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Research paper

On a continuum to anxiety disorders: Adolescents at parental risk for anxiety show smaller rostral anterior cingulate cortex and insula thickness

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ABSTRACT

Background: Having a parent with an anxiety disorder increases the risk of anxiety symptoms and anxiety disorders during the lifespan. Moreover, childhood and adolescence anxiety disorders and symptoms have been linked to a range of brain structure abnormalities. However, to date, no study has investigated brain anatomy in adolescents at high risk based on parental anxiety disorders and in adolescents with an anxiety disorder but without any treatment or therapy.

Methods: Anatomical images from magnetic resonance imaging of 68 adolescents with anxiety disorders without any treatment ($N = 20$), at risk for anxiety because of their parents' anxiety disorders ($N = 21$), and comparison youths ($N = 27$), were analyzed using Freesurfer.

Results: Compared to comparison group, smaller cortical thickness of the rostral anterior cingulate cortex and of the insula was observed in anxious and at-risk groups; smaller amygdala volume was observed in the anxious group only.

Limitations: The age range studied is large (10 to 17 years old). Moreover, this study is cross-sectional. Since adolescence is one of the biggest periods of cerebral reorganization, longitudinal follow-up of these youths would be necessary.

Conclusions: Smaller rostral anterior cingulate cortex and insula cortical thickness appear to be cerebral markers of the risk of developing an anxiety disorder in adolescence. The reduction of the amygdala volume seems to be linked to the onset of the disorder.

1. Introduction

Offspring of anxious parents have an increased risk of developing anxiety disorders, compared to youths of non-anxious parents (Hirshfeld-Becker et al., 2008; Merikangas et al., 1999). Pediatric anxiety is markedly impairing because it is associated with significant alterations in the child's behavior in social and familial interactions and at school (Kessler et al., 1995; Velting and Albano, 2001). Moreover, pediatric anxiety is linked to an increased risk of adult anxiety, depression, bipolar disorder and suicide (Bittner et al., 2007; Duffy et al., 2013; Hirshfeld-Becker et al., 2008; Stein et al., 2001), and is thus connected to high socio-economic costs when it persists into adulthood (Kim-Cohen et al., 2003; Rapee et al., 2009; Waddell et al., 2007).

High-risk family studies represent a powerful strategy to investigate at-risk adolescents. Indeed, anxiety disorders seem in part heritable, and parental history of anxiety is a highly consistent predictor of anxiety disorders among adolescents (Hirshfeld-Becker et al., 2008). Behavioral studies suggest that, similarly to anxious youths, offspring of anxious parents report greater fear, and show exaggerated attention to evocative faces (fearful, angry faces), compared to offspring of non-anxious parents (Bar-Haim et al., 2007; Pérez-Edgar et al., 2007). Increased reactivity to anxiogenic contexts (e.g., induction of darkness in an experimental room, loud sounds) as indexed by larger startle blink reflexes and increased galvanic skin responses or heart rate, have also been observed in at-risk youths compared to offspring of non-anxious parents (Grillon et al., 1998; Merikangas et al., 1999; Turner et al., 2005). Although children of anxious parents have a high risk of developing a disorder themselves, the parental risk of developing anxiety

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disorder suggests environmental (e.g., parental practices) and genetic influences (Ginsburg et al., 2015; Hirshfeld-Becker et al., 2008). Unfortunately, it is very difficult to separate these influences. Recently, the heritability of the gray matter phenotype has been studied in families with separation anxiety disorder (SAD) (Bas-Hoogendam et al., 2018). This study has shown that the volume and cortical thickness of several brain structures seem to vary in the same way across generations of a family at risk of developing SAD. These results suggest a high genetic vulnerability for anxiety disorders and show the importance of studying gray matter volume and cortical thickness in these disorders.

Neural mechanisms underlying anxiety disorders and the risk for anxiety disorders remain unclear. Three structures seem particularly involved in the treatment and regulation of emotional information and linked to anxiety disorders: the amygdala, which seems involved in the processing of emotional information, particularly threatening information (Andersen and Teicher, 2008; Charney, 2004; Davis and Whalen, 2001; Milad and Quirk, 2012; Pine, 2003; Shin et al., 2006; Tottenham and Sheridan, 2010); the subgenual (rostral) anterior cingulate cortex (ACC), which seems implicated in the regulation of emotional information (Etkin et al., 2011); and the insula. Insula seems implicated, among other things, in vigilance during unpredictable threat, modulation of cognitive and affective processing of aversive information and awareness of emotional information (Craig, 2009; Lyoo et al., 2004; Nugent et al., 2006).

Findings from structural neuroanatomical studies in adults are mixed. Indeed, studies generally showed decreased ACC gray matter volume (Asami et al., 2008, 2009; Spampinato et al., 2009; Uchida et al., 2008; Van Tol et al., 2010) and cortical thickness (Frick et al., 2013); whereas some found no differences (Na et al., 2013; Schienle et al., 2011); and some found increased ACC thickness (Bruhl et al., 2013), compared to comparison group. Amygdala volume seemed decreased (Alemany et al., 2013; Asami et al., 2009; Blackmon et al., 2011; Hayano et al., 2009; Irle et al., 2010; Massana et al., 2003; Meng et al., 2013; Spampinato et al., 2009) increased (Baur et al., 2012; Etkin et al., 2009; Machado-de-Sousa et al., 2014; Redlich et al., 2014; Schienle et al., 2011) or with no difference (Bruhl et al., 2013; Kühn et al., 2011; Na et al., 2013; Sobanski et al., 2010; Syal et al., 2012; Uchida et al., 2008, 2003; Van Tol et al., 2010; Yamasue et al., 2008), compared to comparison group. Insula volume (Asami et al., 2009; Lai and Wu, 2013, 2012; Moon et al., 2014; Na et al., 2013) and thickness (Syal et al., 2012) generally decreased with anxiety; but some showed no difference (Schienle et al., 2011; Van Tol et al., 2010); increased volume (Protopopescu et al., 2006; Rosso et al., 2010; Uchida et al., 2008) and/or thickness (Bruhl et al., 2013; Rosso et al., 2010), compared to comparison group. Concomitant depressive disorder, as well as treatment followed by patients with an anxiety disorder could explain the heterogeneity within findings in adult studies on anxiety disorders. Moreover, age at testing and age at which anxiety appeared could also explain discrepancies when anxiety appeared during childhood, as brain structures do not grow in the same way with time. For all these reasons, we were interested in studies in youths only.

Recent structural neuroanatomical studies reported differences in the gray matter volume and cortical thickness of these structures in pediatric anxiety disorders, and youths with anxiety symptoms but no anxiety disorder. Amygdala volume was more often smaller in youths with anxiety disorders (Milham et al., 2005; Mueller et al., 2013), compared to healthy youths. However, its volume was increased with anxiety symptoms in 8-year-old children without an anxiety disorder (Juranek et al., 2006; Qin et al., 2014). These results seem contradictory but may reflect the timing and the development of the amygdala. Indeed, some authors suggested that exposure to a stressor is first linked to an acceleration of the amygdala's development (an increased volume), followed by atrophy, resulting in a larger than normal decrease in amygdala volume later in life, or with the onset of the disorder (Tottenham and Sheridan, 2010; Whittle et al., 2014, 2013). Globally, the anterior cingulate cortex volume did not differ between anxiety disorders and comparison youths (Milham et al., 2005; Mueller et al., 2013), whereas the cortical thickness of the ventromedial prefrontal cortex (including the rostral anterior cingulate cortex) was increased with anxiety levels in adolescents without an anxiety disorder (Ducharme et al., 2013). Insula volume was greater in anxiety disorders relative to comparison youths (Mueller et al., 2013).

The parental risk of developing anxiety disorder has never been studied in relation to the anatomy of these brain structures. Understanding these links between the parental risk of developing a disorder, the appearance of a disorder and the anatomy of these cerebral structures could allow: (1) to provide key insights on the developmental neurobiology of anxiety disorders; (2) to make society and parents aware of the importance of managing their disorder; 3) to implement interventions to learn to deal with anxiety in high-risk families (maybe through the school, in which are already developed strategies to help children to cope with the anxiety in Quebec); (3) to set up research evaluating interventions on the basis of neural alterations and not just symptoms. Identifying interventions that could reverse the neural alterations would allow us to think that the disorder can be avoided in at-risk youth. Reducing anxiety early in its course would prevent anxiety's emergence or present anxiety from becoming chronic and reduce the onset of internalized disorders (anxiety, depression) later in life and the high associated social costs.

The goal of this study was to compare morphometric differences in youths (1) with current anxiety disorders without any treatment (anxious group), (2) without past or current anxiety disorders but at risk for anxiety because of their parents' past or current anxiety disorders (at-risk group), and (3) who are psychiatrically healthy with psychiatrically healthy parents (comparison group). None of the youths had past or current psychiatric disorders or treatment. Based on the previously reported findings in anxious youths (anxiety disorder or symptoms), we hypothesized higher amygdala volume for at-risk adolescents and smaller amygdala volume for anxious adolescents; greater rostral anterior cingulate cortex thickness for at-risk and anxious adolescents; higher insula volume in at-risk and anxious adolescents; compared to comparison youths.

2. Methods

2.1. Participants

All the participants were 10–17 years old. Anxious youths were recruited through the Anxiety Disorders Clinic of the Ste-Justine University Hospital. At-risk youths were recruited through the nonprofit organization Phobies-Zéro, and flyers distributed through medical clinics, mental health hospitals and the community. Comparison youths were recruited in the community. All participants were extensively screened during an experimental session in order to ensure adolescents and their parents met the inclusion and exclusion criteria for this study.

Exclusion criteria for all participants. Adolescents were excluded from the study if they presented: (a) MRI contraindications (e.g., braces); (b) IQ score <70 as assessed by the Wechsler Intelligence Scale for Children IV (Wechsler, 2003); (c) medical illness; (d) past head trauma with loss of consciousness; (e) use of psychotropic medication, or (f) receipt of treatment for psychiatric illness (pharmacological or behavioral).

Youths with current anxiety disorders. Anxious youths needed to meet criteria for current generalized anxiety disorder, panic disorder with or without agoraphobia, separation anxiety disorder, or social phobia based on DSM-IV criteria as evaluated by a semi-structured psychiatric evaluation conducted with the Kiddie Schedule for Affective Disorders and Schizophrenia (Kaufman et al., 1997). The youths and their parents were questioned separately, with the parents being interviewed about their child. Youths with comorbidity between these disorders were included, as these disorders commonly occur together; restricting

inclusion to only one of these illnesses would have severely limited the potential subject pool and the generalization of findings. Anxious youths needed to have clinically significant symptoms of anxiety on the Pediatric Anxiety Rating Scale (PARS, score >9) (Ginsburg et al., 2011), and persistent anxiety over a 14 days period (as re-evaluated by the PARS, at least 2 weeks after the first assessment). Stability of symptoms was monitored to ensure symptoms were not transient. If youths presented any other past or current psychiatric disorders (e.g., depression, bipolar disorders; obsessive-compulsive disorder; post-traumatic stress disorder; Tourette's syndrome; Attention Deficit and Hyperactivity Disorder; oppositional defiant disorder; conduct disorder; suicidal ideation; anorexia, substance abuse; psychosis; or pervasive developmental disorder), they were excluded from the study.

Parents of anxious youths needed to be free of any psychiatric disorders but could meet past or present criteria for generalized anxiety disorder, panic disorder with or without agoraphobia, social phobia or comorbidity between these disorders based on DSM-IV criteria as measured by the semi-structured psychiatric evaluation conducted with the Structured Clinical Interview-I for DSM-IV Axis I Disorders (First et al., 2002). To not include parents with past or current anxiety disorders would have greatly limited the potential subject pool of this research, as these disorders highly aggregate in families. Six youths were not at parental risk of developing anxiety disorders. If parents presented other past or current psychiatric disorders (e.g., depression, except for past depression; bipolar disorders; obsessive-compulsive disorder; post-traumatic stress disorder), their adolescent was excluded from participating in the study. Given the prevalence of depression in the population, the presence of past depression in parents was not a criterion of exclusion.

Unaffected youths at familial risk for anxiety disorders. Youths at risk for anxiety disorders needed to be free from any past or current psychiatric illnesses. One or both of their biological parents needed to meet criteria for past or current generalized anxiety disorder, panic disorder with or without agoraphobia, social phobia or comorbidity between these anxiety disorders based on the DSM-IV criteria. We selected both parents, given that paternal anxiety confers the same risk as maternal anxiety (Connell and Goodman, 2002). If the parents presented other past or current psychiatric disorders (e.g., depression, except past depression; bipolar disorders; obsessive-compulsive disorder; post-traumatic stress disorder), their adolescent was excluded from participating in the study. Given the prevalence of depression in the population, the presence of past depression in parents was not a criterion of exclusion.

Psychiatrically healthy youths. Control youths, as well as both of their parents, needed to be free from past or current psychiatric illnesses, except for parents' past depression. Given the prevalence of depression in the population, the presence of past depression in parents was not a criterion of exclusion.

2.2. Measures

Because socioeconomic status (SES) and puberty changes may influence cognition, brain function and anatomy, both variables were measured and compared between groups. Socio-economic status was assessed using the Hollingshead two-factor index scale (Hollingshead and Redlich, 1958; Miller and Salkind, 2002), and the pubertal stage was assessed using the Tanner puberty stage self-administered scale (Duke et al., 1980; Morris and Udry, 1980). We also measured current anxiety levels using the Screen for Child Anxiety Related Emotional Disorders-Revised (SCARED-R), youth and parent version (parent interviewed about their child) (Martin and Gosselin, 2012).

2.3. Scanning session

A total of 81 youths (37 boys, 44 girls; age range = 10–17 years) met the study's inclusion criteria, and were invited to the scanning session. Structural neuroimaging was performed at the Geriatric University Institute of Montreal (IUGM, Montreal, Canada). The study protocol was approved by the Research Ethics Boards of the CHU Ste-Justine and IUGM, Montreal, Canada. Participants and their parents gave informed assent and consent, respectively, and were compensated for their participation. After removing participants who withdrew their participation before completing the study ($N = 3$), who had cerebral abnormalities ($N = 1$) and who showed important motion artifacts (ghosting, blurring, $N = 9$), a total of 68 youths (32 boys, 36 girls; age range = 10–17 years) were included in the analyses: 20 anxious; 21 at-risk and 27 comparison youths.

2.4. Image acquisition and processing

All scans were performed on a 3 Tesla MRI scanner (Magnetom Tim Trio, Siemens) equipped with a standard head coil. Whole-brain, high-resolution, T1-weighted anatomical images were acquired using an MPRAGE sequence (TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, matrix size = 256 × 256 mm, voxel size = 1 × 1 × 1 mm^3, FOV = 256 mm, 176 slices). The two-dimensional DICOM files of each brain were organized into volumetric three-dimensional files using the MRIcron software package (http://www.mccauslandcenter.sc.edu/mricro/mricron/). FreeSurfer (version 5.1; http://surfer.nmr.mgh.harvard.edu), a fully automated surface-based pipeline, was used to process the T1 images into a common stereotactic space, in which volumes and cortical thickness values could be derived on a participant-by-participant basis (Fischl, 2012). Cortical and sub-cortical segmentation procedure involves the assignment of a neuroanatomical label to each voxel in a MRI volume using voxel intensity, the Desikan-Killiany probabilistic atlas (Desikan et al., 2006), and Bayesian classification rules (Fischl et al., 2002). This technique has previously been shown to be comparable in accuracy to manual tracing (Fischl et al., 2002; Morey et al., 2009). Cortical thickness was automatically quantified within FreeSurfer on a vertex-by-vertex basis, by computing the average shortest distance between the white matter boundary and the pial surface (i.e., the cerebral spinal fluid boundary) at each point on the cortex (Fischl and Dale, 2000). Segmentation boundaries were visually inspected by a trained rater and, if necessary, errors due to segmentation miss-classification were reprocessed. No further exclusion was done.

2.5. Statistical analyses

Demographic characteristics and clinical data. As we can see in Table 1, groups did not differ in terms of sex, age, Tanner stage, IQ, and SES (all *ps* > 0.05). Although there were no significant group differences in terms of age, gender, IQ and SES, these variables were used as covariates of no interest, to ensure that they did not account for any of the findings, as these variables were previously reported to have an influence on brain anatomy, and are usually included as covariates in MRI studies (Burgaleta et al., 2014; Casey et al., 2011; Hanson et al., 2013; Welborn et al., 2009).

Anxiety levels were compared using a one-way Analysis of Covariance (ANCOVA) with the group as the between factor, and age, gender, socio-economic status, intellectual quotient and the total brain volume as covariates of no interest, with SPSS v.20 (Armonk, NY). When a significant effect of the group emerged, findings were decomposed using pairwise comparisons with a Bonferroni correction for multiple comparisons. All groups were compared to each other in this case.

Imaging data. Based on a-priori hypotheses of altered gray matter volumes or cortical thickness in three regions related to anxiety disorder and risk for the disorder, we investigated the rostral anterior cingulate cortex and insula volumes and cortical thickness, as well as subcortical amygda volume, right and left separately for each region.

The main analysis, consisting of a one-way Analysis of Covariance (ANCOVA) with the group as the between factor, and age, gender,

Table 1
Demographic and clinical characteristics of the participants, separately for anxiety (Anxious), at risk for anxiety (At-Risk) and comparison (Controls) groups.[a]

	Anxious	At-Risk	Controls	*p*
N	20	21	27	
Sex (female/male)[b]	10/10	11/10	15/12	0.93
Age (years)[c]	13.40 (2.62)	13.81 (2.21)	13.63 (2.15)	0.85
Tanner stage[b]	3.32 (1.34)	3.57 (1.23)	3.78 (0.93)	0.41
IQ[c]	105 (11.74)	110 (13.55)	108 (10.94)	0.46
SES[c]	26.35 (11.69)	33.33 (14.16)	25.43 (11.71)	0.08
TBV (mm3)[c,e]	1217,870 (80,518)	1224,231 (87,590)	1212,149 (99,962)	0.85
Child diagnostic				
GAD	10	0	0	
PD	3	0	0	
SAD	3	0	0	
SP	3	0	0	
Non specific AD	2	0	0	

N, Number of participants; SES, Socio-Economic Status; TBV, Total Brain Volume; GAD, General Anxiety Disorder; PD, Panic Disorder; SAD, Social Anxiety Disorder; SP, Separation anxiety disorder; AD, Anxiety Disorder.
[a] Means and standard deviations are reported.
[b] Chi-squares for quantitative measures.
[c] ANOVAs with groups as the between-subjects factors.
[d] Higher score corresponds to lower SES.
[e] TBV was calculated as the sum of the volumes of gray matter and white matter.

socio-economic status, intellectual quotient and the total brain volume as covariates of no interest, was performed in SPSS v.20 (Armonk, NY) on the different extracted measures separately (right and left rostral anterior cingulate cortex volume and thickness, right and left insula volume and thickness, right and left amygdala). When a significant effect of the group emerged, findings were decomposed using pairwise comparisons with a Bonferroni correction for multiple comparisons. All groups were compared to each other in this case.

3. Results

3.1. Clinical data

As we can see in Fig. 1 and Table 2, the anxious group showed greater anxiety level compared to comparison youths, whether scores were reported by the adolescents themselves ($F_{2,\ 63} = 9.12$, $p < 0.05$) or their parents ($F_{2,\ 60} = 29.01$, $p < 0.05$), and greater anxiety levels compared to the at-risk group when scores were reported by the parents ($F_{2,\ 60} = 29.01$, $p < 0.05$). The at-risk adolescents showed greater anxiety levels compared to the comparison youths when scores were reported by the adolescents ($F_{2,\ 63} = 9.12$, $p = 0.023$).

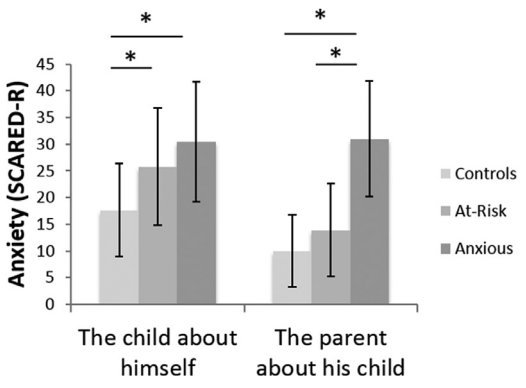

Fig. 1. Symptoms of anxiety in the three groups. Note. SCARED-R, Screen for Child Anxiety Related Emotional Disorders-Revised. *, $p < 0.05$.

3.2. Imaging data

As we can see in Figs. 2 and 3 and Table 2, ANCOVAs revealed main effects of the group in the rostral anterior cingulate cortex, the insula and the amygdala. Findings were decomposed using pairwise comparisons with a Bonferroni correction for multiple comparisons. All groups were compared to each other. Left and right rostral anterior cingulate cortex thickness ($F_{2,\ 60} = 4.8$, $p = 0.044$; $F_{2,\ 59} = 4.07$, $p = 0.02$) were smaller in the anxious group; left rostral anterior cingulate cortex thickness was smaller in the at-risk group ($F_{2,\ 60} = 4.8$, $p = 0.03$), compared to comparison group. Right insula thickness was smaller in the anxious group ($F_{2,\ 60} = 7.96$, $p = 0.002$); left and right insula thickness was smaller in the at-risk group ($F_{2,\ 60} = 4.18$, $p = 0.047$; $F_{2,\ 60} = 7.96$, $p = 0.01$), compared to comparison group. Left amygdala volume was smaller in the anxious group compared to comparison group ($F_{2,\ 60} = 3.7$, $p = 0.03$).

4. Discussion

To our knowledge this study is the first to explore brain anatomy in adolescents who are at parental risk of developing an anxiety disorder, in adolescents with an anxiety disorder but without any medication and in a comparison group. This study is also the first to simultaneously compare gray matter volume and cortical thickness. The results suggest that adolescents at risk for developing an anxiety disorder have similar neural alterations to adolescents with an anxiety disorder. When we compare the three groups, anxiety symptoms, as well as brain differences in each group, it is possible to see some differences between anxious, at-risk for anxiety and comparison youths. The first key finding is that anxiety disorders, as well as those who are at risk for developing anxiety, are related to smaller rostral anterior cingulate cortex and insula thickness. The second key finding concerns the limbic region, with smaller amygdala volume being linked to anxiety disorders.

In accordance with the literature on pediatric anxiety, we found no differences in the anterior cingulate cortex volume in at-risk and anxious groups compared to comparison group. Interestingly, cortical thickness of the rostral anterior cingulate cortex and insula, which had never been studied before, were smaller in at-risk and anxious groups compared to the comparison group, and could reflect brain markers for developing anxiety disorders. The smaller rostral anterior cingulate cortex thickness is contrary to the literature that showed a greater prefrontal cortical thickness (including the rostral anterior cingulate cortex), related to anxiety symptoms in adolescence (Ducharme et al., 2013). Ducharme et al. (2013) proposed that this increase may reflect a biological compensatory mechanism to inhibit limbic system hyperactivity and prevent mood and anxiety disorders; or may be related to a delayed maturation of that structure, ultimately leading to a decrease associated with the onset of a disorder, or the risk of developing one. Smaller anterior cingulate cortex thickness in anxious and at-risk youths in this study could therefore reflect the absence of a compensatory mechanism and/or a pathological decrease in this population, and be a cerebral marker of anxiety disorders and the risk for developing them. Longitudinal follow-up of these youths will be necessary to answer this question.

Normally, amygdala volume seems to increase linearly during adolescence, and present a gradual pruning after that (Eiland and Romeo, 2013; Hu et al., 2013; Scherf et al., 2013; Uematsu et al., 2012; Wierenga et al., 2014). Smaller amygdala volume in relation to anxiety disorders is in accordance with two other studies in pediatric anxiety without treatment and with little comorbidity (Milham et al., 2005; Mueller et al., 2013). Several studies have advanced the hypothesis that exposure to a stressor (Tottenham and Sheridan, 2010), low maternal positive behaviors (Whittle et al., 2014) and psychopathology development in at-risk youths (Whittle et al., 2013) are linked to an acceleration of the amygdala's development, followed by atrophy, or cell death, resulting in a larger than normal decrease in amygdala volume

Table 2
Anxiety and brain differences between the anxiety (Anxious), at risk for anxiety (At-Risk) and comparison (Controls) groups.[a]

	Anxious	At-Risk	Controls	*p*
Child current anxiety level				
SCARED-R (child)[b]	30.39 (11.24)**	25.76 (11.00)*	17.63 (8.68)	< **0.01**
SCARED-R (parent)[b]	31.00 (10.79)**	13.89 (8.66)[#]	9.96 (6.73)	< **0.01**
Rostral Anterior Cingulate Cortex				
Left volume[b]	3368.45 (344.71)	3394.57 (516.42)	3386.11 (578.56)	0.92
Right volume[b]	2563.05 (294.13)	2583.71 (393.92)	2748.07 (567.195)	0.22
Left thickness[b]	3.26 (0.24)*	3.22 (0.20)*	3.41 (0.29)	**0.01**
Right thickness[b]	3.03 (0.11)*	3.06 (0.21)	3.19 (0.25)	**0.02**
Insula				
Left volume[b]	7767.75 (853.78)	7710.90 (973.18)	7454.96 (901.75)	0.28
Right volume[b]	7841.80 (676.28)	7938.00 (976.08)	7625.63 (883.72)	0.43
Left thickness[b]	3.35 (0.14)	3.34 (0.13)*	3.43 (0.17)	**0.02**
Right thickness[b]	3.32 (0.19)	3.34 (0.14)	3.44 (0.16)	**0.001**
Amygdala				
Left volume[b]	1798.15 (180.09)*	1849.00 (175.99)	1908.33 (215.47)	**0.03**
Right volume[b]	1762.65 (229.91)	1802.29 (193.29)	1859.96 (46.35)	0.13

SCARED-R, Screen for Child Anxiety Related Emotional Disorders-Revised.
[a] Means and standard deviations are reported.
[b] ANOVAs with groups as the between-subjects factors, and age, gender, socio-economic status, intellectual quotient and the total brain volume as covariates of no interest.

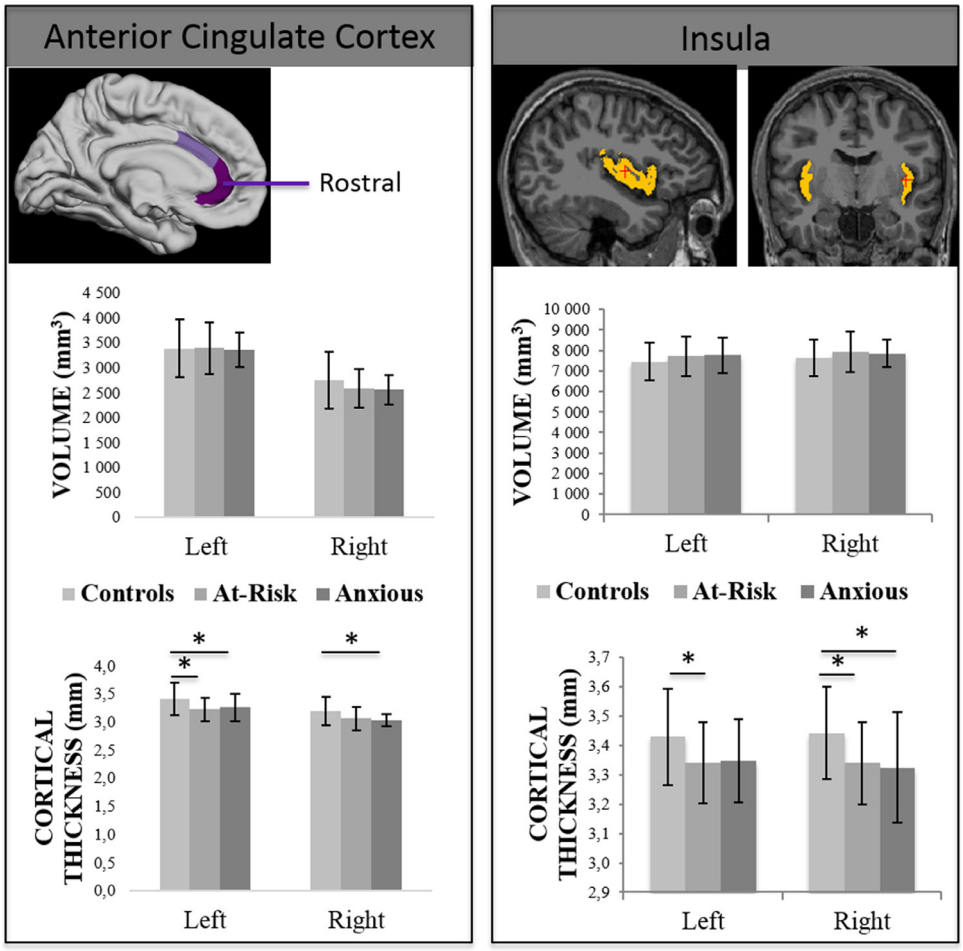

Fig. 2. Volume and thickness differences for the rostral Anterior Cingulate Cortex and the Insula. * $p < 0.05$, Bonferroni corrected.

later (Tottenham and Sheridan, 2010). The smaller amygdala volume observed here could thus be explained by a greater than normal amygdala atrophy, linked to anxiety disorder, measured during the mid-adolescence of our participants. Again, longitudinal follow-up of these youths will be necessary to answer this question.

The results concerning the insula seem contradictory to what is observed in the literature. Indeed, the volume of the insula was greater in young people with an anxiety disorder without medication (Mueller et al., 2013), while we observed a smaller insula thickness in anxious and at-risk youths, compared to comparison youths (see Fig. 3). As volume is the product of cortical thickness and surface area, cortical thickness measurements might be more sensitive than volume when studying cortical anatomy (Winkler et al., 2010). The radial unit hypothesis of cortical development suggests that cortical thickness and

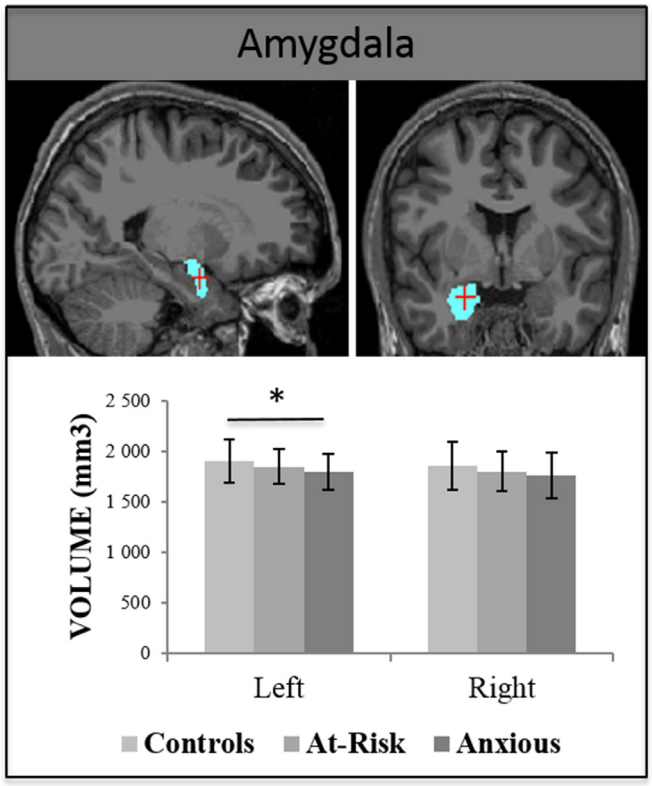

Fig. 3. Volume differences for the Amygdala. * $p < 0.05$, Bonferroni corrected.

surface area measures are genetically and phenotypically independent, and are driven by distinct cellular mechanisms (Panizzon et al., 2009; Rakic, 2000; Winkler et al., 2010). Moreover, cortical thickness alterations seem to emerge earlier than those in surface area (Kelly et al., 2013), and volume differences seem more closely related and driven by surface area rather than cortical thickness differences (Kelly et al., 2013; Panizzon et al., 2009; Winkler et al., 2010). It is therefore not surprising that our results show opposite differences between cortical thickness and volume measures.

Because of the design of this study, it is difficult here to know if cerebral anatomical differences arise from environmental or genetic risk factors in the at-risk youths. Although the results suggest similar neural alterations among anxious youth and youth at risk of developing anxiety disorders, these should be followed longitudinally to see if they develop a disorder in the future. One other limitation of this study is that our age range was large (10 to 17 years old). Since adolescence is one of the biggest periods of cerebral reorganization, it would have been interesting to verify how age might interact with structural findings, with a greater number of samples. Finally, the results should be taken with caution and replicated because of the small sample size.

To conclude, this research suggests similar neural alterations in anxious adolescents and adolescents with a parental risk of developing an anxiety disorder, without comorbidity or medication. More specifically, we showed a relationship between anxiety and the risk for developing anxiety, with smaller rostral anterior cingulate cortex and insula thickness. We also showed a relationship between anxiety disorders and smaller amygdala volume. It is likely that smaller rostral anterior cingulate cortex and insula thickness would be markers of the risk for developing anxiety and of anxiety. The smaller amygdala volume also appears to be a marker of the presence of an anxiety disorder. These results suggest that the onset of a disorder is related to the smaller amygdala volume.

Contributors

Study concept and design: Maheu.
Data acquisition: Chauret, Nassim, Suffren.
Statistical analysis: Suffren.
Interpretation of data and drafting of the manuscript: Suffren, Maheu.
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Conflict of interest

All authors declare that they have no financial interests or potential conflicts of interest.

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