

Elevated Amygdala Perfusion Mediates Developmental Sex Differences in Trait Anxiety

Antonia N. Kaczkurkin, Tyler M. Moore, Kosha Ruparel, Rastko Ciric, Monica E. Calkins, Russell T. Shinohara, Mark A. Elliott, Ryan Hopson, David R. Roalf, Simon N. Vandekar, Efstathios D. Gennatas, Daniel H. Wolf, J. Cobb Scott, Daniel S. Pine, Ellen Leibenluft, John A. Detre, Edna B. Foa, Raquel E. Gur, Ruben C. Gur, and Theodore D. Satterthwaite

ABSTRACT

BACKGROUND: Adolescence is a critical period for emotional maturation and is a time when clinically significant symptoms of anxiety and depression increase, particularly in females. However, few studies relate developmental differences in symptoms of anxiety and depression to brain development. Cerebral blood flow is one brain phenotype that is known to have marked developmental sex differences.

METHODS: We investigated whether developmental sex differences in cerebral blood flow mediated sex differences in anxiety and depression symptoms by capitalizing on a large sample of 875 youths who completed cross-sectional imaging as part of the Philadelphia Neurodevelopmental Cohort. Perfusion was quantified on a voxelwise basis using arterial spin-labeled magnetic resonance imaging at 3T. Perfusion images were related to trait and state anxiety using general additive models with penalized splines, while controlling for gray matter density on a voxelwise basis. Clusters found to be related to anxiety were evaluated for interactions with age, sex, and puberty.

RESULTS: Trait anxiety was associated with elevated perfusion in a network of regions including the amygdala, anterior insula, and fusiform cortex, even after accounting for prescan state anxiety. Notably, these relationships strengthened with age and the transition through puberty. Moreover, higher trait anxiety in postpubertal females was mediated by elevated perfusion of the left amygdala.

CONCLUSIONS: Taken together, these results demonstrate that differences in the evolution of cerebral perfusion during adolescence may be a critical element of the affective neurobiological characteristics underlying sex differences in anxiety and mood symptoms.

Keywords: Adolescence, Amygdala, Anxiety, Cerebral blood flow, Depression, Insula, Perfusion

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Adolescence is a critical developmental period during which sex differences in behavior and appearance intensify. Additionally, increasing evidence suggests that adolescence also signals the emergence of sex differences in brain structure (1–3) and function (4–14). Furthermore, the disproportionate prevalence of anxiety and mood disorders in females also manifests in adolescence (15). Although anxiety is apparent in children, the incidence and diagnostic severity of anxiety and depression increase in adolescence and early adulthood (15–17). However, few studies use large samples to link sex differences in brain development to sex differences in psychiatric symptoms. The current study uses a measure of cerebral blood flow (CBF) to examine such relationships.

Prior studies of the functional neuroanatomic features of anxiety disorders implicate a network of brain regions associated with emotion processing, affect regulation, and salience detection (18–20). Previous functional magnetic resonance imaging (MRI) investigations link both anxiety and depressive disorders to greater activation in the amygdala, insula, and anterior cingulate cortex compared with healthy participants

(21,22). The concordance between findings in anxiety and depressive disorders is supported by factor analyses of clinical psychopathology data, which indicate the presence of an “anxious-misery” dimension of psychopathology encompassing highly comorbid mood and anxiety symptoms (23). The presence of anxious-misery symptoms is associated with anterior cingulate cortex and medial prefrontal cortex dysfunction (24). Additionally, studies link brain activation measured with task-based functional MRI paradigms to both transient state anxiety (25,26) and long-standing trait anxiety (27,28). Trait anxiety in particular is associated with both anxiety and mood symptoms and can be conceptualized as a measure of anxious-misery (29–31). Thus, convergent evidence across multiple studies suggests that anxiety and mood symptoms are associated with abnormalities in affective regions such as the amygdala and insula.

Despite such research, evidence regarding sex differences in affective circuitry relevant to anxiety and mood disorders is relatively limited. One relevant prior study suggested that higher anxiety is associated with greater amygdala responses

SEE COMMENTARY ON PAGE e81

to unattended fearful faces in female participants, but not male participants (32). Similarly, another report documented greater activation in the amygdala and anterior cingulate in women compared with men during fear conditioning (33). However, it remains unknown whether sex differences in brain development may explain in part differential vulnerability to anxiety and mood symptoms in female adolescents. Furthermore, most studies have considered relatively small samples, which limit joint analyses of anxiety, brain development, and sex differences during adolescence.

Prior studies principally have examined affective circuitry using task-based or resting-state functional MRI, which measures changes in evoked or correlated brain function. Research suggests that CBF, which is tightly coupled to regional brain metabolism (34,35), may be a potentially important brain phenotype for understanding developmental sex differences in anxiety and mood symptoms. Previous work in adults has established that women have greater CBF than men (36–38). We recently demonstrated that this sex difference in CBF unfolds with puberty (13). Furthermore, prior research in adults also links regional CBF in affective regions such as the amygdala to symptoms of anxiety and depression (39–42). When considered together, these related findings suggest that age- and sex-related differences in the risk of anxiety and mood disorders may be correlated with corresponding changes in CBF.

Accordingly, in this study we evaluated the hypothesis that emerging sex differences in the perfusion of affective circuits during adolescence mediates sex differences in anxiety. We leveraged a large sample of children, adolescents, and young adults imaged using arterial spin labeled (ASL) MRI as part of the Philadelphia Neurodevelopmental Cohort (PNC) (43–45) to test three specific predictions. First, we predicted that greater levels of anxiety would be associated with greater CBF in affective regions such as the amygdala. Second, following our prior work, we predicted that females would have higher perfusion in these affective regions as adolescence progresses. Third, we examined whether higher CBF in affective regions mediates higher levels of anxiety in females.

METHODS AND MATERIALS

Participants

A total of 1601 youths were imaged as part of the PNC (43–45). Of these participants, 170 were excluded for the following reasons: medical disorders that could impact brain functioning ($n = 73$), nonpsychiatric medication use that could affect central nervous system functioning ($n = 78$), or substantial structural brain abnormalities resulting in frankly abnormal brain anatomy ($n = 20$) (46); one participant was excluded on the basis of two criteria. Of the remaining 1431 participants, children younger than 12 years were not considered in the present analyses ($n = 373$) because of the recommendation of a fifth-grade reading level for the State-Trait Anxiety Inventory (STAI) (47), which was our primary measure of anxiety and mood symptoms. Of these 1058 participants, 183 participants were excluded for missing clinical data, missing STAI data, failure to complete perfusion imaging, or failure to meet image quality assurance protocols; many participants were excluded for multiple reasons. This

yielded a final sample of 875 participants (mean age, 16.5 years; range, 12–23 years; SD, 2.7 years; 398 boys), with similar numbers of male and female participants excluded. Among this final sample, 105 participants (12%) were taking psychotropic medications at the time of imaging. The impact of medication use was evaluated in sensitivity analyses, as described below.

Clinical Assessment

Lifetime psychopathology was determined using DSM-IV Text Revision criteria (48). Demographic data by screening category are summarized in Table 1. As for prior work in this dataset (13), puberty was coded as a categorical variable with three classes: early pubertal (Tanner stages 1–3), midpubertal (Tanner stage 4), and postpubertal (Tanner stage 5); for the number of subjects in each pubertal stage, see Supplemental Table S1. For additional details regarding the clinical assessment, see Supplemental Methods.

Dimensional Characterization of Mood and Anxiety Symptoms

The STAI measures transient, situational levels of anxiety (state anxiety) as well as general and long-standing levels of anxiety (trait anxiety). Past work examining the STAI has shown that trait anxiety comprises a mix of both anxiety and mood symptoms (29–31), consistent with the dimension of anxious-misery (23). To obtain a measure of state and trait anxiety free of response bias shown in previous research (30,49), we conducted separate factor analyses of each scale using a confirmatory bifactor model implemented in MPlus (Muthén & Muthén, Los Angeles, CA) (50–53) (see Supplemental Methods and Supplemental Tables S2 and S3 for factor loadings). Relationships between the summary scores and demographic data such as age, sex, and pubertal phase were explored using linear models; all puberty analyses included age as a covariate. Additionally, we examined how summary scores of state and trait anxiety related to psychopathology screening categories.

Image Acquisition, Preprocessing, and Perfusion Quantification

Image acquisition and processing are reported in detail elsewhere (43,44); see Supplemental Methods for details. Briefly, a custom spin-echo pseudocontinuous ASL sequence was used to measure brain perfusion. After distortion correction, data were processed with FSL (FMRIB Software Library, Oxford, United Kingdom) (54) including skull removal, motion correction, spatial smoothing (6 mm full width at half maximum), and intensity normalization. CBF was quantified from control-label pairs using ASL Toolbox (55). As in a previous study (13), the T1 relaxation parameter was modeled on an age- and sex-specific basis (56). This model accounts for the fact that T1 relaxation time differs according to age and sex and has been shown to enhance the accuracy and reliability of results in developmental samples (57). Participant-level CBF images were coregistered to T1 images using boundary-based registration (58), normalized to the Montreal Neurologic Institute (MNI) 152 1-mm template using the top-performing symmetric image normalization deformable registration included in

Table 1. Summary of Demographic Data by Screening Category

	<i>n</i>	Mean Age (SD), Years	Female (%)	Caucasian (%)	Mean Years Maternal Education (SD)
Typically Developing	236	16.85 (2.88)	47	56.8	14.76 (2.62)
ADHD	140	15.55 (2.36)	42.1	38.6	14.07 (2.45)
Agoraphobia	62	16.39 (2.26)	75.8	29	13.63 (2.15)
Anorexia	10	16.47 (2.27)	70	40	13.1 (1.85)
Bulimia	4	18.31 (1.04)	100	100	15.5 (2.52)
Conduct Disorder	85	16.47 (2.31)	49.4	14.1	12.83 (2.02)
Major Depression	146	17.66 (2.17)	67.8	43.2	13.79 (2.34)
GAD	16	16.86 (2.47)	62.5	56.2	14.19 (2.83)
Mania	8	17.17 (3.06)	75	25	14.14 (1.86)
OCD	29	17.47 (2.42)	72.4	34.5	13.34 (2.33)
ODD	320	16.04 (2.37)	51.6	32.5	13.69 (2.39)
Panic	9	15.5 (3.02)	55.6	33.3	13.11 (1.45)
Specific Phobias	273	16.37 (2.52)	69.6	39.9	13.95 (2.32)
Psychosis Spectrum	302	16.24 (2.78)	54	29.1	13.67 (2.14)
PTSD	125	17.03 (2.55)	70.4	32	13.52 (2.26)
Separation Anxiety	41	16.79 (2.22)	68.3	56.1	14.1 (2.44)
Social Phobia	242	16.28 (2.52)	60.3	34.3	13.79 (2.37)

ADHD, attention-deficit/hyperactivity disorder; GAD, generalized anxiety disorder; OCD, obsessive-compulsive disorder; ODD, oppositional defiant disorder; PTSD, posttraumatic stress disorder.

Because of comorbidity, individual participants may be present in more than one category.

Advanced Normalization Tools (59–61), and downsampled to 2-mm voxels before analysis. All transformations were concatenated so that only one interpolation was performed.

Group-Level Analyses

As a first step, to identify regions where perfusion was related to summary scores of state or trait anxiety, we conducted a whole-brain voxelwise analysis. Both linear and nonlinear age effects were modeled flexibly using penalized splines within a generalized additive model (62,63). Furthermore, to control for confounding effects of brain structure on estimates of CBF (64), gray matter density calculated using Atropos (59) was modeled on a voxelwise basis.

$$\text{CBF}_{\text{vox}} = \text{trait anxiety} + \text{state anxiety} + \text{sex} + \text{spline}(\text{age}) \\ + \text{spline}(\text{age}, \text{by} = \text{sex}) + \text{gray matter density}_{\text{vox}} \\ + \text{in-scanner motion}$$

where vox = voxels. Type I error for voxelwise whole-brain analyses was controlled using AFNI (Analysis of Functional NeuroImages, Bethesda, MD) AlphaSim (65) with a minimum voxel significance of $z > 2.58$ and a corrected cluster significance of $p < .0001$ (minimum cluster size $k = 146$).

Supplementary analyses tested alternative models and covariates such as excluding participants taking psychoactive medication, including race and maternal education as covariates, examining the effect of the motion covariate, modeling state and trait in separate models, and using raw STAI scores that were not factor analyzed to remove response bias. For details regarding group level analyses, see [Supplemental Methods](#). Additionally, we examined whether regions associated with dimensional symptom severity were linked specifically to certain diagnostic groups with at least 100 participants (see [Supplemental Methods](#) for details); results are reported as covariate-adjusted effect sizes (Cohen's d).

Analyses of Interactions With Age and Puberty

Based on our previous work in this dataset showing that CBF is higher in females than males during puberty (13), we next evaluated whether interactive effects with age and sex were present within regions identified by the voxelwise analysis as anxiety relevant. Analyses included modeling interactions between anxiety and age, between sex and age, and between anxiety and pubertal stage (while controlling for age). For additional details regarding each of these analyses, see [Supplemental Methods](#). To control for multiple comparisons across clusters, we used the false discovery rate (FDR; $Q < 0.05$).

Mediation Analysis

As described below, the above analyses revealed that post-pubertal young women have higher trait anxiety and also that higher CBF in affective regions was associated with greater trait anxiety. Accordingly, we investigated whether higher levels of anxiety in postpubertal females were mediated by higher CBF. The indirect effect of sex on trait anxiety through the proposed mediator (perfusion) was tested using both the Sobel test and bootstrapping procedures (66) (see [Supplemental Methods](#) for details).

Amygdala Resting State Functional Connectivity

As a final step, to further evaluate the functional significance of increased left amygdala perfusion, we conducted a seed-based functional connectivity analysis in a sample of 592 participants who also received resting-state imaging as part of the PNC, using previously detailed methods (67–70) (see [Supplemental Methods](#) for details). Group-level analyses used the same procedures, models, and type I error correction as for ASL data.

RESULTS

Postpubertal Young Women Have Higher Trait Anxiety

Sex differences in trait anxiety levels were found in postpubertal adolescents, with females endorsing higher levels of trait anxiety than males ($t_{449} = 2.50, p = .013$). No significant sex differences were found for trait anxiety in the early-pubertal or midpubertal groups ($p \geq .194$). Female participants also endorsed higher levels of state anxiety in both the postpubertal group ($t_{449} = 2.09, p = .038$) and the midpubertal group ($t_{274} = 2.18, p = .030$), but not in the early-pubertal group ($p = .54$). As displayed in [Supplemental Figure S1](#), the levels of state and trait anxiety were high across all screening diagnostic categories (which because of comorbidity are not mutually exclusive categories) compared with those of typically developing youth.

Trait But Not State Anxiety Is Associated With Elevated Perfusion of Affective Regions

We used voxelwise mass-univariate analyses to test the prediction that greater levels of anxiety would be associated with greater CBF in affective circuitry. Even while controlling for state anxiety, higher levels of trait anxiety were associated with increased perfusion in a network of regions involved in emotion processing, including the left amygdala, bilateral insula, and bilateral fusiform gyrus ([Figure 1](#); see [Table 2](#) for a complete list of regions). The dorsal anterior cingulate was significant at a slightly more liberal cluster threshold ([Supplemental Table S4](#)).

State anxiety was associated with decreased perfusion in lateral occipital cortex ($k = 206$, maximum $z = 3.6$, peak MNI coordinates: $-40, -86, -10$), but this result was not stable in supplementary analyses. In contrast, trait anxiety results were consistent over a range of models, subsamples, and covariates. Specifically, trait anxiety results were similar when state and trait anxiety were analyzed in separate models ([Supplemental Table S5](#)), when participants taking psychotropic medications were excluded and participant race and maternal education were also included in the model ($n = 766$; [Supplemental Table S6](#)), and when raw total scores from STAI were used instead of factor scores ([Supplemental Table S7](#)). Notably, these results could not be attributed to motion: CBF in the left amygdala was related to motion (see [Supplemental Figure S2](#)), but motion was not related to trait anxiety ($r = -.01, p = .86$).

We additionally examined whether elevated amygdala perfusion was associated with specific screening diagnostic categories. Notably, differences in amygdala perfusion between screening diagnostic categories and typically developing youth showed small to medium effect sizes for all categories ($d = 0.15-0.30$). See [Supplemental Table S8](#) for covariate-adjusted Cohen's d for each screening category.

Brain Regions Associated With Trait Anxiety Show Developmental Sex Differences

In our prior work in this dataset, we demonstrated developmental sex differences in cerebral perfusion, whereby CBF becomes higher in females than males as adolescence progresses ([13](#)). Accordingly, we next tested the prediction that there would be prominent sex differences in the regions

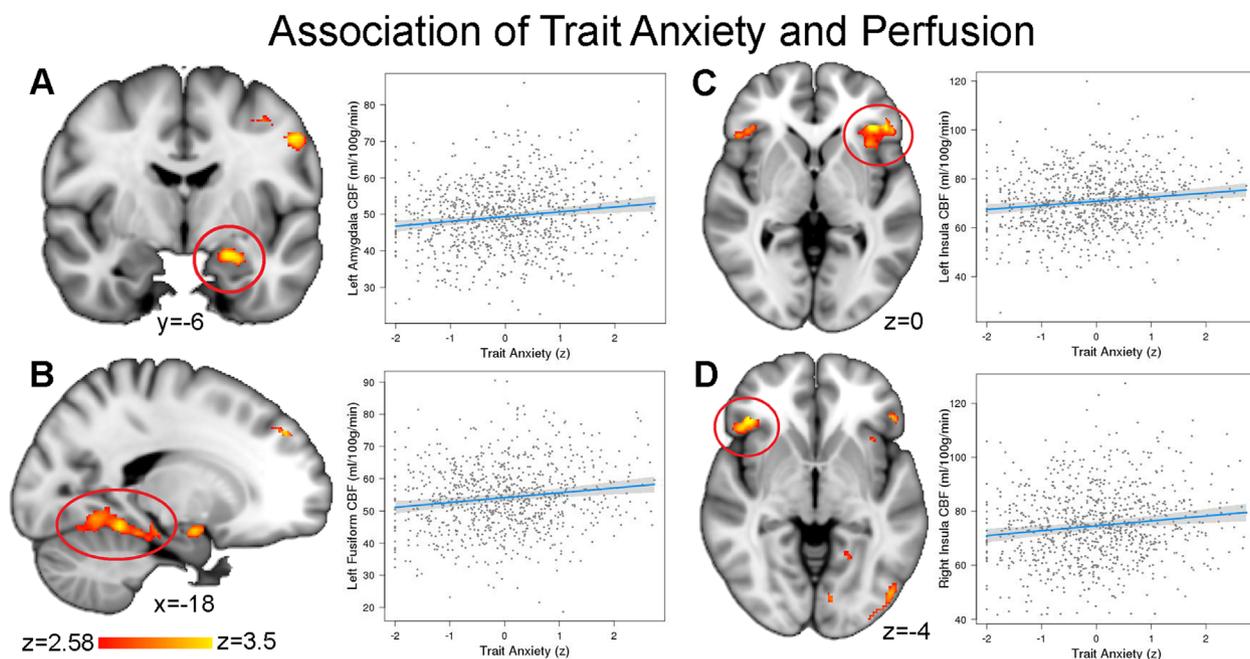


Figure 1. Elevated perfusion in affective regions is associated with trait anxiety. Higher perfusion was associated with higher trait anxiety in the (A) left amygdala, (B) fusiform gyrus, (C) left anterior insula, and (D) right anterior insula (see [Table 2](#) for a complete list). Images thresholded at $z > 2.58$, cluster corrected $p < .0001$. CBF, cerebral blood flow.

Table 2. Regions Where Trait Anxiety Was Associated With Increased Perfusion

Region	k	Maximum z	Peak MNI Coordinates		
Left Fusiform	1036	4.39	-36	-58	-14
Left Insula	560	4.00	-44	30	2
Left Precentral Gyrus	275	3.70	-48	-10	38
Left Frontal Pole	263	3.82	-26	44	36
Right Insula	218	3.99	46	30	-8
Left Inferior Lateral Occipital Cortex	193	3.62	-38	-88	-8
Left Amygdala	168	3.63	-20	-4	-16
Right Frontal Pole	148	3.75	30	40	-12

MNI, Montreal Neurological Institute.

Clusters considered significant if $z > 2.58$, $k > 146$ in a sample of 875 subjects.

identified by their significant association between CBF and trait anxiety. As expected, perfusion in these regions showed a divergent pattern between females and males in adolescence (Figure 2 and Table 3): whereas perfusion declined throughout adolescence in males, perfusion displayed a quadratic or increasing pattern in females. As a result, perfusion was greater in female participants than in male participants during the postpubertal period, but not in other pubertal stages. These results demonstrate that regions where trait anxiety is associated with CBF show marked sex differences in their developmental patterns.

Association Between Perfusion and Trait Anxiety Increases With Development

Next, we investigated how the association between trait anxiety and CBF differs with age and puberty within those

regions. Multiple comparison-corrected results revealed significant trait anxiety-by-age interactions in the left amygdala ($t_{864} = 2.66$, $p_{FDR} = .017$), left insula ($t_{864} = 2.50$, $p_{FDR} = .017$), and right insula ($t_{864} = 2.40$, $p_{FDR} = .017$; Figure 3). No other regions had a significant trait anxiety-by-age interaction. These results suggest that the association between trait anxiety and perfusion of affective regions becomes stronger with age. Next, in clusters where a significant anxiety-by-age effect was present, we examined whether this effect could be attributable specifically to puberty, while controlling for age. Notably, there was a significant trait anxiety-by-puberty interaction in the left amygdala after correction for multiple comparisons ($t_{854} = 2.48$, $p_{FDR} = .039$). As displayed in Figure 4A, this interaction suggests that the relationship between amygdala perfusion and anxiety is higher in later pubertal stages in this sample, above and beyond the effects of age. No significant interactions between trait anxiety and puberty were found in the left or right insula ($p_{FDR} \geq .073$). All sensitivity analyses revealed the same significant pattern of results.

Elevated Levels of Anxiety in Postpubertal Young Women Is Mediated by Amygdala Perfusion

Having established that postpubertal young women have higher levels of both anxiety and amygdala perfusion, we tested the prediction that amygdala perfusion mediates postpubertal sex differences in trait anxiety. Mediation analysis revealed a significant indirect effect (95% confidence interval, 0.02–0.12; $z = 2.29$, $SE = 0.03$, $p = .022$), suggesting that higher trait anxiety levels in postpubertal young women may be mediated in part by higher perfusion in the left amygdala (Figure 4B). The significance of this mediation effect is

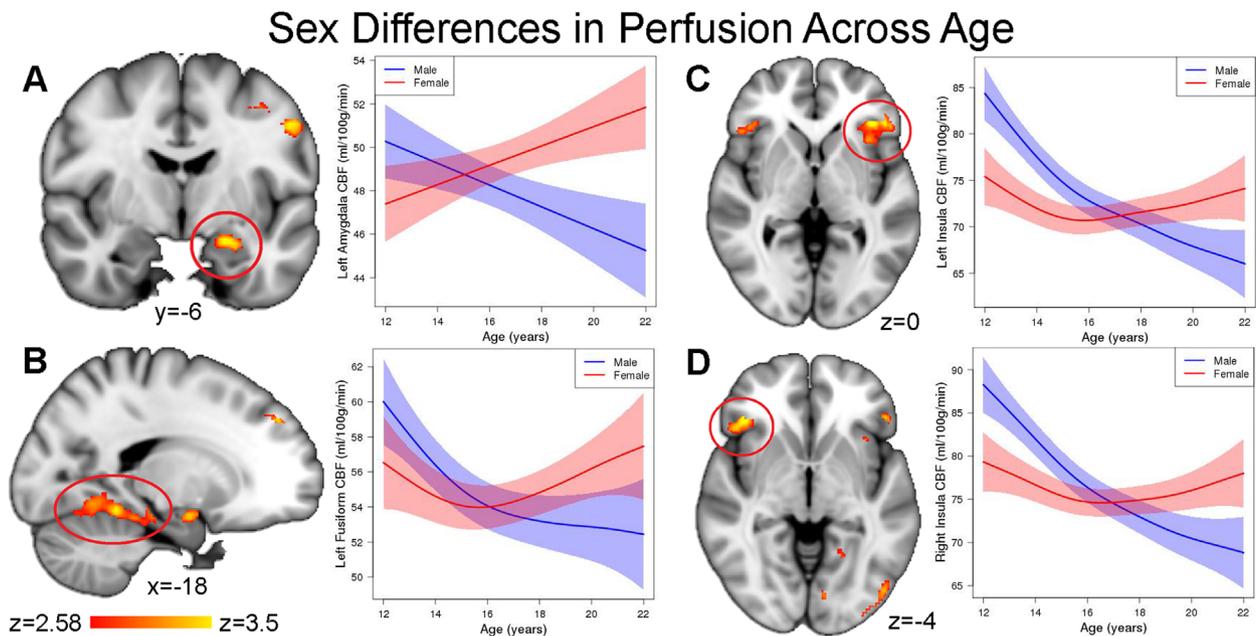


Figure 2. Regions where cerebral blood flow (CBF) is linked to trait anxiety also show marked developmental sex differences. CBF declined over time in males (blue), whereas perfusion increased in females (red) in the (A) left amygdala, (B) fusiform gyrus, (C) left anterior insula, and (D) right anterior insula (also see Table 3).

Table 3. Developmental Sex Differences (Age-by-Sex Interaction) in Regions Where a Significant Association Between Trait Anxiety and Perfusion Was Present

Region	<i>F</i>	<i>p</i>	<i>p</i> _{FDR}
Left Fusiform	9.05	.003	.003 ^a
Left Insula	29.82	<.001	<.001 ^a
Left Precentral Gyrus	20.57	<.001	<.001 ^a
Left Frontal Pole	2.86	.055	.055
Right Insula	25.45	<.001	<.001 ^a
Left Inferior Lateral Occipital Cortex	12.70	<.001	.001 ^a
Left Amygdala	14.81	<.001	<.001 ^a
Right Frontal Pole	4.02	.006	.007 ^a

*p*_{FDR}, false discovery rate-corrected *p* value.

^aSignificant at $\alpha < .05$.

emphasized by the fact that when controlling for mean perfusion in the left amygdala, the relationship between sex and trait anxiety became nonsignificant.

Trait Anxiety Is Associated With Left Amygdala–Insula Connectivity

A seed analysis using the left amygdala cluster where CBF was associated with anxiety revealed that higher levels of trait anxiety were associated with increased connectivity with the left insula ($k = 153$; maximum $z = 3.49$; peak MNI coordinates, $-40, 6, -10$; see Figure 5). No other clusters achieved significance. Additionally, there were no age-by-sex, anxiety-by-age, or anxiety-by-puberty interactions within this cluster.

DISCUSSION

The current study related sex differences in perfusion in the amygdala, insula, and other regions to sex differences in trait anxiety that emerge in adolescence. Leveraging a large sample of youth imaged as part of the PNC, we delineate five interrelated results describing how the development of brain perfusion may relate to anxiety. First, as expected, sex differences in anxiety were apparent, with postpubertal female participants showing higher levels of trait anxiety than postpubertal male participants. Second, trait anxiety was associated with greater perfusion in a network of regions including the amygdala, anterior insula, and fusiform cortex. This effect was present even while accounting for in-scanner state anxiety. Third, these regions showed substantial developmental sex differences, with female participants demonstrating higher perfusion than male participants in the postpubertal period. Fourth, across both male and female participants, the relationship between trait anxiety and perfusion in affective regions increased in strength with age and puberty. Finally, higher trait anxiety levels observed in postpubertal female participants were mediated by elevated perfusion of the left amygdala. Taken together, these results suggest a new mechanism for understanding sex differences in anxiety and mood symptoms.

The results of this study are convergent with and extend results from prior studies that link elevated perfusion to clinically diagnosed anxiety disorders (39,40) or to task-induced anxiety or stress (41,42). Trait anxiety encompasses

symptoms of both anxiety and depression (30,49,71) and thus may be conceptualized as a measure of anxious misery, which is identified consistently by factor analyses of large-scale studies of psychopathology (23). Remarkably, the regions we found associated with trait anxiety are localized within the social cognition network and are critical for processes including face perception, emotion identification, detection of social threat, and emotion regulation (72–75). Furthermore, many of the regions implicated including the anterior insula belong to the cingulo-opercular network (76–78), which frequently has been implicated in anxiety disorders (79).

Prior research has demonstrated that CBF is tightly linked to regional metabolism, and thus local neural activity (80,81). Current results suggest that heightened activity in affective circuitry associated with anxiety may result in higher regional metabolism and CBF. Along with prior reports from clinical samples (39,40), these results suggest that perfusion in affective regions may reflect the presence of long-standing symptoms of anxiety, and thus may be a candidate biomarker for longitudinal studies of development, drug discovery, and clinical trials of novel interventions for ameliorating anxiety. Furthermore, connectivity analyses showed that the left amygdala connectivity with the left insula was also associated with higher trait anxiety, suggesting that amygdala–insula coupling is an important circuit associated with trait anxiety.

As suggested by previous work in adults (82), sex differences were marked. As in our prior work (13), CBF diverged with pubertal stage, such that female participants showed higher perfusion than male participants by the end of adolescence. Although the PNC did not include hormonal measures such as circulating androgen or estrogen levels, based on animal models (83,84) and studies of ovarian hyperstimulation in the context of fertility treatment (85,86), we speculate that such developmental sex differences may be associated with rising estrogen levels in females. The amygdala has both estrogen and androgen receptors (87–89), and the influx of estrogen enhances the acquisition of fear conditioning in female mice (83,84). Therefore, the amygdala may be particularly susceptible to the hormonal changes that occur during pubertal development. Likewise, developmental sex differences also may be influenced by the increase in testosterone in boys, as testosterone reduces anxious behaviors in rodents (90,91).

In addition to the presence of developmental sex differences, the relationship between perfusion in affective regions and trait anxiety was stronger in the older adolescents and young adults. Thus, perfusion of affective regions may be associated with differential risk in specific developmental epochs. Of note, greater trait anxiety was associated with higher perfusion only in the left amygdala and not the right amygdala in the current study. This lateralization of amygdala perfusion is consistent with previous work showing greater activity in the left but not the right amygdala in response to negative emotional information (82,92). Furthermore, prior studies of major depression also showed greater left amygdala activity (93), which may suggest a potential common marker for anxious-misery symptoms across disorders.

Although increased amygdala perfusion was associated with higher anxiety in the postpubertal period in both male

Age by Trait Anxiety Interaction on CBF

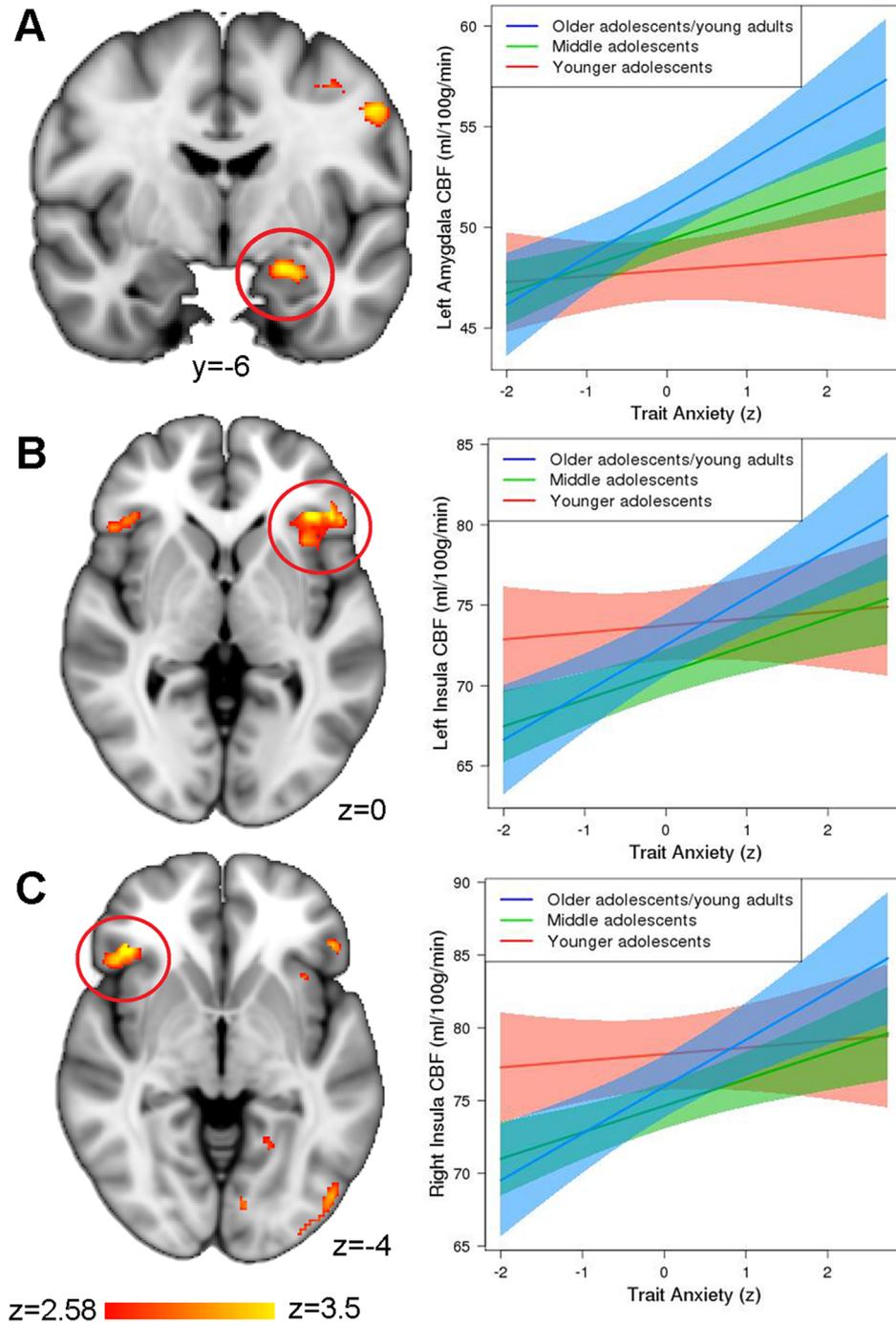


Figure 3. The association between trait anxiety and regional perfusion becomes stronger with increasing age. An age-by-trait anxiety interaction was significant in the (A) left amygdala, (B) left anterior insula, and (C) right anterior insula. CBF, cerebral blood flow.

and female participants, postpubertal females have both higher anxiety and higher perfusion. We tested the link between these effects using a mediation analysis and found that higher trait anxiety levels in postpubertal young women were mediated by higher perfusion in the left amygdala. This suggests that greater perfusion in affective regions in

postpubertal females may represent an important developmental vulnerability to trait anxiety. Intriguingly, during adolescence females also display enhanced development of social cognition, demonstrating clear superiority over males (8). Future studies could evaluate whether elevated perfusion in affective regions during adolescence may allow for

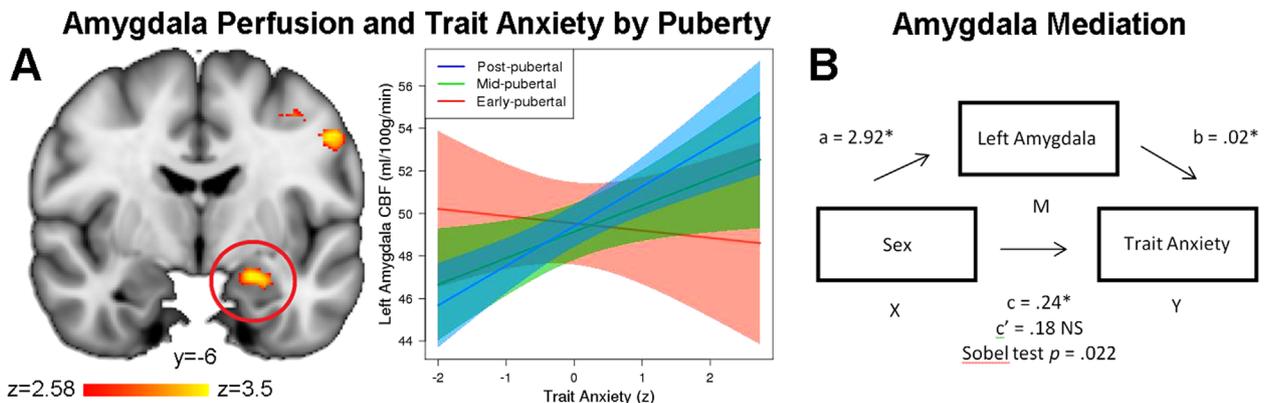


Figure 4. Amygdala perfusion mediates trait anxiety in postpubertal females. **(A)** The relationship between amygdala perfusion and anxiety increases with pubertal stage (early, mid, or postpubertal), above and beyond effects of age. **(B)** The relationship between higher trait anxiety levels in postpubertal females was mediated by higher perfusion in the left amygdala. Mediation results show the unstandardized regression coefficients for the postpubertal group in the left amygdala; when controlling for mean perfusion in the left amygdala, the relationship between sex and trait anxiety becomes nonsignificant (c'). CBF, cerebral blood flow.

improvements in social cognition while also conferring vulnerability to anxiety and mood disorders.

Examination of specific screening diagnostic categories demonstrated that elevated CBF in the amygdala was relatively similar across diagnostic categories, with small to medium effect sizes. Posttraumatic stress disorder showed the largest effect size, consistent with previous research demonstrating that posttraumatic stress disorder shows a closer relationship with the anxious-misery dimension than with the fear dimension (94). However, all diagnostic categories (including oppositional defiant disorder and attention-deficit/hyperactivity disorder) showed similar effect sizes, suggesting that these findings may potentially be indicative of general psychopathology, rather than being specific to anxiety or depression. It should be noted that the observed effect sizes may result in part from the use of a community-based sample with lower levels of psychopathology than treatment-seeking clinical populations. However, this approach provides enhanced power to investigate important dimensional effects that are present across traditional diagnostic categories.

Several limitations of the present work should be noted. First and foremost, direct causal effects regarding sex differences cannot be inferred from the results of this study. Sex differences in brain functioning are the result of a complex interaction between both genetics and environmental factors (95,96). The associations described here are not “sexually dimorphic,” consistent with research showing that brains show a mosaic of male and female features rather than being sexually dimorphic (97). Perfusion of affective regions is a continuous variable, with highly overlapping distributions among male and female participants. Indeed, the association between anxiety and perfusion was present for both sexes in this cross-sectional sample. Nonetheless, the presence of significant sex differences in perfusion does suggest particular relevance for females and may represent a potentially important mechanism for sex differences in anxiety and mood disorders.

Second, although the PNC benefitted from a large sample size that allowed examination of interactive effects of age, sex, and anxiety, cross-sectional data limit the ability to infer developmental patterns. Future research would benefit from longitudinal designs that can better assess changes in the

Left Amygdala Seed Connectivity with Left Insula

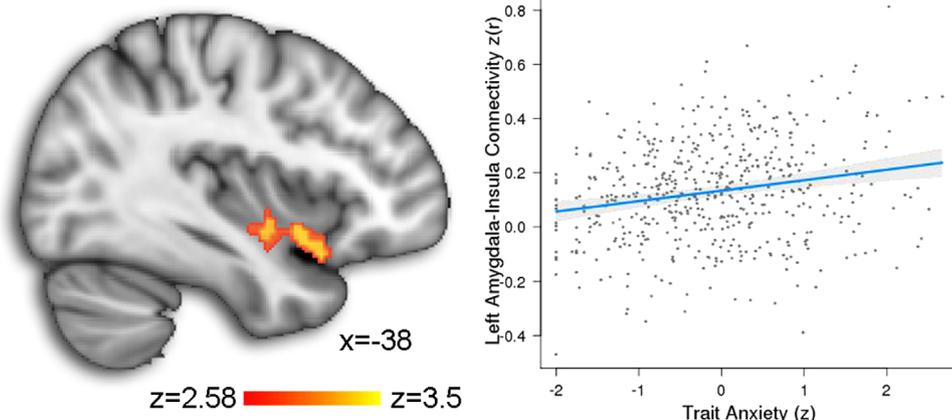


Figure 5. Higher levels of trait anxiety were associated with increased connectivity between the left amygdala seed and the left insula. Analyses were conducted in a sample of $n = 592$ Philadelphia Neurodevelopmental Cohort participants who also received a resting-state functional connectivity scan.

relationship between trait anxiety and perfusion over time and that can account for the temporal precedence between variables. Third, the pubertal assessment included in the PNC does not allow us to fully disentangle chronologic age and puberty. Future studies should gather measures of circulating hormones and also should assess menstrual phase, which may impact functional brain phenotypes (98). Finally, although generalizability of these results is enhanced by the use of a representative community sample, it would also be useful to investigate the association between anxiety and perfusion in clinically ascertained samples or in samples of children at risk for psychopathologic characteristics because of environmental stressors or family history.

These limitations notwithstanding, our data suggest a novel mechanism for sex differences in anxiety and mood symptoms, which are associated with high morbidity and mortality in females worldwide. To the degree that elevated perfusion of affective brain regions may represent a risk phenotype for subsequent clinical disorders, it may aid in early identification of youth at risk for disabling symptoms. Through incorporation within clinical trials, elevated perfusion may become a useful biomarker for treatment of anxiety and mood disorders.

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ARTICLE INFORMATION

From the Departments of Psychiatry (ANK, TMM, KR, RC, MEC, RH, DRR, EDG, DHW, JCS, EBF, REG, RCG, TDS), Biostatistics and Epidemiology (RTS, SNV), Radiology (MAE, JAD, REG, RCG), Neurology (JAD), University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; Philadelphia Veterans Administration Medical Center (JCS, RCG), Philadelphia, Pennsylvania; and Emotion and Development Branch, Intramural Research Program, National Institute of Mental Health (DSP, EL), Bethesda, Maryland.

Address correspondence to Theodore D. Satterthwaite, M.D., M.A., Department of Psychiatry, Hospital of the University of Pennsylvania, 34th and Spruce Street, 10th floor, Gates Building, Philadelphia, PA 19104; E-mail: sattertt@upenn.edu.

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