 4 (Table S6).

## fMRI results

Task effect results can be found in Supplementary Data.

***Age-by-sex longitudinal effects.*** For the POS*vs*NEU contrast, no regions passed the corrected level of significance for the whole-brain analysis. For the NEG*vs*NEU contrast, a significant age-by-sex interaction was found in the right precentral gyrus (centered at $x = 38$, $y = -12$, $z = 56$; $k = 966$, $t = 3.94$), encompassing portions of the right postcentral gyrus and the right superior parietal lobule (Figure 2A, left). For the *a priori* amygdala, a significant group-by-age interaction was centered at $x = 26$, $y = 0$, $z = -16$; $k = 67$, $t = 3.94$ in the whole-brain analysis (Figure 2A, right) for NEG*vs*NEU. A small volume correction was applied to the amygdala using an anatomical ROI [defined using the AAL set of templates in PickAtlas (Maldjian *et al.*, 2003)] to determine whether the interaction passed voxel-wise statistics at $P < 0.05$, corrected. Small volume correction revealed a voxel-wise significance of $P = 0.004$ (corrected).

When quantifying the significance of this effect for each sex in SPSS, *post hoc* linear mixed model analyses revealed a significant decrease in the right precentral gyrus activation with age

in males [$F(1, 43.56)=4.53$, $P=0.04$], with females showing a non-significant increase in activation with age [$F(1, 42.04)=2.43$, $P=0.13$] (Figure 2B, left). In the right amygdala, there was also a significant decrease in activation with age in males [$F(1, 43.38)=9.64$, $P=0.003$], again with females showing a non-significant increase in activation with age [$F(1, 40.24)=1.35$, $P=0.25$] (Figure 2B, right). The contribution of each component (Negative *vs* Rest, Neutral *vs* Rest) by sex can be found in Supplementary Data.

This linear mixed model was repeated after the 13 participants reporting any substance use were removed (eight males, five females; Table S1). The significant interaction remained for both the amygdala [$F(1, 47.09)=4.84$, $P=0.03$] and the precentral gyrus [$F(1, 50.25)=5.64$, $P=0.02$]. *Post hoc* analyses showed trends toward significance in decreased activation with age for males in the amygdala [$F(1, 23.47)=3.88$, $P=0.06$] and precentral gyrus [$F(1, 24.80)=3.72$, $P=0.06$], and non-significant increases with age for females in both the amygdala [$F(1, 23.58)=1.54$, $P=0.23$] and precentral gyrus [$F(1, 25.62)=2.84$, $P=0.13$].

***Group differences at Time 1.*** In SPSS, an independent samples *t*-test revealed a significant group difference for NEG*vs*NEU at Time 1 for both the amygdala, $t(34)=-2.16$, $P=0.038$, and the precentral gyrus, $t(34)=-2.55$, $P=0.02$. For each ROI, males had greater mean activation at Time 1 (amygdala: $0.37 \pm 0.75$; precentral: $0.05 \pm 0.32$) than females (amygdala: $-0.35 \pm 1.20$; precentral: $-0.43 \pm 0.72$). At Time 4, there was a trend toward significance for the amygdala, $t(14)=1.72$, $P=0.07$, and the precentral gyrus, $t(14)=1.88$, $P=0.08$, with females having greater mean activation at Time 4 (amygdala: $0.12 \pm 0.49$; precentral: $0.08 \pm 0.35$) than males (amygdala: $-0.45 \pm 0.67$; precentral: $-0.42 \pm 0.66$). This was repeated after the five participants reporting any substance use prior to Time 1 were removed, and the significant group difference still remained [amygdala, $t(21)=-2.18$, $P=0.04$; precentral gyrus, $t(21)=-2.64$, $P=0.02$], with males having greater mean activation at Time 1 for each ROI (amygdala: $0.53 \pm 0.79$; precentral: $0.12 \pm 0.33$) than females (amygdala: $-0.33 \pm 1.02$; precentral: $-0.52 \pm 0.72$).

**Table 2.** YSR linear mixed model results

| YSR measure | A | | B | | | |
|---|---|---|---|---|---|---|
| | Interaction | | Male | | Female | |
| | *F* | *P*-value | *F* | *P*-value | *F* | *P*-value |
| Internalizing problems* | 8.84 | 0.01* | 5.25 | 0.03* | 8.17 | 0.01* |
| Anxious behavior* | 6.66 | 0.01* | 6.70 | 0.01* | 4.86 | 0.04* |
| Somatic complaints | 2.85 | 0.10 | | | | |
| Withdrawn† | 3.65 | 0.06† | 0.95 | 0.34 | 5.62 | 0.03† |
| Externalizing problems | 1.26 | 0.27 | | | | |
| Aggressive behavior | 1.25 | 0.27 | | | | |
| Rule-breaking behavior | 1.97 | 0.17 | | | | |

In A, * indicates significance at $P<0.05$; † indicates a trend toward significance.
In B, * indicates which group(s) drove significance in A, † indicates which group drove trend in A.

***Brain and behavior associations.*** *YSR.* There was a trend towards a significant association between amygdala activation and subjective experience of anxiety problems [$F(1, 94.03)=3.45$, $P=0.07$], where increased amygdala activation was associated with higher anxiety scores. This was the only YSR scale to have a significant association with the amygdala.

*Negative memory bias.* There was no significant association between amygdala activation and negative memory bias [$F(1, 23.39)=0.05$, $P=0.82$].

## Discussion

This longitudinal study examined sex differences in the neural development of children and adolescents at risk for problem substance use in response to a negative word task. Here we found that activation in the right amygdala and right precentral gyrus significantly decreased across age in males, whereas in females activation remained steady.

The amygdala is involved in the detection and processing of emotion, particularly negative emotion (for review, see Costafreda *et al.*, 2008; Garcia-Garcia *et al.*, 2016), and augments the neural representation of emotional stimuli based on current needs, goals and values (Cunningham and Brosch, 2012). The amygdala also evaluates the subjective salience and relevance of stimuli (Adolphs, 2010; Pessoa and Adolphs, 2010), particularly social and emotional stimuli. Throughout adolescence, the salience and relevance of these stimuli can change, reflected in amygdala activation increases and decreases (Scherf *et al.*, 2013). The precentral gyrus, on the other hand, is primarily a

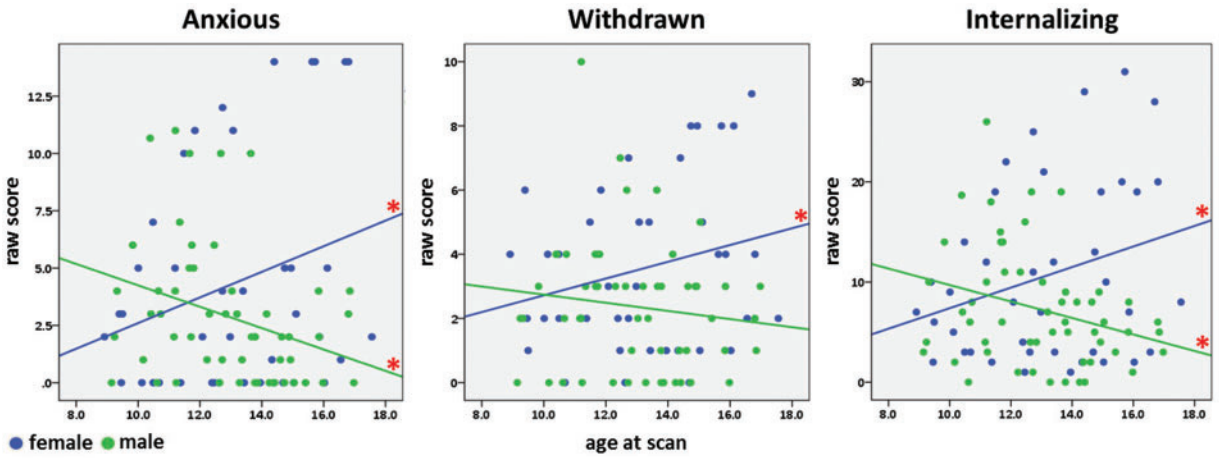


**Fig. 1.** Significant YSR scores. Longitudinal linear age effects are depicted in the scatterplots for anxious and withdrawn raw scores, subscales which make up the broader internalizing raw score. Female scores (blue) significantly increase across age for anxious, withdrawn, and internalizing. Male scores (green) significantly decrease across age for anxious and internalizing. Red asterisks indicate $P<0.05$.

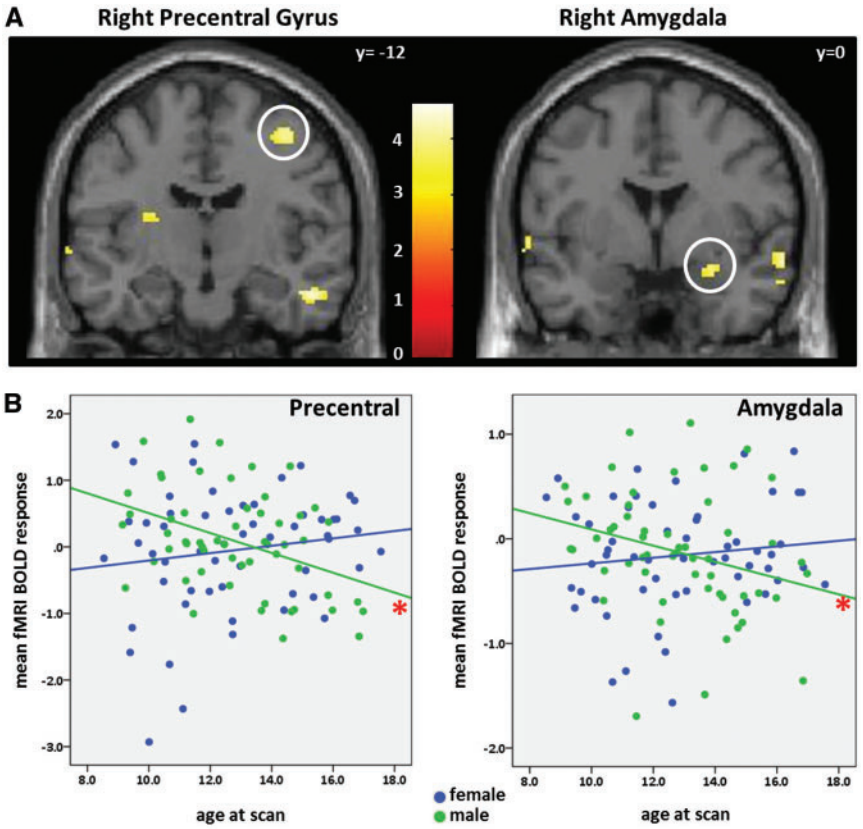


**Fig. 2.** Negative *vs* neutral words. (A) This contrast revealed a significant group difference for linear age in the whole-brain longitudinal analysis in the right precentral gyrus at a statistical threshold of $P < 0.05$, FDR-corrected (top left, white circle). The amygdala was chosen as an *a priori* region of interest, and passed a pre-selected threshold of $P < 0.001$, uncorrected (top right, white circle). Color bar represents *t*-values and the *y*-coordinate is in MNI space. (B) Extracted values from the precentral gyrus and amygdala, depicted in scatterplots with mean fMRI activation on the *y*-axis and age at scan on the *x*-axis. All time points are depicted. Males (green) show a significant decrease in right precentral and right amygdala activation with age, whereas females (blue) show a persistence of activation with age. Red asterisks indicate $P < 0.05$.

motor region but consistently activates during emotion perception and experience (de Gelder *et al.*, 2004; Hajcak *et al.*, 2007); damage to this area can cause emotion recognition deficits (Adolphs *et al.*, 2000). Both the precentral and postcentral gyrus are part of the core brain network involved in discriminating between the basic emotions (Saarimaki *et al.*, 2015). The pre-supplementary motor area, located on a portion of the precentral gyrus, in particular is involved in processing emotional words presented both visually and verbally (Beauregard *et al.*, 1997; Isenberg *et al.*, 1999; Crosson *et al.*, 2002; Kensinger and Schacter, 2006; Warren *et al.*, 2006; Hinojosa *et al.*, 2014). In general, the motor cortices in the precentral gyrus seemingly play a significant role in motor preparation related to emotion (Frijda 1986; Mazzola *et al.*, 2013).

Although previous studies have demonstrated co-activation and functional connectivity between amygdala and cortical motor-related regions during the perception of emotional expressions (de Gelder *et al.*, 2004; Grosbras and Paus, 2006; Pichon *et al.*, 2008, 2009; Van den Stock *et al.*, 2011; Conty *et al.*, 2012; Grezes *et al.*, 2013), a recent diffusion tensor imaging study provides evidence for a direct pathway between the amygdala and motor cortices, including the precentral gyrus, formerly illustrated only in cats, monkeys and rats (Grezes *et al.*, 2014). The authors suggest that ipsilateral connections from the amygdala to these motor regions provide a direct mechanism by which the amygdala could influence, rather than trigger, complex and subtle motor behaviors. Here, the amygdala and precentral gyrus exhibit similar BOLD patterns to one another across development in at-risk males (significant decrease in activation) and in females (sustained activation), suggesting this limbic-motor interaction is sex dependent in the context of affect processing. These differences in affective processing relative to family history could be one factor that leads to sex differences in substance use risk in adolescents.

Amygdala responsiveness is greater in healthy adolescents than in healthy adults, indicating that amygdala activation should decrease with age (Baird *et al.*, 1999; Monk *et al.*, 2003; Guyer *et al.*, 2008; Pfeifer *et al.*, 2011; Somerville *et al.*, 2011), in agreement with what at-risk adolescent males exhibit here. The sustained activation in at-risk females across adolescence, on the other hand, must be interpreted with caution; one possible explanation is that this reflects hyper-vigilance to negative stimuli. This theory builds on previous research showing a greater amygdala response in females compared to males for negative stimuli in adulthood (Stevens and Hamann, 2012; Andreano *et al.*, 2014). It is also possible that the findings reflect a sex difference in amygdala habituation, although this was not directly tested here. Prior reports suggest the degree to which there are sex differences in the habituation of the amygdala response to negative stimuli may vary based on valence, arousal and image novelty (for meta-analyses, see Wager *et al.*, 2003; Sergerie *et al.*, 2008; Stevens and Hamann, 2012; Garcia-Garcia *et al.*, 2016). For example, adult females have been shown to have a more persistent amygdala response than males to

negative stimuli that are familiar but not those that are novel, and persistence in amygdala response was further associated with greater negative affect (Andreano *et al.*, 2014). To the extent that emotional words may be considered familiar stimuli, the present findings may be due to greater habituation of amygdala response in males across development compared with a lack of habituation in females.

Importantly, the amygdala may have a larger impact on affective behavior in females than in males. For example, Blanton *et al.* (2010) found that larger amygdala volume was correlated with poorer emotional control in females but not males, hypothesized to result in a greater risk for the development of anxiety and depression. Foster *et al.* (2015) posit a developmental cascade to explain how the early emergence of internalizing symptoms (i.e. anxiety, depression) in adolescent females could exacerbate alcohol use problems later in life. Alcohol use first begins as a way to cope with negative emotions stemming from depression and anxiety; in turn, alcohol use magnifies internalizing problems by negatively impacting psychosocial development, which may then increase alcohol problems even more (Foster *et al.*, 2015). In this study, the YSR scores anxious and withdrawn, components of the broader internalizing score, significantly increase across age for females but not males, consistent with the view that internalizing and negative affect may mediate substance use risk in females. Moreover, Kuntsche and Muller (2012) showed adolescent females were twice as likely as males to report trying alcohol for the first time in order to cope with negative emotions. Together, these results lend evidence to the theory that females may be more vulnerable to SUD risk through an emotion processing pathway that includes the amygdala.

The exact role of the precentral gyrus in this pathway is less clear. Even though the right amygdala and right precentral gyrus show similar patterns of activation across development in males and females, it is unknown how the precentral gyrus contributes to SUD risk. However, it is likely that amygdala hyper-vigilance is involved, as previous studies have demonstrated that the amygdala can trigger and inhibit motor responses and/or modulate motor programs in emotionally specific contexts (LeDoux, 2000; Sagaspe *et al.*, 2011). Additionally, El Zein *et al.* (2015) demonstrated that healthy individuals with high anxiety show hyper-vigilance to potentially threatening stimuli by way of earlier electroencephalogram signals in the motor cortex compared to low anxiety individuals. Thus, it is possible that emotion processing in at-risk adolescent females influences both the amygdala and motor-related regions. Future research is needed to specifically examine the role of the precentral gyrus in affect processing during the developmental period—both late childhood and early adolescence—particularly via connectivity analyses.

There are several limitations to note. First, results should be interpreted carefully, as a significant decrease across age was found in the amygdala and precentral gyrus only for at-risk males, yet results are discussed from the framework of at-risk females. There is evidence for this framework from both the YSR results and the broader literature, but given the small sample size, only tentative conclusions can be drawn. Furthermore, these analyses focused on average amygdala activation to valenced stimuli across each scan session and therefore no conclusions can be drawn regarding a role of habituation. This will be an important area for future work. Second, this study focused on at-risk individuals only, which precludes these results from generalizing to males and females who have no family history of substance use. Future studies would need a non-high risk control group in order to interpret a gender comparison between high-risk and control participants. Third, the maximum age here is 17.6 years; it is unknown what neural and behavioral changes would occur with respect to both groups as they continue to develop. Potential follow-up studies should capture these subsequent outcomes, particularly as these participants move into substance use and abuse. Finally, the greater amount of substance use reported in males than females throughout the study could be a confound; however, the supplementary analyses run without participants reporting substance use suggests that this is not likely.

To conclude, this study revealed developmental differences in the functional brain response of at-risk males and females to negative emotional stimuli. These neural distinctions may underlie sex differences in behavioral risk trajectories for SUDs, such as negative emotionality and vulnerability to internalizing disorders in adolescent females. This study suggests there may be sex-specific neural and behavioral patterns visible in females at-risk for developing SUDs, which could potentially be useful for predicting future problem use.

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## Supplementary data

Supplementary data are available at *SCAN* online.

*Conflict of interest*. None declared.

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