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Developmental sex differences in resting state functional connectivity of amygdala sub-regions

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Abstract

During adolescence, considerable social and biological changes occur that interact with functional brain maturation, some of which are sex-specific. The amygdala is one brain area that has displayed sexual dimorphism, specifically in socio-affective (superficial amygdala [SFA]), stress (centromedial amygdala [CMA]), and learning and memory (basolateral amygdala [BLA]) processing. The amygdala has also been implicated in mood and anxiety disorders which also display sex-specific features, most prominently observed during adolescence. Using functional magnetic resonance imaging (fMRI), the present study examined the interaction of age and sex on resting state functional connectivity (RSFC) of amygdala sub-regions, BLA and SFA, in a sample of healthy adolescents between the ages 10-16 years (n=122, 71 boys). Whole-brain, voxel-wise partial correlation analyses were conducted to determine RSFC of bilateral BLA and SFA seed regions, created using the Eickhoff-Zilles maximum probability maps based on cytoarchitectonic mapping and FMRIB's Integrated Registration and Segmentation Tool (FIRST). Monte Carlo simulation was implemented to correct for multiple comparisons (threshold of 53 contiguous voxels with a *z*-value ≥ 2.25). Results indicated that with increasing age, there was a corresponding decrease in RSFC between both amygdala sub-regions and parieto-occipital cortices, with a concurrent increase in RSFC with medial prefrontal cortex (mPFC). Specifically, boys and girls demonstrated increased coupling of mPFC and left and right SFA with age, respectively; however, neither sex showed increased connectivity between mPFC and BLA, which could indicate relative immaturity of fronto-limbic networks that is similar across sex. A dissociation in connectivity between BLA- and SFA- parieto-occipital RSFC emerged, in which girls had weaker negative RSFC between SFA and parieto-occipital regions and boys had weaker

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negative RSFC of BLA and parieto-occipital regions with increased age, both standing in contrast to adult patterns of amygdala sub-regional RSFC. The present findings suggest relative immaturity of amygdala sub-regional RSFC with parieto-occipital cortices during adolescence, with unique patterns in both sexes that may support memory and socio-affective processing in boys and girls, respectively. Understanding the underlying normative functional architecture of brain networks associated with the amygdala during adolescence may better inform future research of the neural features associated with increased risk for internalizing psychopathology.

Keywords

resting state functional connectivity; amygdala; adolescence; sex differences

1. Introduction

Structural and functional connectivity between the amygdala and cortical brain regions undergoes dramatic maturation across adolescence (Qin et al., 2012, Gabard-Durnam et al., 2014). Resting state functional connectivity (RSFC) refers to the coupling of spontaneous blood oxygen level-dependent (BOLD) signal, as measured with functional magnetic resonance imaging (fMRI), in discrete brain regions or networks. Positive functional connectivity between regions is thought to reflect patterns of synchronous activity or increased communication. Limbic structures, including the amygdala, demonstrate emerging functional and structural maturity by early adolescence (Giedd et al., 1996, Ostby et al., 2009, Wierenga et al., 2014); however, prefrontal cortical brain regions display a protracted rate of development that extends into the third decade of life (Giedd et al., 1996, Gogtay et al., 2004). As such, it is believed that amygdalar functioning is not down-regulated effectively by medial prefrontal cortex (mPFC) and rostral anterior cingulate cortex (rACC) during adolescence (i.e. Dual Systems Model), which can manifest as heightened emotional reactivity typical of this developmental period (Hare et al., 2008, Perlman and Pelphrey, 2011, McRae et al., 2012, Gee et al., 2013). An imbalance in maturity of frontal and limbic brain regions is likely insufficient, however, to explain the range of behavior in adolescents. Additionally, there are sex differences in the structural development of the amygdala during adolescence (Giedd et al., 1996) in which males demonstrate significant increases in volume that females do not (Giedd et al., 1996), as well as in prefrontal cortices, with girls peaking in gray matter volume approximately two years earlier than boys (Lenroot et al., 2007). These sex-specific structural developmental trajectories may impact concomitant functional connectivity of these brain regions. Previous studies in adults have reported sex differences in amygdala sub-region shape and volume (Kim et al., 2011, Kim et al., 2012) and in RSFC of non-limbic brain regions using a variety of analytic methods (Kilpatrick et al., 2006, Biswal et al., 2010, Tian et al., 2011, Casanova et al., 2012, Satterthwaite et al., 2014). Furthermore, atypical functional connectivity of amygdala-mPFC neurocircuitry has been shown to underlie disrupted emotional and cognitive ability during psychopathologic states, such as schizophrenia, bipolar disorder, and mood disorders (Anand et al., 2005, Das et al., 2007, Henry et al., 2008, Wang et al., 2009, Berking and Wupperman, 2012, Cisler and Olatunji, 2012), many of which emerge in late adolescence and display sex-specific onset and progression of illness.

Examination of the functional interactions of amygdala-cortical neurocircuitry is complicated by the fact that the amygdala is not one homogenous structure (LeDoux, 2003, Price, 2003, Amunts et al., 2005). The amygdala can be subdivided into basolateral (BLA), centromedial (CMA) and superficial (SFA) nuclei, each with distinct functional connections to the cortex supporting different brain functions. The BLA facilitates associative learning processes, like fear conditioning, through afferent projections from the frontal cortex and other subcortical regions (LeDoux, 2003, Phelps and LeDoux, 2005). The CMA is critical in the generation of behavioral responses through projections to the brainstem, striatum, and regions of the cortex (LeDoux, 2003). Finally, the SFA is relevant for olfactory (Price, 2003, Heimer and Van Hoesen, 2006) and affective processes (Bzdok et al., 2013a). Previous work has found distinct functional connectivity patterns, as measured with RSFC, across amygdalar nuclei (Roy et al., 2009, Li et al., 2012, Qin et al., 2012, Gabard-Durnam et al., 2014), specifically different patterns of age-dependent positive connectivity between amygdalar nuclei and ventromedial PFC (vmPFC), temporal, and subcortical regions, as well as negative connectivity with parietal and occipital cortices (Qin et al., 2012, Gabard-Durnam et al., 2014). However, only one study performed a secondary analysis to examine sex differences in adolescents, which did not yield a significant effect of sex (Gabard-Durnam et al., 2014).

The current study examined sex differences in age-dependent RSFC of amygdalar nuclei in a relatively large adolescent sample. Previous studies attempting to characterize developmental differences in amygdalar RSFC have appropriately used samples with broad age ranges spanning childhood and adulthood (Qin et al., 2012, Gabard-Durnam et al., 2014); however, sex differences may be obscured when collapsing data across a variety of developmental stages. Given the dynamic nature of adolescent brain development and sex differences in amygdalar and frontal lobe gray matter maturation (Giedd et al., 1996, Lenroot et al., 2007), additional examination of amygdalar RSFC during adolescence may better address whether sex differences in amygdala sub-nuclei RSFC exist over the span of this period. Previous research has shown coupling of mPFC and all amygdala sub-regions with increasing age (Roy et al., 2009, Qin et al., 2012, Gabard-Durnam et al., 2014), while different studies have also shown protracted prefrontal cortical development through adolescence, with girls showing relative maturity as compared to boys (Giedd et al., 1996, Lenroot et al., 2007). In light of this research, we hypothesized a positive relationship between age and RSFC of amygdalar sub-regions and mPFC across the sampled age range that would be stronger in girls, compared to boys. Sex differences in the developmental trajectory of RSFC of amygdala sub-regions may provide insight on the mechanisms that support sex differences in the onset and progression of mental illness.

2. Materials and Methods

2.1. Participants

Data from an ongoing adolescent neurodevelopment protocol were used for this study. Participants with anatomical magnetic resonance imaging (MRI) and resting state functional (RSFC) MRI data, acceptable amygdala sub-nuclei region of interest (ROI) masks (see *2.5 Definition of amygdala sub-region ROIs)* and limited head movement (see *2.4 Motion*

Correction) were included in functional connectivity analyses. The total sample included 122 adolescents (boys = 71) between the ages of 10 and 16 years. A restricted age range was employed to capture developmental effects and sex differences in amygdalar functional connectivity specific to adolescence.

Written assent and consent from children and their parents, respectively, were obtained in accordance with the Oregon Health & Science University (OHSU) Institutional Review Board. Exclusionary criteria included current (past 12 month) diagnosis of DSM-IV psychiatric disorders, significant substance use (>10 lifetime alcoholic drinks or >2 drinks/occasion, >5 uses of marijuana, any other drug use, or >4 cigarettes per day), neurological illness, significant head trauma, chronic medical problems affecting the central nervous system, prenatal exposure to drugs or alcohol, reported history of psychotic disorders in biological parents, current pharmacological treatment that may affect neural function (e.g. psychoactive medication), the inability of a parent to provide family history information, left-handedness (Edinburgh Handedness Inventory, (Oldfield, 1971)), pregnancy, and MRI contraindications (e.g. braces, irremovable ferrous material).

Once eligibility was established, youth were administered the Wechsler Abbreviated Scale of Intelligence (Weschler, 1999) – short form and the self-rated Pubertal Development Scale (PDS) (Petersen, 1988). Self-report on the PDS has been shown to correlate moderately with other measurements of pubertal status, like Tanner's Sexual Maturation Scale (Bond et al., 2006). Parents of youth were administered the Hollingshead Index of Social Position (Hollingshead, 1975) to determine socioeconomic status (SES), which is based on occupation and educational attainment of each parent.

2.2 Image acquisition

Participants were scanned on a Siemens Trim Trio 3.0 Tesla MRI scanner at the Advanced Imaging Research Center at OHSU. One high-resolution T1-weighted MPRAGE sequence of 9 minutes and 14 seconds was acquired (TR = 2300 ms, TE = 3.58 ms, orientation = sagittal, 256×256 matrix, resolution = 13 mm). BOLD-weighted functional images were collected (along the anterior-posterior commissure) using T2*-weighted echo planar imaging (TR = 2500 ms, TE = 30 ms, flip angle = 90 degrees, FOV = 240 mm^2, 36 slices covering the entire brain, slice thickness = 3.8 mm, resolution = 3.75 × 3.75 × 3.8 mm). Two runs of 4 minutes and 17 seconds of resting state BOLD data were acquired, during which participants were instructed to stay still and fixate on a white cross in the center of a black screen projected from the head of the scanner and viewed with a mirror mounted on 12-channel head coil. The resting state runs were separated by a 10-minute task that was the same for every participant. Following completion of the scan, youth confirmed wakefulness during resting state scans.

2.3 Image processing

Data processing followed commonly used procedures to reduce spurious noise and artifacts (Fair et al., 2007, Fair et al., 2009, Fair et al., 2012, Mills et al., 2012, Costa Dias et al., 2013). In order, these steps included 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. Resting state runs were then concatenated and underwent subsequent processing together. Anatomical images were transformed into 3 mm^3 voxels in standard Talairach space (Talairach and Tournoux, 1988) and used for co-registration of functional data into the same atlas space. Proper co-registration was confirmed by visual inspection (A.C.). Functional data underwent further processing, including temporal band-pass filtering to remove high-frequency noise (0.009 Hz < f < 0.08 Hz), detrending, regression of white matter and ventricular signal from amygdalar ROIs, global signal regression from the whole brain, and regression of white matter, ventricular and whole-brain signal derivatives.

2.5 Definition of amygdala sub-region ROIs

ROIs of amygdala sub-regions were created using cytoarchitectonic maps implemented in the Analysis of Functional NeuroImages (AFNI) suite (Cox, 1996). Bilateral BLA, CMA and SFA ROIs were created in AFNI, based on the Eickhoff-Zilles maximum probability map from post-mortem analysis (Amunts et al., 2005). Although cytoarchitectonic maps are precise microstructural tools, they are based on adult samples that may not be representative of adolescent populations. Therefore, to account for developmental, as well as individual differences, a model-based segmentation method was implemented using FMRIB's Integrated Registration and Segmentation Tool (FIRST) (Patenaude et al., 2011) to create subject-based amygdala ROIs based on grey and white matter boundaries present on participant T1-weighted images. Bilateral ROIs of the entire amygdala were created and visually inspected to confirm accurate segmentation (M.D.R. and G.A.). Final ROIs constituted the overlap from maximum probability maps and FIRST amygdala masks to confirm precise localization of amygdala sub-regions. No voxel corresponded to more than one ROI. On average, BLA ROIs had larger voxel counts (905.1 ± 232.4), followed by SFA (180.8 ± 85.6) and CMA (0.9 ± 2.2), corresponding to anatomical size. However, most CMA voxel counts were quite small and in many cases zero, so we chose not to include CMA ROIs in functional connectivity analyses. BLA size, based on voxel count, was larger in left compared to right hemisphere (t_{120} = 33.41, p < 0.001), while right SFA voxel count was larger than left SFA (t_{120} = −10.71, p < 0.001). Further, voxel counts were not statistically different by sex for any sub-region (all t_{120} < 0.48, p > 0.05). Functional connectivity maps were created from processed RSFC data by correlating average BOLD signal from each sub-region with every voxel in the brain.

2.4 Motion correction

Strict motion correction procedures were applied to resting state functional maps, due to the sensitivity of RSFC data to head motion (Power et al., 2012, Satterthwaite et al., 2012, Van Dijk et al., 2012). TRs with signal intensities exceeding absolute values of 8 (or 0.8% signal change), as measured by the variance of the signal change from the average signal (DVAR), were excluded to limit the effect of head movement on MRI signal (Shannon et al., 2011, Power et al., 2012). DVAR values, which are based in BOLD signal intensity, differ across datasets for a variety of reasons (e.g. blurring kernel size, frequency filter or data acquisition characteristics) (Power et al., 2014), effectively eliminating the need for a standard threshold. Upon examination of the present data and processing methods, which excluded blurring altogether, a threshold of 0.8% was determined to be appropriate. Based on the

DVAR algorithm, frames were removed using a threshold of 40%, such that participants with greater than 40% of frames removed (80 of 200 TRs), were excluded from analyses entirely (Fair et al., 2012, Power et al., 2012, Cservenka et al., 2014). This threshold was employed to ensure that all participants had a minimum of 120 TRs, or approximately 5 minutes of resting state data for analyses, shown to yield sufficiently high sensitivity (77%) for detecting true functional connections (Smith et al., 2011). A final measure, fframewise displacement (FD) (Fair et al., 2012, Power et al., 2012), was measured post-hoc to confirm the remaining volumes had equivalent head movement between males and females. The FD method indexes head movement relative to adjacent volumes and is based on the following scalar formula: $FDi = |\Delta dix| + |\Delta diy| + |\Delta diz| + |\Delta \alpha i| + |\Delta \beta i| + |\Delta \gamma i|)$, where $\Delta dix = d(i-1)x - dix$. Using this formula, an FD remaining mean variable was calculated for every individual, representing the degree of micro-movement (in the range of millimeters) of the previously scrubbed (DVAR) data. Sex differences in FD remaining mean were assessed with an independent samples *t*-test and correlations between FD remaining mean and age were determined with Pearson's correlation. To further confirm our findings were not confounded by head movement, we performed a supplementary analysis using the FD measurement itself for motion censoring (see **Supplementary Methods**).

2.6 Data analysis

2.6.1 Demographic data—Demographic data were examined for normality and outliers (2.5 standard deviations from the mean) within the whole sample and by sex using IBM SPSS Statistics 20 (Armonk, NY: IBM Corp.). No variables exceeded absolute skew/kurtosis values of 2.0, and thus were considered normally distributed. Sex differences for IQ, SES, and age were assessed with independent samples *t*-tests, and PDS sex differences were examined with a Mann-Whitney *U* test.

2.6.2 Imaging data—Using functional connectivity maps, a whole-brain, voxel-wise partial correlation that included age, sex (dummy coded), and sex-by-age was implemented with in-house software using 4dfp tools developed at Washington University as previously utilized (Fair et al., 2012) to assess amygdala sub-region RSFC. Due to a residual correlation between FD remaining mean and age, this variable was included as a covariate in the analyses (see *3. Results*). A Monte Carlo simulation was implemented to account for multiple comparisons (threshold of 53 contiguous voxels with a *z*-value ≥ 2.25). This analysis was conducted four times, once for each sub-region (bilateral BLA and SFA). For each amygdala sub-region, the outcome of these analyses provided effects of a sex-by-age interaction (with age, sex, and FD remaining mean in the model). Values from significant clusters in sex-by-age analyses for each sub-region were extracted and plotted to confirm the interaction effect. The unique effects of age (controlling for sex, sex-by-age, and FD remaining mean) and sex (controlling for age, sex-by-age, and FD remaining mean), were also obtained and are reported in **Supplementary Data**.

2.6.3 Post-hoc Analyses—To account for potential confounds of puberty, post-hoc analyses were conducted using Fisher's Z-transformed correlation coefficients extracted from cortical brain regions that were functionally connected with amygdala sub-regions. The partial correlation models used in the RSFC analyses (**2.6.2 Imaging data**) were

reconstructed in SPSS Statistics 20 (Armonk, NY: IBM Corp.); puberty was then included in the models as an additional covariate to determine if RSFC results remained significant.

3. Results

Boys and girls did not differ statistically by age, IQ or SES, but did differ in pubertal development, although on average, boys and girls reported the same pubertal stage (see Table 1). Age distribution by sex was also statistically similar (see **Supplementary Figure 6**). Prior to data scrubbing, FD values were statistically similar in boys and girls (t_{120} = −1.91, p = 0.06) and FD was negatively correlated with age (r^2 = −0.22, p = 0.02). Following data scrubbing, there were no sex differences in FD (i.e. FD remaining mean) (t_{120} = 0.27, p = 0.79), but age was still negatively correlated with FD (r^2 = −0.31, p = 0.001); therefore, FD remaining mean values were included as a covariate in RSFC analyses.

Although examination of group differences by sex is informative, given the age range of our sample, the interactions between age and sex in amygdala sub-region RSFC was pursued. This examination revealed a dissociation of RSFC of BLA, in which girls had positive RSFC at younger ages that decreased over adolescent development into negative RSFC, while boys had negative RSFC at younger ages that increased over development to positive RSFC. Several sex-by-age interactions in amygdala-cortical connectivity were identified. In left BLA, an interaction of sex and age was seen in left inferior parietal lobule (IPL) and right angular gyrus (AG) that displayed the pattern described above: increased positive connectivity with age in boys and increased negative connectivity with age in girls. In right BLA, an interaction was observed in left superior frontal gyrus (SFG), IPL, precuneus (PC), middle occipital gyrus (MOG) and postcentral gyrus with the same dissociation as left BLA RSFC patterns across the sexes. Patterns of RSFC in the sex-byage interaction of SFA with cortical brain regions diverged, however, in some cases. In left SFA an interaction was seen in right lingual gyrus (LG), left SFG, right cuneus and right culmen, and in all these regions, except left vmPFC, girls had increased positive connectivity with age, and boys had increased negative connectivity with age. Connectivity between left SFA and vmPFC followed the patterns observed in RSFC of BLA with cortical brain regions. Finally, in right SFA, connectivity with left postcentral gyrus also followed the same patterns of RSFC observed with BLA, while connectivity with left dorsomedial PFC (dmPFC) showed the opposite pattern, with girls having increased positive connectivity with age and boys having increased negative connectivity with age (Figures 1-3 and Table 2).

3.1 Post-hoc Analyses

The effect of puberty on amygdala sub-regional RSFC was examined separately post-hoc. Introducing puberty into partial correlation analyses did not significantly change the aforementioned RSFC results (all $r^2 \geq 0.52$, $p \leq 0.001$).

4. Discussion

Analysis of positive and negative resting state functional connectivity (RSFC) in an early- to mid-adolescent sample demonstrated significant age-by-sex interactions in RSFC between

amygdala sub-regions and other cortical brain regions. Both SFA and BLA sub-regions showed decreased connectivity with parieto-occipital cortices and increased connectivity with mPFC, which corresponds with adult RSFC patterns of amygdala sub-regions (Roy et al., 2009, Qin et al., 2012, Gabard-Durnam et al., 2014), albeit with varying effects by sex. Girls and boys exhibited opposing RSFC connectivity patterns that only partially reflect adult functional connectivity of parieto-occipital cortices with sub-regions of the amygdala, in which SFA RSFC is more mature in girls, while BLA RSFC is more mature in boys. Additionally, in contrast to our hypothesis, functional connectivity between amygdala sub-regions and mPFC was not more mature in girls. Rather, with age, boys showed coupling between vmPFC and BLA, while girls showed coupling between dmPFC and SFA, which may indicate relative immaturity of both male and female adolescent fronto-limbic networks in comparison to adults.

4.1 Age-by-sex effects

Analysis of the interactions of sex and age on RSFC of amygdala sub-regions and other cortical brain regions revealed a consistent pattern that differentiated male and female adolescents. Specifically, at younger ages, girls had more positive RSFC between BLA and posterior-occipital cortex that diminished to negative connectivity by the age of 14 years, reflecting increased decoupling of these regions with age. On the other hand, boys showed increased coupling between similar regions with age, with a shift from negative to positive connectivity, also around the age of 14 years. The opposite pattern was observed with functional connectivity of SFA and parieto-occipital brain regions: with age, boys had more robust negative RSFC, while girls had more positive RSFC between SFA and parieto-occipital cortex. Although the groups were not matched on pubertal status, the effect of puberty was examined post-hoc, and this maturational gap did not statistically affect the results of the present analyses. Negative functional connectivity, or segregation and specialization of networks (Rubinov and Sporns, 2010), has been observed between amygdalar sub-regions and more dorsal and posterior brain regions in both adults (Roy et al., 2009) and in a cross-sectional sample spanning childhood and early adulthood (Gabard-Durnam et al., 2014). Therefore, the present results support an interpretation of increased maturity of limbic-parietal-occipital networks supported by BLA in girls and SFA in boys that may underlie distinct cognitive and behavioral profiles.

In contrast, functional connectivity of mPFC-amygdala RSFC appears to be similarly integrated in male and female adolescents, with a few notable distinctions. Specifically, increased positive connectivity between left SFA and left vmPFC with age was observed in boys, while girls showed increased positive connectivity between right SFA and a more dorsal mPFC region. These findings are in line with the Dual Systems Model that posits increasing top-down regulation of the limbic system via mPFC with age (Galvan et al., 2006, Steinberg, 2010); however, the regional specificity of the present findings slightly deviate from this model. Our findings are specific to SFA and lateralized by sex, with functional connections to different regions of the mPFC. Many studies in rodents and humans have functionally differentiated the dorsal and ventral mPFC, and there is general concensus that dmPFC supports cognitive processing, while vmPFC supports more emotional processing (Euston et al., 2012, Bzdok et al., 2013b) or internally-directed activity

(Raichle et al., 2001). The vmPFC is a component of the "ventral affective system" described in the Dual Systems Model, which posits an imbalance in access to affective versus cognitive control brain regions leading to ineffective top-down regulation of the affective system by the cognitive control system (Steinberg, 2010). The relatively delayed maturation of cognitive control regions favors emotional processing by regions like the amygdala and vmPFC in early adolescence; however, with increasing age and maturity, cognitive control brain regions like dorsolateral PFC and dmPFC more effectively regulate limbic regions. Increased coupling between SFA and dmPFC in girls may indicate a more mature pattern of connectivity facilitating top-down regulation by dmPFC. An alternative interpretation is that RSFC between SFA and both ventral and dorsal mPFC support different components of social cognition. The mPFC has been strongly implicated in social cognition and also displays functional segregation along the dorsal-ventral axis (Amodio and Frith, 2006, Pfeifer and Allen, 2012, Bzdok et al., 2013b); therefore, distinct RSFC of SFA sub-regions with vmPFC and dmPFC in boys and girls may functionally relate to different aspect of social cognition. A recent study comparing dmPFC and vmPFC function with meta-analytic connectivity modeling found that vmPFC is primarily involved in bottom-up, approach/avoidance, and evaluation-related processing, while dmPFC was mostly involved in top-down and metacognition-related processing in social cognition (Bzdok et al., 2013b). More research is necessary to confirm behavioral relevance of the RSFC patterns observed in the present study.

Connectivity of the amygdala, as well as its sub-regions, has been studied in human and animal models (Pitkanen et al., 2000, Cunningham et al., 2002, Roy et al., 2009, Bach et al., 2011, Gabard-Durnam et al., 2014), and more recently in children (Qin et al., 2012) and across adolescence (Gabard-Durnam et al., 2014). These and other studies suggest that the amygdala is positively connected with subcortical and limbic regions, and that functional connectivity between these brain regions is stabilized early in childhood development (Gabard-Durnam et al., 2014); however, functional coupling between other cortical regions may be delayed. Previous research examining RSFC of amygdala sub-regions found increased positive connectivity with age (10 – 25 years) between all sub-regions and the mPFC (Gabard-Durnam et al., 2014), which may indicate that integration of the fronto-limbic network is part of a general developmental course. Due to the lack of coupling between BLA and mPFC, the current sample may be displaying some immaturity in fronto-limbic connectivity. The age range of the current sample (10-16 years) is more representative of the adolescent period, therefore he lack of RSFC between mPFC and BLA may also suggest that fronto-limbic connectivity is primarily supported by RSFC between mPFC and SFA during adolescence. Connectivity between mPFC and BLA may be delayed and strengthen later in adolescence or early adulthood. Given that the SFA supports processing of emotional, olfactory and social stimuli (Goossens et al., 2009), its coupling with the mPFC may confer an advantage that supersedes coupling of mPFC with BLA, which supports emotional learning and memory processes, during adolescence. This is interpretation is speculative, however, and requires additional study to verify.

Although all sub-regions are relevant for emotional processing (Ball et al., 2007, Hurlemann et al., 2008, Bzdok et al., 2013a), BLA is additionally important for memory function (Bzdok et al., 2013a); therefore, males demonstrating positive RSFC between parieto-

occipital and BLA may report problems with memory functions. An alternative, and more parsimonious interpretation, is that males rely on a functional coupling between BLA and parieto-occipital brain regions for memory functions during this early- to mid-adolescent period, which is supported by research in adults and adolescents (Darki and Klingberg, 2014, Hill et al., 2014). Cortical regions functionally coupled with bilateral BLA include areas that are important for memory, such as the IPL (Rama et al., 2004) and PC (Lundstrom et al., 2005). Furthermore, SFA is more functionally connected with parieto-occipital cortical regions in females as compared to males. The SFA is the most conserved amygdala sub-region and it is thought to play an important role in social communication via olfactory (Bzdok et al., 2013a) and social cues (Goossens et al., 2009, Bzdok et al., 2013a); therefore, increasing RSFC between this sub-region and the parieto-occipital cortex could indicate that adolescent girls have relatively compromised socio-emotional processing. This interpretation is supported by studies conducted in adults showing functional connectivity of parieto-occipital cortex with SFA is linked with childhood behavioral inhibition (Roy et al., 2014) and current social inhibition (Blackford et al., 2014). A different study showed that administration of allopregnanolone reduced connectivity between the amygdala and parietal cortex, which was also correlated with reductions in negative affect (Sripada et al., 2014). However, these effects have not been confirmed in adolescents. Notably, studies that have examined amygdalar RSFC in children and adolescents with major depressive disorder have found increased connectivity of the amygdala with parietal and occipital cortices (Cullen et al., 2014, Jacobs et al., 2014, Pannekoek et al., 2014); therefore, it is possible that functional coupling of SFA and parieto-occipital regions is present in healthy girls, but may partly account for the increased risk for depression in females. Alternatively, girls may rely on regions such as the cuneus and LG, which are implicated in emotional face processing (Bremner et al., 2004, Kitada et al., 2010, Kret et al., 2011), for socio-emotional processing as a distinct mechanism supporting neural functional connectivity patterns that in turn manifest into sexually dimorphic social behavior. One study in adults found that negative connectivity between SFA and the temporoparietal junction is positively related to harm avoidance and that this relationship is more robust in women, compared to men (Li et al., 2012), demonstrating that functional decoupling of SFA and parietal cortex is relevant for social communication and behavior, but that behavioral outcomes may differ depending on sex. If decoupling of SFA and parieto-occipital cortices is indeed the norm in adulthood as some studies suggest (Roy et al., 2009, Gabard-Durnam et al., 2014), then the sexually dimorphic trajectories of amygdala sub-regional connectivity observed in the current sample may give way to mature patterns observed in adults; however, research comparing sex differences in adolescents and adults will be necessary to confirm this hypothesis.

4.2 Strengths and limitations

The present study carefully examined the interaction between age and sex in RSFC of superficial and basolateral amygdalae in a sample of adolescents. The intersection of sex and age is particularly relevant during this period of development, as striking biological and social changes take place that influence neurophysiologic function, often in a sexually dimorphic fashion. The large sample size, careful delineation of amygdala sub-regions, and meticulous assessment of head motion and subsequent data scrubbing (see *2.4 Motion Correction* and **Supplementary Data**) inspires confidence in the reported results. However,

some limitations of the study should be noted. For example, although the data processing and motion censoring approach employed by the current study is appropriate for the stated aims, we tested the robustness of our findings by adjusting the functional connectivity processing steps and employing a different method of motion censoring in a supplementary analysis (see **Supplementary Methods**), as suggested by prior reports (Hallquist et al., 2013, Power et al., 2014). The results of this analysis show consistency with the reported findings, albeit some findings, potentially due to reduced power, remained trend like and did not reach significance. Second, due to our strict delineation of amygdala sub-regions (see *2.5 Definition of amygdala sub-region ROIs*), we could not confidently ascertain that CMA ROIs indeed corresponded to anatomical CMA. Because the CMA is the smallest nucleus of the amygdala, many participants had CMA ROIs with as little as one voxel and as many as ten corresponding to this region; the majority of participants had zero voxels that confidently corresponded to the CMA (48% in left CMA and 100% in right CMA). As such, RSFC analyses were not conducted with either left or right CMA seed regions, which limits direct comparisons with existing amygdala sub-region RSFC studies (Roy et al., 2009, Li et al., 2012, Qin et al., 2012, Gabard-Durnam et al., 2014). Of these studies, two used the same Eickhoff-Zilles maximum probability maps (Amunts et al., 2005) to determine sub-regions; however, they did not account for developmental and individual variation in anatomy by masking with model-based (Patenaude et al., 2011) segmented amygdalar ROIs (Qin et al., 2012, Gabard-Durnam et al., 2014), as was done in the current study. Accounting for developmental differences in brain maturation is crucial when studying young populations. Previous studies have shown that throughout the span of adolescence, different brain regions reach peak gray matter volume at distinct rates (Giedd, 2004, Gogtay et al., 2004, Creze et al., 2014), with concurrent changes in neurophysiology (Whitford et al., 2007). Due to the dynamic nature of brain development, we believe a more stringent classification of amygdala sub-regions outweigh the benefits of including a CMA ROI in our analyses.

An additional limitation of this study is its cross sectional design, which is not ideal for capturing developmental effects. While the current study included a relatively large sample, future research should attempt to utilize longitudinal designs to confirm the present findings. Direct comparisons with adult samples may also provide valuable insight about RSFC patterns that are unique to the adolescent period. The current study design also limits conclusions about causal relationships of age and sex on functional connectivity of the brain. Our measurements reflect a snapshot of brain RSFC over a sample of male and female adolescents at varying ages, which provide information about the developmental course of functional connectivity as a function of sex. Lastly, our measure of puberty shares only modest concordance with clinician evaluations of pubertal girls (Brooks-Gunn et al., 1987), which we did not have access to, and a moderate agreement with self-reported Tanner staging (Bond et al., 2006), limiting our interpretation of true pubertal effects. However, we can conclude that sex differences in our measure of puberty did not statistically impact age-by-sex RSFC results.

4.3 Conclusions

The current study demonstrated sex-specific trajectories of amygdala sub-region RSFC in a large sample of early- to mid-adolescents. Our hypothesis predicting increased coupling

between fronto-limbic regions in girls was not supported. Rather, both sexes showed comparable patterns of functional coupling, which is in accordance with a healthy developmental trajectory (Roy et al., 2009, Qin et al., 2012, Gabard-Durnam et al., 2014). However, there was a laterality effect that differentiated the sexes in frontal-limbic RSFC. Specifically, boys showed functional coupling between left SFA and vmPFC, while girls showed coupling between right SFA and dmPFC. With increasing age, boys also displayed more mature decoupling of SFA and parieto-occipital cortex, compared to girls who showed immature functional coupling between these regions. Conversely, girls had more mature decoupling of BLA and parieto-occipital cortical regions, while boys showed immature functional coupling between these same areas. Overall, these results indicate a sex-specific dissociation in amygdala sub-region RSFC with cortical brain regions, which underscores the importance of both the examination of sex differences in adolescent samples and of amygdalar function by sub-region, potentially in the contexts of socio-emotional processing and with populations at risk for, or suffering from, depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Amygdalar functional coupling with parieto-occipital cortex decreases with age.
- Amygdalar functional coupling with medial frontal cortex increases with age.
- Boys have more integration of basolateral amygdala and parieto-occipital cortex.
- Girls have more integration of superficial amygdala and parieto-occipital cortex.
- Adolescents show integration of superficial amygdalae and medial frontal cortex.

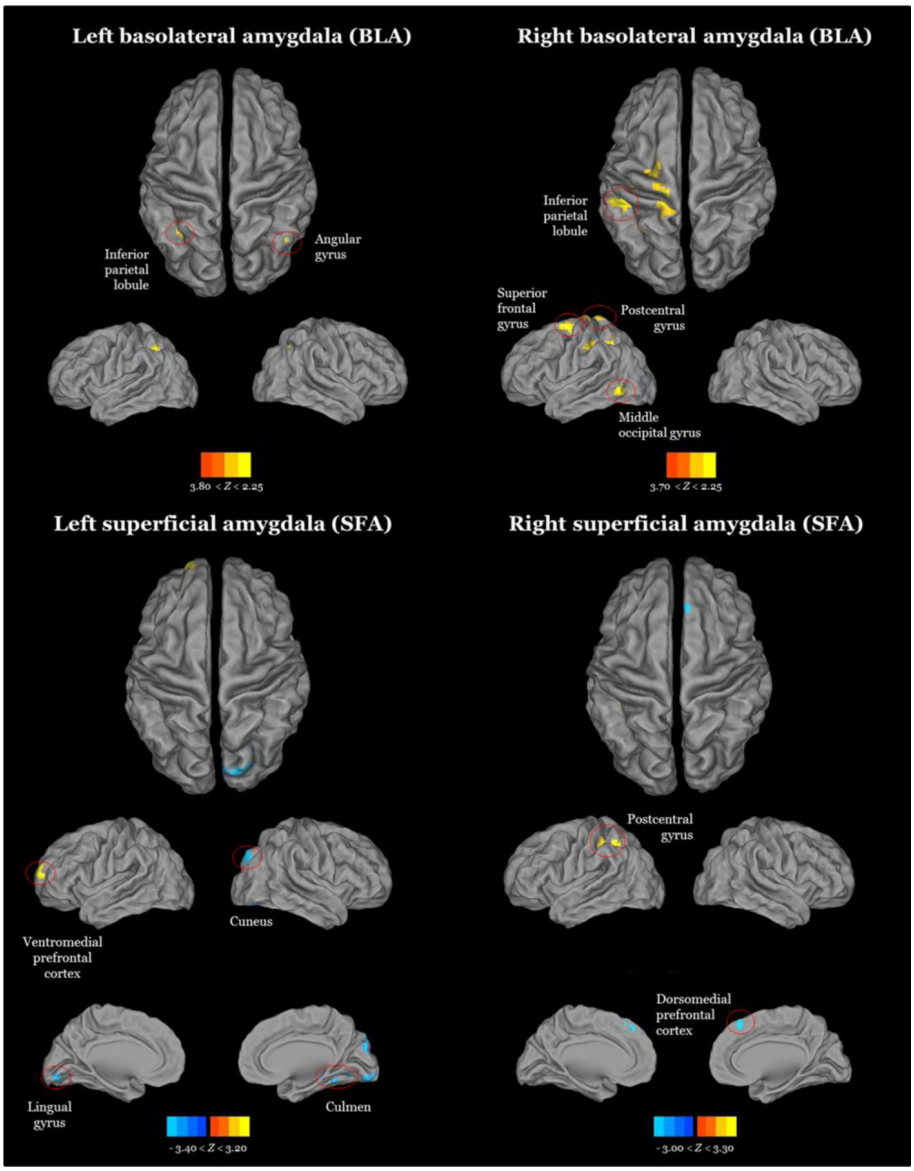

Figure 1.

Dorsal and lateral surface mapped results showing significant age-by-sex interactions (blue: negative, yellow: positive) in the coupling of amygdala sub-nuclei and other brain regions. *Top left*: Age-by-sex interaction with the left basolateral amygdala. *Top right*: Age-by-sex interaction with the right basolateral amygdala. *Bottom left*: Age-by-sex interaction with the left superficial amygdala. *Bottom right*: age-by-sex interaction with the left basolateral amygdala. All findings underwent Monte Carlo multiple comparisons correction ($z \geq 2.25$).

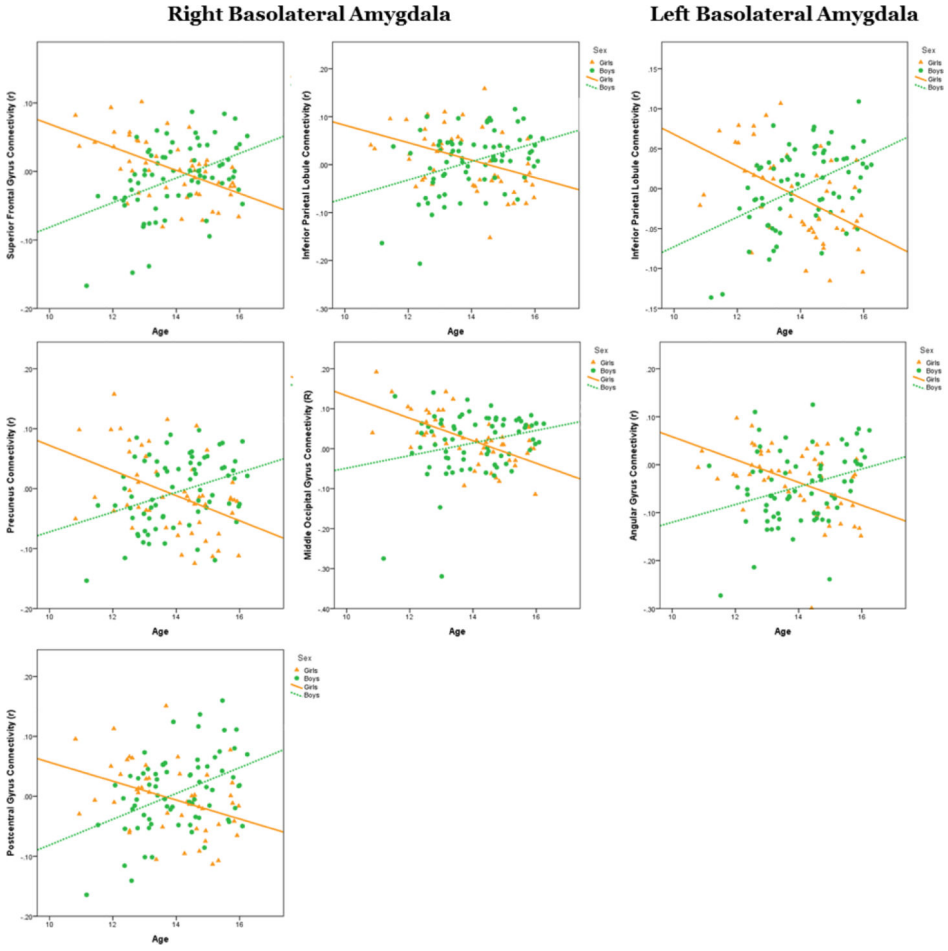

Figure 2.

Fisher's Z transformed *R*-values from brain regions with significant functional connectivity age-by-sex interactions with left basolateral amygdala (left) and right basolateral amygdala (right) were extracted and plotted against age and by sex.

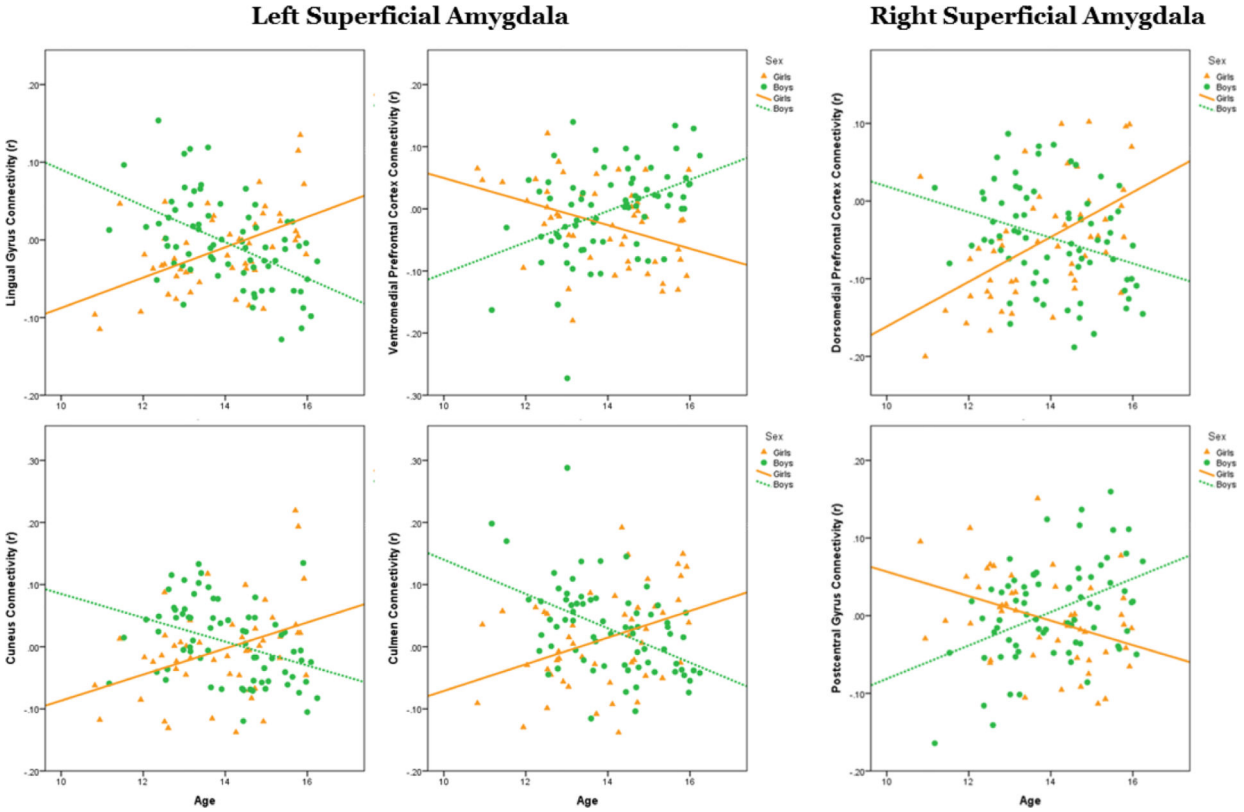

Figure 3.

Fisher's Z transformed *R*-values from brain regions with significant functional connectivity age-by-sex interactions with left superficial amygdala (left) and right superficial amygdala (right) were extracted and plotted against age and by sex.

Table 1

Demographics

	Girls (n=51)	Boys (n=71)	Statistic
Age (years)	13.8 (1.4)	14.1 (1.2)	t = 0.90
[a]IQ	113.3 (9.3)	113.3 (11.0)	t = 0.04
[b]SES	27.6 (12.3)	29.6 (13.3)	t = 0.85
[c]Puberty	3.8 (0.8)	3.1 (0.9)	U = 4.80**

[a]Wechsler Abbreviated Scale of Intelligence

[b]Hollingshead Index of Social Position; larger values indicate lower socioeconomic status (middle class corresponds to 32-47 range)

[c]Crockett Pubertal Development Scale; Values range from 1-5, with larger values referring to more advanced pubertal development.

**Indicates $p < .001$.

Table 2

Age-by-Sex changes in functional connectivity (RSFC) with amygdala sub-regions.

Structure	BA	Voxels (mm^3)	Peak Talairach coordinates (x, y, z)	Boys Direction of *positive* FC change with increasing age	Boys Direction of *negative* FC change with increasing age	Girls Direction of *positive* FC change with increasing age	Girls Direction of *negative* FC change with increasing age	Z-score
Left Basolateral								
L Inferior Parietal Lobule	7	132	−32, −69, 21	↑			↑	2.66
R Angular Gyrus	39	57	34, −51, 30	↑			↑	2.67
Right Basolateral								
L Superior Frontal Gyrus	6	161	−32, −9, 39	↑			↑	2.71
L Precuneus	7	86	−28, −51, 39	↑			↑	2.79
L Middle Occipital Gyrus	19	67	−56, −57, −18	↑			↑	2.63
L Inferior Parietal Lobule	40	107	−32, −27, 30	↑			↑	2.73
L Postcentral Gyrus		57	−16, −21, 69	↑			↑	2.67
Left Superficial								
R Lingual Gyrus	18	110	32, −81, −21		↑	↑		−2.58
L Ventromedial Prefrontal Cortex	10	70	−20, 57, 12	↑			↑	2.69
R Cuneus	19	123	4, −87, 18					−2.58
R Culmen		88	16, −39, −21		↑	↑		−2.61
Right Superficial								
L Postcentral Gyrus	3	129	−28, −27, 30		↑	↑		2.63
R Dorsomedial Prefrontal Cortex	8	70	2, 39, 39		↑	↑		−2.91

L=Left, R=Right.