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Reduced fronto-amygdalar connectivity in adolescence is associated with increased depression symptoms over time

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Abstract

Depression is common among adolescents, affecting greater than 12% of youth in a given year. Studies have shown aberrant amygdala connectivity in depressed adolescents, compared with controls; however, no studies have examined whether these abnormalities precede and heighten risk for depressive symptom expression. This study used resting state functional connectivity (RSFC) magnetic resonance imaging to examine neurobiological markers of escalating depression symptoms in adolescents (ages 12–16 years; free from psychopathology at baseline). Of a large sample of adolescents, 18 showed ± 1 S.D. increase in depression scale t-scores over time (“escalators”; time to escalation ranging from 6 to 54 months in follow up) and were matched and compared to 19 youth showing stable CDI scores over time (“controls”). Whole-brain analyses on baseline RSFC data using an amygdala seed region-of-interest (ROI) showed that controls had greater RSFC, relative to escalators, between the right amygdala and left inferior frontal and supramarginal gyrus and right mid-cingulate cortex. Additionally, relative to escalators, control youth had less RSFC between the left amygdala and cerebellum. Findings suggest a possible neurobiological marker of increasing depressive symptoms during adolescence, characterized in part by reduced fronto-limbic connectivity, suggesting a premorbid deficiency in top-down emotional regulation.

Keywords

Resting state; fMRI; Functional connectivity; Risk; Limbic

1. Introduction

Adolescence is a developmental period characterized by social, physical, hormonal, and neural changes (Casey et al., 2008; Luciana, 2013). These diverse changes, and their different developmental timelines, are thought to create a unique vulnerability to psychopathology that is specific to this time in life (Blakemore and Choudhury, 2006; Casey et al., 2008; Konrad et al., 2013; Ladouceur et al., 2012). One such example is an increased

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incidence of depression. Major depressive disorder (MDD) is common among adolescents, affecting over 12% of youth in a given year (SAMSHA, 2015). Furthermore, adolescent-onset MDD is associated with more severe and chronic depression across the lifespan, as well as increased suicide rates (Zisook et al., 2007), the latter of which is the second leading cause of mortality among those aged 10–24 years (Heron, 2016). Given the dramatic increase in rates of MDD that occurs during the adolescent years (Hankin et al., 1998), identifying potential neurobiological biomarkers and underpinnings of risk for MDD is crucial for ultimately informing prevention efforts.

The prefrontal cortex and limbic system both undergo major neurodevelopment and reorganization during adolescence, which affects the ways in which adolescents assess, process, and evaluate both risk and emotion (Heller and Casey, 2016; Steinberg, 2005). The amygdala, a key brain structure within the limbic system, detects and responds to threats and aids in the formulation of emotional responses (Baxter and Murray, 2002; LeDoux, 2003; Ochsner et al., 2012) and responds differentially to emotional stimuli in adolescents when compared to adults (Casey, 2015). The prefrontal cortex (PFC) is one of the last brain regions to develop and is broadly responsible for higher order cognitive functions, including planning and cognitive control (Blakemore and Choudhury, 2006; Fuster, 2001). The PFC also plays a major role in emotional behavior when working in tandem with the limbic system (Fuster, 2001). More specifically, the functionally connected circuits of the amygdala and PFC have been shown to be the principal neural correlates of emotional regulation and processing (Ochsner et al., 2002; Ochsner and Gross, 2005), circuitry showing developmental change during adolescence (Gabard-Durnam et al., 2014).

Grounded in existing knowledge of emotional neural circuitry and associated developmental changes in the brain, several neuroimaging studies have investigated the neural correlates of depression in adolescent populations. Utilizing task-based functional magnetic resonance imaging (fMRI), these studies have reported abnormal amygdala activation, including both hyper- and hypo-active amygdala response to emotional stimuli in un-medicated depressed adolescents, as well as regions of atypical PFC brain response, during emotion processing tasks (Henje Blom et al., 2015; for review, see Hulvershorn et al., 2011; Keresztes et al., 2014). In addition to task-based fMRI studies of adolescent depression implicating regional abnormalities in amygdala and PFC functioning, more recently, studies have begun to examine resting state functional connectivity of the amygdala in depressed adolescents. Resting state functional connectivity (RSFC) magnetic resonance imaging allows the examination of functional connections in the brain, in the absence of external task demands, by correlating temporally synchronous spontaneous blood-oxygen-level dependent (BOLD) activity (Fox and Raichle, 2007; van den Heuvel and Hulshoff Pol, 2010). Though widespread differences in functional connectivity have been observed between depressed adolescents and controls using this technique (Bebko et al., 2015; Connolly et al., 2017; Pannekoek et al., 2014; Rzepa and McCabe, 2016; Sacchet et al., 2016), several studies have demonstrated fronto-amygdalar hypo-connectivity in adolescents with MDD compared to healthy controls, including reduced RSFC between the right amygdala and the left frontal pole and right anterior cingulate cortex (Pannekoek et al., 2014) and between the right amygdala and ventromedial and bilateral dorsolateral PFC (Connolly et al., 2017). Another study examining children with MDD, with and without maternal MDD, showed reduced

RSFC between the amygdala and bilateral dorsolateral PFC in both groups compared to controls (Luking et al., 2011). Thus, aberrant fronto-amygdalar RSFC may be a neural substrate of altered emotional processing, either as a function of the depressed state or as an etiological risk factor leading to depressive symptomatology, given the role of this circuitry in emotional regulation. Examining premorbid functional connectivity between the amygdala and prefrontal regions in adolescents who later show an increase in depression symptoms could provide unique insight into the neural correlates of developmental risk for psychopathology, specifically adolescent-onset MDD.

While a few studies have examined amygdala task-related brain response (Swartz et al., 2015) and RSFC (Luking et al., 2011) in at-risk children and adolescents with familial depression, and one study showed increased subgenual anterior cingulate cortex to amygdala RSFC in adolescents who develop depression (Davey et al., 2015), to our knowledge, no studies have examined whether atypical neural features entirely precede depressive symptom expression. The present study addresses this gap by using RSFC to examine potential neurobiological connectivity markers of later escalating depression symptoms in an adolescent sample. Based on previous findings of abnormalities in functional connectivity of the limbic system in depressed adolescents (Bebko et al., 2015; Connolly et al., 2017; Pannekoek et al., 2014), we compared baseline whole-brain, seed-based amygdala RSFC of youth who showed a significant increase in depression symptoms over time to that of well-matched youth who showed a stable mood presentation. We hypothesized weaker premorbid fronto-amygdalar functional connectivity in those adolescents who later showed an increase in depressive symptoms. This investigation is crucial to identifying potential neurobiological markers of adolescent-onset depressive symptom expression.

2. Method

2.1. Study participants

Adolescents, ages 12–16 years, were recruited through the community as part of a larger, ongoing longitudinal study of adolescent neurodevelopment (Alarcon et al., 2015; Cservenka et al., 2014; Cservenka and Nagel, 2012). Following informed consent and assent, all youth and a parent/legal guardian completed separate comprehensive screening interviews to assess eligibility. Exclusionary criteria at baseline included major medical conditions or injury affecting central nervous system functioning, prenatal exposure to drugs or alcohol, personal alcohol/drug use (> 10 lifetime alcoholic drinks or > 2 drinks per occasion, > 10 lifetime uses of marijuana, any other drug use, or > 4 cigarettes per day), as assessed by the Brief Lifetime version of the Customary Drinking and Drug Use Record (CDDR) (Brown et al., 1998), current diagnoses of DSM-IV Axis I psychiatric disorder (including learning disability) as assessed by the computerized NIMH Diagnostic Interview Schedule for Children - Predictive Scales (DISC-PS-4.32b) (Lucas et al., 2001; Shaffer et al., 2000), report of psychotic disorder in a biological parent as assessed by the Family History Assessment Module (Rice et al., 1995), current use of psychotropic medication, left handedness, and MRI contraindications.

After establishing eligibility, youth were administered a baseline battery of neuropsychological assessments and questionnaires. This battery included the two-subtest

form of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) to assess intellectual functioning, the Childhood Depression Inventory (CDI) (Kovacs, 1985) to assess current (past two weeks) depression symptoms and severity, and the State Anxiety Subscale from the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) to assess state anxiety at the time of the baseline scan. Socioeconomic status was estimated using parent-report via the Hollingshead Index of Social Position (Hollingshead, 1957). Pubertal development was assessed through self-report using the Pubertal Development Scale (Petersen et al., 1988). Following baseline study visits, youth were contacted every three months and administered quarterly follow-up telephone interviews. During these telephone interviews, youth were asked to complete the CDI and the CDDR (Brown et al., 1998) to assess recent (past 90 days) alcohol, drug, and tobacco use.

Based on these quarterly telephone interviews of approximately 175 participants, youth who showed a 10-point increase in depression t-scores (one standard deviation) from baseline, as assessed by the CDI, were included in the sample (escalators; $n=18$, females=10). Although a full diagnostic assessment of MDD was not completed, 7 escalator youth had an intermediate or positive diagnosis on the DISC MDD module at some point during the follow-up period. Escalators showed this increase in depression scores at varying times throughout follow up, ranging from 6 months to 54 months. These youth were carefully matched and compared to youth from the larger sample who showed stable CDI scores over time (controls; $n=19$, females=11). Specifically, escalators and controls were matched on baseline CDI scores, age, sex, puberty, IQ, time in follow up, and alcohol and drug use at baseline. All procedures were approved by the Oregon Health & Science University (OHSU) Institutional Review Board.

2.2. MRI data acquisition

Youth were scanned at baseline on a Siemens Tim Trio 3.0 T MRI scanner at the Advanced Imaging Research Center at OHSU. One high-resolution T1-weighted anatomical image was acquired for co-registration of functional data (repetition time (TR) = 2300 ms, echo time (TE) = 3.58 ms, orientation = sagittal, 256×256 matrix, resolution 13 mm, 9:14 min). Resting state functional MRI (fMRI) data were acquired with two blood-oxygen level dependent (BOLD)-weighted images (TR = 2500 ms, TE = 30 ms, flip angle = 90° , field of view = 240 mm^2 , slices = 36, slice thickness = 3.8 mm, resolution = $3.75 \times 3.75 \times 3.8 \text{ mm}$, 4:17 min/run), during which participants were instructed to stay still and fixate on a white cross in the center of a black screen. Lying in a supine position on the scanner bed, youth were able to view visual stimuli with a mirror mounted on a 12-channel head coil reflecting a projection from the head of the scanner. Afterwards, youth confirmed wakefulness during resting state scans.

2.3. MRI data processing

Data processing followed previously published procedures (Alarcon et al., 2015), including slice time correction, debanding, rigid body head motion correction with regression of 3 translational and 3 rotational parameters, and signal normalization to a mode value of 1000 (Alarcon et al., 2015; Costa Dias et al., 2013; Fair et al., 2009, 2012). Anatomical images were resampled into 3 mm^3 Talairach space (Talairach and Tournoux, 1988) and used for co-

registration of functional data into the same atlas space. Data smoothing was not included in image processing. Functional data underwent further processing (Power et al., 2014), including image detrending, multiple regression across concatenated runs including whole-brain global signal, white matter signal, cerebral spinal fluid and their derivatives, as well as 24 motion-related regressors ($R^2 R_t - 1 R_{t-1}^2$, where $R = [X \ Y \ Z \ \text{pitch} \ \text{yaw} \ \text{roll}]$, t = current timepoint and $t-1$ = preceding timepoint) (Friston et al., 1996) for a total of 30 motion regressors, and band-pass filtering (0.009–0.08 Hz). Global signal regression was used in the current analyses following several reports noting its merits in reducing global artifacts and robustly dealing with in-scanner movement, especially when used in combination with motion censoring (Burgess et al., 2016; Power et al., 2015; Yan et al., 2013).

2.4. Definition of amygdala seed regions

Bilateral amygdala regions of interest (ROIs) were created using FSL's Juelich histological atlas, which is based on stereotaxic, probabilistic maps of amygdala laterobasal, centromedial, and superficial subregions (Amunts et al., 2005). For right and left amygdala ROIs, subregions were combined and thresholded at 50% probability, as has been done previously (Roy et al., 2009). Overlap between amygdala ROIs and individual anatomy was confirmed through visual inspection (H.S.). Functional connectivity maps of amygdala connectivity were created by correlating average BOLD signal from each amygdala ROI (left and right) with every voxel in the brain.

2.5. Motion censoring

Amygdala functional connectivity maps were censored with an FD motion threshold of 0.3 mm (uncensored segments of data with fewer than 5 contiguous frames were removed) (Power et al., 2014). The FD method indexes head movement relative to adjacent volumes and is based on the following scalar formula: $FD_i = |dix| + |diy| + |diz| + |\alpha_i| + |\beta_i| + |\gamma_i|$, where $dix = d(i-1)x - dix$. The 0.3 mm FD threshold was selected to ensure that all participants had a minimum of 120 frames, or approximately 5 min of resting state data for analyses, which has been shown to yield sufficiently high sensitivity (77%) for detecting true functional connections (Smith et al., 2011). While frames exceeding an FD threshold of 0.3 mm were censored from the data, mean remaining FD was calculated for every individual, representing the degree of micro-movement (in the range of millimeters) in the censored data.

2.6. Data analysis

2.6.1. Demographic data—Demographic variables, as well as remaining mean FD (following censoring), were compared between the two groups using independent samples *t*-tests. The distribution of males and females across escalators and matched controls was compared with a chi-square test. All statistical analyses were completed in IBM SPSS Statistics 20 (IBM Corp, 2011).

2.6.2. RSFC data—Whole-brain, voxel-wise analyses were conducted with amygdala-seeded functional connectivity maps obtained from baseline RSFC data. Correlation coefficients (*r*-values) from connectivity maps were Fisher Z transformed to improve

normality prior to analyses. Amygdala functional connectivity maps between groups were compared with an independent samples *t*-test, assuming unequal variance, using in-house software that implements 4dfp tools developed at Washington University and has been utilized previously (Alarcon et al., 2015; Fair et al., 2012). To correct for multiple comparisons, the same in-house software determined a whole-brain corrected cluster threshold using Monte Carlo simulation for the level of smoothness applied to the data (in this case no smoothing was applied), as opposed to estimated from the data itself, for two levels of voxel/cluster-wise statistical correction ($Z > 2.25$, $p < 0.05$; $Z > 3.00$, $p < 0.01$). This method of correction avoids inflated false positives observed with other statistical packages (Eklund et al., 2016). This analysis was conducted twice, once for each amygdala ROI. Values from significant clusters were extracted and plotted to visualize group differences and conduct post-hoc analyses.

2.6.3. Post-hoc analyses—Post-hoc MANCOVA was conducted in SPSS to confirm group differences in RSFC, while statistically controlling for potential confounds, including remaining motion in the data (following censoring), baseline CDI scores, and baseline STAI scores. Additionally, exploratory post-hoc regression analyses were conducted among escalators to examine whether RSFC differences observed between groups were associated with CDI change scores or alcohol use at follow up.

3. Results

3.1. Demographic characteristics

At baseline, youth who showed an increase in CDI scores (“escalators”) and youth with stable CDI scores (“controls”) were matched on all demographic variables listed in Table 1, including age, sex, pubertal maturity, IQ, SES, baseline State STAI scores, baseline alcohol and substance use, and baseline CDI scores. Escalator and control groups were also matched on follow up time in the study ($t_{35} = 0.21$, $p = 0.84$); however, escalators showed a statistically significant 1 S.D. *t*-score increase in depression scores ($t_{17} = -10.32$, $p < 0.001$) at varying times throughout enrollment in the study, ranging from 6 months to 54 months. The groups remained matched on demographic variables at follow up; however, in addition to increasing depressive symptoms, escalators showed a trend-level increase in alcohol use ($t_{35} = -1.88$, $p = 0.07$) as compared to control youth (Table 1).

3.2. RSFC independent samples *t*-tests

Independent samples *t*-tests were run to compare right and left amygdala connectivity between escalators and matched controls. These analyses revealed that control youth had stronger baseline RSFC relative to escalators, between the right amygdala and left inferior frontal and supramarginal gyri and right mid-cingulate cortex (voxel/cluster threshold $p < 0.05$). Additionally, relative to escalators, control youth had weaker baseline RSFC between the left amygdala and the left cerebellar vermis (voxel/cluster threshold $p < 0.05$) (Fig. 1, Fig. 2, and Table 2). At a more rigorous voxel/cluster threshold ($p < 0.01$), only the group difference in right amygdala to left inferior frontal gyrus connectivity remained.

3.3. Effects of covariates

Residual motion, as measured with mean FD remaining following motion censoring, did not significantly differ by group ($t_{35} = -1.46, p = 0.15$), nor did the amount of censored resting state data available for analyses ($t_{35} = 1.77, p = 0.09$). Post-hoc MANCOVA revealed that significant group differences in right and left amygdala RSFC Z values remained in all clusters after covarying for mean FD remaining, baseline CDI scores, and baseline STAI scores (all $F(1,32) = 4.16, p = 0.008$).

3.4. CDI change scores

Total change in CDI scores from baseline to follow up, among escalators only, was regressed against RSFC Z values extracted from clusters of significant group difference. Among escalators, CDI change scores were not significantly associated with RSFC Z values (all p 's > 0.05). Time from baseline to 1 S.D. t-score increase in depression symptoms among escalators was also not significantly correlated with Fisher Z values where group differences in connectivity were detected (all p 's > 0.05).

3.5. Lifetime alcohol use

Given the trend-level difference in alcohol use between controls and escalators at follow up ($p = 0.07$), with escalators reporting more use, total number of lifetime drinks at follow up was regressed against RSFC Z values extracted from clusters that displayed a significant group difference in RSFC to determine if shared RSFC differences were associated with both increased depression symptoms, as well as increased alcohol use. Among escalators, lifetime drinks at follow up was not significantly associated with functional connectivity between regions of group difference (all p 's > 0.05).

4. Discussion

The goal of the current study was to compare baseline amygdala RSFC in youth who escalated in depressive symptoms over time (escalators) compared to a group of well-matched youth who showed stable depression scores (controls). Based on previous research showing reduced RSFC between the amygdala and prefrontal regions in depressed adolescents compared with healthy controls (Connolly et al., 2017; Pannekoek et al., 2014), we hypothesized weaker connectivity between these brain regions in escalators compared to controls. To our knowledge, this is the first study to examine whether amygdala RSFC abnormalities precede depressive symptom expression. Since the rates of MDD increase substantially during the adolescent years (Hankin et al., 1998), this work may shed light on potential neurobiological markers and underpinnings of risk for increasing depression symptoms during this time.

As hypothesized, escalators showed baseline differences in fronto-amygdalar RSFC compared to controls, consistent with the literature examining RSFC in depressed adolescents (Connolly et al., 2017; Kerestes et al., 2014; Pannekoek et al., 2014), suggesting atypical connectivity between the limbic system and regulatory prefrontal regions may be indicative of premorbid deficits in emotional processing and regulatory circuitry in youth who later escalate in depressive symptoms, rather than solely inherent to the disorder or

expression of symptomatology themselves. Specifically, the current study found weaker connectivity between the right amygdala and left lateral inferior frontal and supramarginal gyri in escalators, as compared to controls. Previous research has demonstrated that the inferior frontal gyrus, and in particular the ventrolateral PFC, plays an important role in cognitive control and may help to regulate emotional responses (Ochsner and Gross, 2005; Wager et al., 2008). Further, a recent meta-analysis supports existing theory that reappraisal of negative emotional responses occurs via a distributed network, including PFC modulation of semantic representations in regions of lateral temporal cortex, such as the supramarginal gyrus, which in turn influences the emotional responsivity of the amygdala (Buhle et al., 2014; Ochsner et al., 2012). Accordingly, one recent study in adults found that depression was associated with altered task-based activity, during an emotionally-valenced attention task, in both the inferior frontal and supramarginal gyri (Beevers et al., 2010), further highlighting this network in depression. Together with our findings, results from previous studies supporting amygdala, ventrolateral PFC, and supramarginal gyri involvement in both emotional regulation/reappraisal and depression suggest dysfunction in this circuitry may be important to consider as potential neurobiological underpinnings for increasing depression symptom presentation during adolescence.

In addition to less fronto-amygdalar and amygdala to supramarginal gyri connectivity among escalators, we also demonstrated that escalators showed less RSFC between the right amygdala and mid-cingulate cortex. The mid-cingulate cortex has also been implicated in emotion processing and regulation in patients with MDD. Compared to healthy controls, adult patients with MDD show greater activation of the midcingulate while passively viewing negative (versus neutral) pictures (Anand et al., 2005). Moreover, one study reported decreased activation of the mid-cingulate cortex by women with MDD and women at risk for MDD (by way of family history of MDD), compared to controls, while failing to suppress negative thoughts, which was an experimental manipulation meant to simulate ruminative processes (Carew et al., 2013). Indeed, using quantitative meta-analysis, rumination has been shown to negatively correlate with gray matter volume in the midcingulate cortex (Kuhn et al., 2012). These studies lend support for the role for the mid-cingulate cortex in networks involved in processing and/or regulating negative emotional stimuli in individuals with MDD and at high risk for developing MDD, and suggest that atypical premorbid amygdala connectivity with this region may contribute to the escalation of depression symptoms.

Finally, escalators showed increased connectivity between the left amygdala and the left cerebellar vermis compared to control youth. While the cerebellum's role in motor control is highly established (McLeod and Vand der Meulen, 1968), recent studies also have begun to investigate the cerebellum's role in cognition and emotion (Schutter and van Honk, 2005; Stoodley, 2012). Importantly, cerebellar activation has been shown during the processing of emotional stimuli, most prominently in negatively-valenced emotional stimuli, suggesting that the cerebellum plays a role in emotional processing and regulation (Stoodley, 2012). RSFC studies have also demonstrated negative connectivity between the amygdala and cerebellum (Gabard-Durnam et al., 2014; Roy et al., 2009), with increasing negative connectivity with development (Gabard-Durnam et al., 2014). Thus, our increased RSFC between the amygdala and cerebellum in escalators may be functioning to suboptimally

compensate for reduced regulatory connectivity with the PFC. Taken together, these findings suggest that atypical cerebellar connectivity with the amygdala may also be a potential neurobiological risk marker of emotional dysregulation.

While this study is the first to examine neural abnormalities in youth who escalate in depression symptom severity, limitations and future directions must be considered. First, though not formally assessed, most escalators in this study do not yet appear to meet diagnostic criteria for MDD. Therefore, we cannot infer that these findings show definite neurobiological markers or underpinnings of risk for diagnostic levels of depression, particularly since the change in depressive symptoms from baseline to follow up did not directly correlate with RSFC values from the clusters that differed between escalators and controls. Further research is needed to investigate premorbid amygdala connectivity and deficits in emotion regulation prior to symptom expression in samples of clinically depressed adolescents. Second, the escalators in this sample showed increases in depression symptoms at varying times from baseline. Some youth showed increased depression scores six months after their baseline visit, while others showed increases almost four years after baseline. While time to depressive symptom escalation was not related to any of the RSFC findings, and the groups did not differ with regard to time in follow up, we cannot rule out potential confounds of developmental changes. Future work should include a sample of adolescents matched on the timing of measurements. Third, due to the small sample size, not all results survived our most conservative level of statistical correction for multiple comparisons, suggesting that we may have been somewhat underpowered with the current number of participants. Further, we were unable to analyze potential sex differences in amygdala connectivity prior to depressive symptom expression. Given the vast literature suggesting sex differences in adolescent depression (Hamilton et al., 2014; Nolen-Hoeksema and Girgus, 1994), as well as sex differences in amygdala connectivity during adolescence (Alarcon et al., 2015), this is a notable future direction. Fourth, MDD symptoms were only assessed using the CDI (Kovacs, 1985). The use of a single measure to assess the increase in MDD symptoms over time may not have captured the full scope or magnitude of change in MDD symptoms. Future work should use multiple measures, ideally including both self-report and clinician report/diagnosis, to assess MDD symptomatology. Finally, future studies should utilize a prospective longitudinal study design to examine potential neural abnormalities both before and after depression symptom expression, as well as the comorbid expression of other psychopathology and risk behaviors. Notably, youth who escalated in CDI scores over time in this sample also showed a trend-level increase in alcohol use at follow-up compared to controls. Previous research has documented an internalizing pathway to alcohol use, suggesting that internalizing symptoms and problems with emotional regulation may contribute to ultimate alcohol use (Hussong et al., 2011). While our RSFC differences between escalators and controls were not significantly related to increased alcohol consumption at follow-up, it is important to acknowledge that the current study may not be pinpointing a neurobiological risk marker specific to depression per se, but rather may suggest phenotypic overlap in neurobiological markers of risk for both increased depression symptoms and alcohol use. Importantly, neuroimaging studies have begun to publish longitudinal findings with regard to depression symptom expression in adolescents (Davey et al., 2015; Strikwerda-Brown et al., 2015), providing an optimistic indication of the

feasibility and importance of prospective longitudinal study design in defining the neurobiological underpinnings of risk for psychopathology during this important developmental time.

In conclusion, this examination revealed that youth who later escalated in depressive symptoms had aberrant RSFC compared to control youth, prior to the onset of any depression symptoms. Our hypothesis that youth who escalate in depression symptoms over time would show weaker premorbid fronto-amygdalar functional connectivity was supported, as well as findings implicating broader emotional regulatory networks. Overall, results suggest a potential neurobiological marker of increasing depressive symptom expression during adolescence, possibly by way of diminished control and regulation of emotional responding. Future longitudinal work of this nature is necessary to better inform prevention and treatment efforts.

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References

- Alarcon G, Cservenka A, Rudolph MD, Fair DA, Nagel BJ. Developmental sex differences in resting state functional connectivity of amygdala sub-regions. *Neuroimage*. 2015; 115:235–244. [PubMed: 25887261]
- Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ, Habel U, Schneider F, Zilles K. Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *Anat. Embryol*. 2005; 210:343–352. [PubMed: 16208455]
- Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, Mathews VP, Kalnin A, Lowe MJ. Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. *Biol. Psychiatry*. 2005; 57:1079–1088. [PubMed: 15866546]
- Baxter MG, Murray EA. The amygdala and reward. *Nat. Rev., Neurosci*. 2002; 3:563–573. [PubMed: 12094212]
- Bebko G, Bertocci M, Chase H, Dwojak A, Bonar L, Almeida J, Perlman SB, Versace A, Schirda C, Travis M, Gill MK, Demeter C, Diwadkar V, Sunshine J, Holland S, Kowatch R, Birmaher B, Axelson D, Horwitz S, Frazier T, Arnold LE, Fristad M, Youngstrom E, Findling R, Phillips ML. Decreased amygdala-insula resting state connectivity in behaviorally and emotionally dysregulated youth. *Psychiatry Res*. 2015; 231:77–86. [PubMed: 25433424]
- Beevers CG, Clasen P, Stice E, Schyner D. Depression symptoms and cognitive control of emotion cues: an fMRI study. *Neuroscience*. 2010; 167:97–103. [PubMed: 20116416]
- Blakemore SJ, Choudhury S. Development of the adolescent brain: implications for executive function and social cognition. *J. Child Psychol. Psychiatry*. 2006; 47:296–312. [PubMed: 16492261]
- Brown SA, Myers MG, Lippke L, Tapert SF, Stewart DG, Vik PW. Psychometric evaluation of the Customary Drinking and Drug Use Record (CDDR): a measure of adolescent alcohol and drug involvement. *J. Stud. Alcohol Drugs*. 1998; 59:427–438.
- Buhle JT, Silvers JA, Wager TD, Lopez R, Onyemekwu C, Kober H, Weber J, Ochsner KN. Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. *Cereb. Cortex*. 2014; 24:2981–2990. [PubMed: 23765157]
- Burgess GC, Kandala S, Nolan D, Laumann TO, Power JD, Adeyemo B, Harms MP, Petersen SE, Barch DM. Evaluation of denoising strategies to address motion-correlated artifacts in resting-state

- functional magnetic resonance imaging data from the Human Connectome Project. *Brain Connect.* 2016; 6:669–680. [PubMed: 27571276]
- Carew CL, Milne AM, Tatham EL, MacQueen GM, Hall GB. Neural systems underlying thought suppression in young women with, and at-risk, for depression. *Behav. Brain Res.* 2013; 257:13–24. [PubMed: 24055881]
- Casey BJ. Beyond simple models of self-control to circuit-based accounts of adolescent behavior. *Annu. Rev. Psychol.* 2015; 66:295–319. [PubMed: 25089362]
- Casey BJ, Getz S, Galvan A. The adolescent brain. *Dev. Rev.* 2008; 28:62–77. [PubMed: 18688292]
- Connolly CG, Ho TC, Blom EH, LeWinn KZ, Sacchet MD, Tymofiyeva O, Simmons AN, Yang TT. Resting-state functional connectivity of the amygdala and longitudinal changes in depression severity in adolescent depression. *J. Affect. Disord.* 2017; 207:86–94. [PubMed: 27716542]
- IBM Corp. Released. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp; 2011.
- Costa Dias TG, Wilson VB, Bathula DR, Iyer SP, Mills KL, Thurlow BL, Stevens CA, Musser ED, Carpenter SD, Grayson DS, Mitchell SH, Nigg JT, Fair DA. Reward circuit connectivity relates to delay discounting in children with attention-deficit/hyperactivity disorder. *Eur. Neuropsychopharmacol.* 2013; 23:33–45. [PubMed: 23206930]
- Cservenka A, Fair DA, Nagel BJ. Emotional processing and brain activity in youth at high risk for alcoholism. *Alcohol Clin. Exp. Res.* 2014; 38:1912–1923. [PubMed: 24890898]
- Cservenka A, Nagel BJ. Risky decision-making: an fMRI study of youth at high risk for alcoholism. *Alcohol Clin. Exp. Res.* 2012; 36:604–615. [PubMed: 22250647]
- Davey CG, Whittle S, Harrison BJ, Simmons JG, Byrne ML, Schwartz OS, Allen NB. Functional brain-imaging correlates of negative affectivity and the onset of first-episode depression. *Psychol. Med.* 2015; 45:1001–1009. [PubMed: 25162634]
- Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc. Natl. Acad. Sci. USA.* 2016; 113:7900–7905. [PubMed: 27357684]
- Fair DA, Cohen AL, Power JD, Dosenbach NU, Church JA, Miezin FM, Schlaggar BL, Petersen SE. Functional brain networks develop from a "local to distributed" organization. *PLoS Comput. Biol.* 2009; 5:e1000381. [PubMed: 19412534]
- Fair DA, Nigg JT, Iyer S, Bathula D, Mills KL, Dosenbach NU, Schlaggar BL, Mennes M, Gutman D, Bangaru S, Buitelaar JK, Dickstein DP, Di Martino A, Kennedy DN, Kelly C, Luna B, Schweitzer JB, Velanova K, Wang YF, Mostofsky S, Castellanos FX, Milham MP. Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data. *Front. Syst. Neurosci.* 2012; 6:80. [PubMed: 23382713]
- Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci.* 2007; 8:700–711. [PubMed: 17704812]
- Friston KJ, Williams S, Howard R, Frackowiak RS, Turner R. Movement-related effects in fMRI time-series. *Magn. Reson. Med.: Off. J. Soc. Magn. Reson. Med. / Soc. Magn. Reson. Med.* 1996; 35:346–355.
- Fuster JM. The prefrontal cortex-an update: time is of the essence. *Neuron.* 2001; 30:319–333. [PubMed: 11394996]
- Gabard-Durnam LJ, Flannery J, Goff B, Gee DG, Humphreys KL, Telzer E, Hare T, Tottenham N. The development of human amygdala functional connectivity at rest from 4 to 23 years: a cross-sectional study. *Neuroimage.* 2014; 95:193–207. [PubMed: 24662579]
- Hamilton JL, Hamlat EJ, Stange JP, Abramson LY, Alloy LB. Pubertal timing and vulnerabilities to depression in early adolescence: differential pathways to depressive symptoms by sex. *J. Adolesc.* 2014; 37:165–174. [PubMed: 24439622]
- Hankin BL, Abramson LY, Moffitt TE, Silva PA, McGee R, Angell KE. Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *J. Abnorm. Psychol.* 1998; 107:128–140. [PubMed: 9505045]
- Heller AS, Casey BJ. The neurodynamics of emotion: delineating typical and atypical emotional processes during adolescence. *Dev. Sci.* 2016; 19:3–18. [PubMed: 26548573]
- Henje Blom E, Connolly CG, Ho TC, LeWinn KZ, Mobayed N, Han L, Paulus MP, Wu J, Simmons AN, Yang TT. Altered insular activation and increased insular functional connectivity during sad

- and happy face processing in adolescent major depressive disorder. *J. Affect. Disord.* 2015; 178:215–223. [PubMed: 25827506]
- Heron SF. Deaths: leading causes for 2014, National vital statistics reports. *Cent. Dis. Control Prev. (CDC)*. 2016
- Hollingshead, AB. Two Factor Index of Social Position. Privately Published; New Haven, CT: 1957.
- Hulvershorn LA, Cullen K, Anand A. Toward dysfunctional connectivity: a review of neuroimaging findings in pediatric major depressive disorder. *Brain Imaging Behav.* 2011; 5:307–328. [PubMed: 21901425]
- Hussong AM, Jones DJ, Stein GL, Baucom DH, Boeding S. An internalizing pathway to alcohol and substance use disorders. *Psychol. Addict. Behav.* 2011; 25:390–404. [PubMed: 21823762]
- Kerestes R, Davey CG, Stephanou K, Whittle S, Harrison BJ. Functional brain imaging studies of youth depression: a systematic review. *NeuroImage Clin.* 2014; 4:209–231. [PubMed: 24455472]
- Konrad K, Firk C, Uhlhaas PJ. Brain development during adolescence: neuroscientific insights into this developmental period. *Dtsch Arztebl Int.* 2013; 110:425–431. [PubMed: 23840287]
- Kovacs M. The children's depression inventory (CDI). *Psychopharmacol. Bull.* 1985; 21:995–998. [PubMed: 4089116]
- Kuhn S, Vanderhasselt MA, De Raedt R, Gallinat J. Why ruminators won't stop: the structural and resting state correlates of rumination and its relation to depression. *J. Affect. Disord.* 2012; 141:352–360. [PubMed: 22497878]
- Ladouceur CD, Peper JS, Crone EA, Dahl RE. White matter development in adolescence: the influence of puberty and implications for affective disorders. *Dev. Cogn. Neurosci.* 2012; 2:36–54. [PubMed: 22247751]
- LeDoux J. The emotional brain, fear, and the amygdala. *Cell. Mol. Neurobiol.* 2003; 23:727–738. [PubMed: 14514027]
- Lucas C, Zhang H, Fisher P, Shaffer D, Regier D, Narrow W, Bourdon K, Dulcan M, Canino G, Rubio-Stipec M, Lahey B, Friman P. The disc Predictive scales (DPS): efficiently screening for diagnoses. *J. Am. Acad. Child Adolesc. Psychiatry.* 2001; 40:443–449. [PubMed: 11314570]
- Luciana M. Adolescent brain development in normality and psychopathology. *Dev. Psychopathol.* 2013; 25:1325–1345. [PubMed: 24342843]
- Luking KR, Repovs G, Belden AC, Gaffrey MS, Botteron KN, Luby JL, Barch DM. Functional connectivity of the amygdala in early-childhood-onset depression. *J. Am. Acad. Child Adolesc. Psychiatry.* 2011; 50:1027–1041. e1023. [PubMed: 21961777]
- McLeod J, Vand der Meulen J. Cerebellar control of alpha motoneurone function. *Proc. Aust. Assoc. Neurol.* 1968; 5:19–24. [PubMed: 5715703]
- Nolen-Hoeksema S, Girgus JS. The emergence of gender differences in depression during adolescence. *Psychol. Bull.* 1994; 115:424–443. [PubMed: 8016286]
- Ochsner KN, Bunge SA, Gross JJ, Gabrieli JDE. Rethinking feelings: an fMRI study of the cognitive regulation of emotion. *J. Cogn. Neurosci.* 2002; 14:1215–1229. [PubMed: 12495527]
- Ochsner KN, Gross JJ. The cognitive control of emotion. *Trends Cogn. Sci.* 2005; 9:242–249. [PubMed: 15866151]
- Ochsner KN, Silvers JA, Buhle JT. Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Ann. NY Acad. Sci.* 2012; 1251:E1–E24. [PubMed: 23025352]
- Pannekoek JN, van der Werff SJ, Meens PH, van den Bulk BG, Jolles DD, Veer IM, van Lang ND, Rombouts SA, van der Wee NJ, Vermeiren RR. Aberrant resting-state functional connectivity in limbic and salience networks in treatment-naïve clinically depressed adolescents. *J. Child Psychol. Psychiatry.* 2014; 55:1317–1327. [PubMed: 24828372]
- Petersen A, Crockett L, Richards M, Boxer A. A self-report measure of pubertal status: reliability, validity, and initial norms. *J. Youth Adolesc.* 1988; 17:117–133. [PubMed: 24277579]
- Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage.* 2014; 84:320–341. [PubMed: 23994314]

- Power JD, Schlaggar BL, Petersen SE. Recent progress and outstanding issues in motion correction in resting state fMRI. *Neuroimage*. 2015; 105:536–551. [PubMed: 25462692]
- Rice JP, Reich T, Bucholz KK, Neuman RJ, Fishman R, Rochberg N, Hesselbrock VM, Nurnberger JI, Schuckit MA, Begleiter H. Comparison of direct Interview and family history diagnoses of alcohol dependence. *Alcohol. Clin. Exp. Res.* 1995; 19:1018–1023. [PubMed: 7485811]
- Roy AK, Shehzad Z, Margulies DS, Kelly AM, Uddin LQ, Gotimer K, Biswal BB, Castellanos FX, Milham MP. Functional connectivity of the human amygdala using resting state fMRI. *Neuroimage*. 2009; 45:614–626. [PubMed: 19110061]
- Rzepa E, McCabe C. Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology. *J. Psychiatr. Res.* 2016; 82:40–47. [PubMed: 27459031]
- Sacchet MD, Ho TC, Connolly CG, Tymofiyeva O, Lewinn KZ, Han LK, Blom EH, Tapert SF, Max JE, Frank GK, Paulus MP, Simmons AN, Gotlib IH, Yang TT. Large-scale hypoconnectivity between resting-state functional networks in unmedicated adolescent major depressive disorder. *Neuropsychopharmacology*. 2016; 41:2951–2960. [PubMed: 27238621]
- SAMSHA. Center for behavioral health statistics and quality. *Natl. Surv. Drug Use Health*. 2015 2014 2015.
- Schutter D, van Honk J. The cerebellum on the rise in human emotion. *Cerebellum*. 2005; 4:209–204.
- Shaffer D, Fisher P, Lucas C, Dulcan M, Schwab-Stone M. NIMH diagnostic Interview schedule for children version IV (NIMH disc-IV): description, differences from previous versions, and reliability of some common diagnoses. *J. Am. Acad. Child Adolesc. Psychiatry*. 2000; 39:28–38. [PubMed: 10638065]
- Smith JF, Pillai A, Chen K, Horwitz B. Effective connectivity modeling for fMRI: six issues and possible solutions using linear dynamic systems. *Front. Syst. Neurosci.* 2011; 5:104. [PubMed: 22279430]
- Spielberger, CD., Gorsuch, RL., Lushene, R., Vagg, PR., Jacobs, GA. *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press, Inc; Palo Alto, CA: 1983.
- Steinberg L. Cognitive and affective development in adolescence. *Trends Cogn. Sci.* 2005; 9:69–74. [PubMed: 15668099]
- Stoodley C. The cerebellum and cognition: evidence from functional imaging studies. *Cerebellum*. 2012; 11:352–365. [PubMed: 21373864]
- Strikwerda-Brown C, Davey CG, Whittle S, Allen NB, Byrne ML, Schwartz OS, Simmons JG, Dwyer D, Harrison BJ. Mapping the relationship between subgenual cingulate cortex functional connectivity and depressive symptoms across adolescence. *Soc. Cogn. Affect. Neurosci.* 2015; 10:961–968. [PubMed: 25416726]
- Swartz JR, Williamson DE, Hariri AR. Developmental change in amygdala reactivity during adolescence: effects of family history of depression and stressful life events. *Am. J. Psychiatry*. 2015; 172:276–283. [PubMed: 25526599]
- Talairach, J., Tournoux, P. *Co-planar Stereotaxic Atlas of the Human Brain-3-Dimensional Proportional System: An Approach to Cerebral Imaging*. Georg Thieme, Stuttgart; New York: 1988.
- van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur. Neuropsychopharmacol.* 2010; 20:519–534. [PubMed: 20471808]
- Wager TD, Davidson ML, Hughes BL, Lindquist MA, Ochsner KN. Neural mechanisms of emotion regulation: evidence for two independent prefrontal-subcortical pathways. *Neuron*. 2008; 59:1037–1050. [PubMed: 18817740]
- Wechsler, D. *Wechsler Abbreviated Scale of Intelligence*. Psychological Corporation; San Antonio, TX: 1999.
- Yan CG, Craddock RC, Zuo XN, Zang YF, Milham MP. Standardizing the intrinsic brain: towards robust measurement of inter-individual variation in 1000 functional connectomes. *Neuroimage*. 2013; 80:246–262. [PubMed: 23631983]
- Zisook S, Rush AJ, Lesser I, Wisniewski SR, Trivedi M, Husain MM, Balasubramani GK, Alpert JE, Fava M. Preadult onset vs. adult onset of major depressive disorder: a replication study. *Acta Psychiatr. Scand.* 2007; 115:196–205. [PubMed: 17302619]

Fig. 1.

Whole-brain analyses on baseline RFSC data were conducted using an atlas-based seed region-of-interest (ROI) that encompassed the right and left whole amygdala. (A) Control youth showed greater RSFC (yellow), relative to escalator youth, between the right amygdala and left inferior frontal, supramarginal gyrus, and right mid-cingulate cortex ($Z > 2.25$, $p < 0.05$). (B) Control youth showed less RSFC (blue) between the left amygdala and the left cerebellar vermis ($Z > 2.25$, $p < 0.05$) relative to escalator youth ($Z > 2.25$, $p < 0.05$).

Fig. 2.

Bar graphs illustrating significant differences between control and escalator youth in RSFC Fisher Z values (with standard error bars). (A) Control youth showed greater RSFC, relative to escalator youth, between the right amygdala and left inferior frontal, supramarginal gyrus, and right cingulate cortex. (B) Control youth showed less RSFC between the left amygdala and the left cerebellar vermis relative to Escalator youth.

Table 1

Participant Demographics.

	Escalators	Control	Statistic	P Value
Number	18	19		
Age in Years (SD)	13.51 (1.51)	13.84 (1.17)	$t_{35} = 0.88$	0.39
Gender	8M/10F	8M/11F	$X^2_1 = 0.02$	0.89
IQ (SD)^a	111.22 (13.84)	115.11 (9.79)	$t_{35} = 0.99$	0.33
Socioeconomic Status (SD)^b	27.06 (11.43)	29.21 (12.86)	$t_{35} = 0.54$	0.59
PDS Crockett Stage (SD)^c	3.33 (1.19)	3.63 (0.96)	$U = 146.0$	0.46
Months in Follow Up (SD)	31.17 (15.95)	30.16 (13.95)	$t_{35} = 0.21$	0.84
Baseline Alcohol Use (SD)^d	0 (0)	0.11(0.32)	$t_{35} = 1.42$	0.17
Follow-Up Alcohol Use (SD)^d	0.50 (0.51)	0.26 (0.45)	$t_{35} = -1.88$	0.07
Baseline Marijuana Use (SD)^d	0.06 (0.24)	0.05 (0.23)	$t_{35} = 0.04$	0.97
Follow-Up Marijuana Use (SD)^d	13.72 (31.04)	71.37 (211.36)	$t_{35} = 1.14$	0.26
Baseline CDI scores (SD)^e	41.39 (4.84)	44.21 (7.67)	$t_{35} = 1.33$	0.19
Follow-Up CDI Scores (SD)^e	59.22 (8.79)	45.95 (6.83)	$t_{35} = -5.14$	0.00
Baseline STAI Scores (SD)^f	41.41 (5.05)	45.28 (8.73)	$t_{34} = 1.63$	0.11

^aWechsler Abbreviated Scale of Intelligence, 2-subscale version (Wechsler, 1999).^bHollingshead Index of Social Position (Hollingshead, 1957); larger values indicate lower socioeconomic status.^cPubertal Development Scale Crockett Stage (Petersen et al., 1988), scores range from 1 to 5, with higher scores reflecting greater maturity.^dAlcohol Use Reported as number of lifetime drinks; Marijuana Use reported as times used throughout lifetime.^eChildren's Depression Inventory (Kovacs, 1985); t-scores under 60 are considered mild or sub-clinical depression severity.^fState-Trait Anxiety Inventory (STAI; Spielberger et al., 1983); only State Anxiety was measured, t-scores under 50 are considered mild or sub-clinical.

Table 2

Resting state functional connectivity of bilateral whole amygdala in control youth relative to escalator youth.

Structure	BA	Voxels (mm ³)	Peak Talairach Coordinates (x,y,z)	Z-score
Right Amygdala: Control > Escalators				
L Middle Frontal Gyrus	47	90	-40, 36, -12	2.88
R Mid-Cingulate Gyrus	24	71	20, -9, 27	2.53
L Inferior Frontal Gyrus	45	58	-46, 21, 3	2.73
L Supramarginal Gyrus	40	54	-56, -48, 12	2.64
Left Amygdala: Control < Escalators				
L Cerebellar Vermis		74	-20, -54, -24	-2.42

L = left, R = right, BA = Brodmann Area.