

Amygdala volume correlates positively with fearfulness in normal healthy girls

Ellen A. A. van der Plas,^{1,2} Aaron D. Boes,² John A. Wemmie,² Daniel Tranel,³ and Peg Nopoulos²

¹University of Iowa Neuroscience Program, ²Department of Psychiatry, and ³Department of Neurology, University of Iowa Carver College of Medicine, Iowa City 52242, Iowa

Research into the neural underpinnings of fear and fear-related pathology has highlighted the role of the amygdala. For instance, bilateral damage to the amygdaloid complex is associated with decreased appreciation of danger and recognition of fear in humans, whereas enlarged amygdala volume is associated with internalizing syndromes. It is unknown whether amygdala volume and fearfulness are related in the absence of pathology. We examined the correlation between normal fearfulness and amygdala morphology in 116 healthy children and adolescents (60 boys, 56 girls, age 7–17 years). Fearfulness was measured using the parent ratings on the Pediatric Behavior Scale and amygdala volumes were determined by manual tracing. We found a positive correlation between right amygdala volume in girls ($r = 0.29$). This relationship was more robust and present bilaterally when analyses were limited to girls with a positive nuclear family history of depression (for left $r = 0.63$; for right $r = 0.58$). In boys there was no significant relationship which may suggest that biological mechanisms differ between sexes. Given the role of enlarged amygdala volume in pathology, these findings may indicate that variation in amygdala morphology marks susceptibility to internalizing disorders.

Keywords: amygdala volume; fearfulness; endophenotype; sex differences

INTRODUCTION

Fearfulness is a continuously distributed temperamental trait in the population (Lesch *et al.*, 1996) and high levels of fearfulness are particularly common in girls (Smider *et al.*, 2002; Else-Quest *et al.*, 2006; Zahn-Waxler *et al.*, 2008). A fearful or inhibited temperament, characterized by shyness and withdrawal in response to novelty (Kagan *et al.*, 1988), has a biological basis and is heritable (Else-Quest *et al.*, 2006; Zahn-Waxler *et al.*, 2008). Pathological fear, such as anxiety disorders and post-traumatic stress disorder, can be found at the extreme end of the continuum (Khan *et al.*, 2005; Zahn-Waxler *et al.*, 2008). Thus, fearfulness represents a spectrum of behavior in which the extreme ends of the spectrum constitute psychopathology with abnormally low fearfulness manifesting as extreme novelty seeking, whereas abnormally high fearfulness would represent anxiety disorders (Khan *et al.*, 2005).

A key region of the brain that is known to play a crucial role in processing fear is the amygdala [e.g. (Adolphs *et al.*, 1995; Davis, 1992)]. It mediates the ability to associate emotional significance to a formerly neutral stimulus (Nestler *et al.*, 2002). Subsequently, the amygdala triggers a host of adaptive responses to threatening stimuli, for example, by regulating the magnitude and duration of serotonergic

responses (Saudou *et al.*, 1994; Lesch *et al.*, 1996), which in turn trigger behavioral inhibition (Davis, 1992).

Different lines of research have established the role of the amygdala in fear at a behavioral level. Specifically, bilateral amygdala damage in humans is associated with impaired recognition, recall and visual imagery of fearful facial expressions (Adolphs *et al.*, 1995), and impaired classical fear conditioning (Bechara *et al.*, 1995). In the absence of the amygdala complex, patients do not seem to appreciate danger to the same extent as healthy comparisons participants (Tranel *et al.*, 2006). For example, patients with bilateral amygdala damage judged faces who were rated as untrustworthy and unapproachable by healthy subjects, as more approachable and more trustworthy (Adolphs *et al.*, 1998; Tranel *et al.*, 2006). Similarly, monkeys with bilateral amygdala damage show a marked decrease in freezing behavior to formerly threatening stimuli (Machado and Bachevalier, 2008). The role of the amygdala in fear processing has also been supported by studies using functional imaging (Morris *et al.*, 1996; Tavares *et al.*, 2008) and by direct electrical stimulation (Lanteaume *et al.*, 2007).

The amygdala is also implicated in the neurobiology of pathologic fear. That is, disorders that are commonly related to a fearful temperament such as major depressive and anxiety disorder (De Bellis *et al.*, 2000; Pezawas *et al.*, 2005; Zahn-Waxler *et al.*, 2008) are associated with greater amygdala volume (De Bellis *et al.*, 2000; van Elst *et al.*, 2000). This is particularly true in the early stages of the disease (Frodl *et al.*, 2003). Furthermore, functional imaging studies have shown higher metabolism in the amygdala in subjects

Received 14 May 2009; Accepted 24 November 2009

Advance Access publication 11 February 2010

This research was supported by the National Institute of Dental and Craniofacial Research (E014399).

Correspondence should be addressed to Ellen van der Plas, Department of Psychiatry, University of Iowa Carver College of Medicine, 200 Hawkins Drive, W285 GH, Iowa City 52242, Iowa. E-mail: ellen-vanderplas@uiowa.edu

with major depressive disorder (Tavares van Elst *et al.*, 2008; Fales *et al.*, 2009). Interestingly, evidence from studies with rats suggests that hypertrophy of amygdala neurons is related to increased anxiety (Vyas *et al.*, 2003), suggesting pathological enhancement of function (McEwen, 2003).

Thus, previous studies showed that structural abnormalities of the amygdala are associated with behavioral outcomes, with decreased appreciation of danger in the absence of the amygdala complex (Adolphs *et al.*, 1998; Machado and Bachevalier, 2008; Tranel *et al.*, 2006) and pathological fear with enlarged amygdala volume (Tebartz van Elst *et al.*, 1999; De Bellis *et al.*, 2000; Frodl *et al.*, 2003). However, it is unknown whether there is a direct correlation between amygdala volume and fearfulness in the absence of pathology. Given the results of previous research described above, one may expect smaller amygdala volume to be associated with decreased fearfulness and enlarged amygdala volume with increased fearfulness. If this relationship is indeed present, it raises an intriguing question: can we identify structural variation within the 'normal' range of fearfulness that marks an individual's vulnerability to develop pathology (Gottesman and Gould, 2003). Furthermore, disease susceptibility is commonly highly correlated with genetic liability (Gottesman and Gould, 2003; Zahn-Waxler *et al.*, 2008). It is therefore important to examine whether a potential relationship between amygdala morphology and fearfulness is modulated by genetic liability to internalizing disorders.

The aims of the current study were to address the following questions: (i) does the volume of the amygdala have a direct relationship with differences in normal fearfulness and (ii) is there any modulation of this relationship by genetic liability to anxiety disorders? Since internalizing disorders tend manifest during adolescence and early adult years (Zahn-Waxler *et al.*, 2008) we addressed our research questions in healthy children and adolescents (age 7–17 years). We predicted a positive correlation between amygdala volume and fearfulness, which is based on evidence that damage is related with decreased fearfulness, whereas enlargement is associated with increased fearfulness. This

relationship was expected to be more robust in participants with a genetic liability for internalizing disorders.

METHOD

Participants

The sample consisted of 116 healthy children and adolescents (60 boys, 56 girls, age 7–17 years; see Table 1 for more details). This sample was not gathered specifically for the purposes of the current study aims, but subjects were recruited as a comparison group for a study of brain structure and function in children and adolescents with oral clefts (Nopoulos *et al.*, 2007). Exclusionary criteria included: any serious medical condition, psychiatric diagnosis, neurologic disorder, learning disorder or special services needs.

Demographics

Demographic data included sex, age, IQ, parental socio-economic status (SES) and family history of psychiatric disorders. IQ was estimated using the full-scale Wechsler Intelligence Scale for Children (Wechsler, 1991) and SES was determined using a modified Hollingshead scale of 1–5, with a lower number corresponding to a higher social class (Hollingshead, 1975). Finally, family history of psychiatric disease was obtained using a standardized questionnaire. The participant's parent was instructed to list all the relatives that had received a formal psychiatric diagnosis, and to indicate the relationship of the relative to the child. The questionnaire asks for the presence of specific diagnoses including depression, learning disabilities, autism, mental retardation, schizophrenia, bipolar affective disorder, substance abuse or incarceration. In addition the number of relatives, their relationship to the participant and the nature of their disorder (e.g. treatment, duration) were recorded. Anxiety disorders were not explicitly inquired about, but instead listed within a category designated as 'other'.

Behavioral measure

The behavioral measure used in the present study was the Pediatric Behavior Scale (PBS) that is adapted from

Table 1 Descriptive demographic data

Measure	Boys			Girls		
	All boys (<i>n</i> = 60)	(–) Nuclear FHD (<i>n</i> = 44)	(+) Nuclear FHD (<i>n</i> = 16)	All girls (<i>n</i> = 56)	(–) Nuclear FHD (<i>n</i> = 41)	(+) Nuclear FHD (<i>n</i> = 15)
Age						
Range	7.75–17.92	7.75–17	8.08–17.92	7.08–17.58	7.08–17.58	7.17–14.83
Mean (s.d.)	12.15 (2.71)	12.03 (2.51)	12.46 (3.28)	12.51 (2.89)	12.80 (3.14)	11.71 (1.95)
IQ						
Mean (s.d.)	113 (16.65)	111.45 (17.39)	117.06 (14.21)	108.45 (14.53)	110.10 (14.33)	103.64 (14.54)
SES						
Mean (s.d.)	2.32 (0.57)	2.32 (0.61)	2.31 (0.48)	2.28 (0.52)	2.18 (0.44)	2.53 (0.64)

FHD, family history of depression: (–), negative; (+), positive; IQ, intelligence quotient; SES, parental socio-economic status.

the Child Behavior Checklist (CBCL) (Achenbach and Edelbrock, 1983). In the current study, a 30-item screening version of the long-form PBS was used.

The PBS is used for evaluating emotional and behavioral problems, and it assesses opposition–aggression, hyperactivity–inattention, depression–anxiety and physical health. Items in each scale do not cross-load to any significant degree (<0.30) with other PBS scales. For each participant, a parent rated the child's behavior on a four-point Likert scale (0–3), with a higher score indicating more problems.

The behaviors of interest in the current study include items related to the spectrum of anxiety, with the following individual questions: (i) fearful, anxious or worried, (ii) self-conscious or easily embarrassed and (iii) afraid to try new things for fear of making mistakes. Note that the PBS is a proxy to assess whether participants were fearful; it is not appropriate for a formal psychiatric evaluation.

The PBS scales can be used in children aged 6–16 years, and normative data were collected on 600 Iowa children. The scales were obtained from a four-factor solution with all eigenvalues greater than one. A comprehensive review of other parent rating scales, and in particular the CBCL was used to guide the format of the items (Lindgren and Koepl, 1987). The internal consistency coefficient (established in a sample of 106 children) of the depression/anxiety scale from which we derived our fearfulness items is 0.84 (Lindgren and Koepl, 1987; McCarthy *et al.*, 2002). Finally, the interrater reliability for the depression/anxiety scale is 0.73 (Lindgren and Koepl, 1987).

MRI acquisition

Magnetic resonance imaging scans were obtained using a 1.5 T General Electric SIGNA System (GE Medical Systems, Milwaukee, WI, USA). Three-dimensional (3D) T1-weighted images were acquired in the coronal plane using a spoiled grass sequence with the following parameters: 1.5 mm coronal slices, 40° flip angle, 24 ms repetition time (TR), 5 ms echo time (TE), two numbers of excitations (NEX), 26 cm field of view (FOV) and a 256 × 192 matrix. The proton density (PD) and T2-weighted images were acquired with the following parameters: 3.0 mm coronal slices, 36 ms TE (for PD) or 96 ms TE (for T2), 3000 ms TR, 1 NEX, 26 cm FOV, 256 × 192 matrix and an echo train length = 1.

Image processing

MRI data were processed using BRAINS2 (Brain Research: Analysis of Images, Networks and Systems) (Magnotta *et al.*, 2002). T1-weighted images were bias-field corrected and re-sampled to 1.01 mm³ voxels. The anterior–posterior axis of the brain was realigned parallel to the anterior–posterior commissure line. The interhemispheric fissure was aligned by selecting points along the fissure in the coronal and axial views. T2- and PD-weighted images were aligned to the spatially normalized T1-weighted image (Magnotta *et al.*, 2002)

to allow the use of a multimodal discriminant classifier. The resulting classified image was used for the application of an artificial neural network that creates an automated brain mask (Woods *et al.*, 1992). This mask was visually inspected and manually edited by trained, reliable technicians. The resulting intracranial volume (ICV) mask includes all brain tissue and both internal and surface cerebrospinal fluid.

Amygdala volumes were generated by manual tracing in the BRAINS2 environment, and the Mai atlas (Mai *et al.*, 2007) was used as the main anatomical reference. Tracing was done in the coronal plane. For further details of the anatomical boundaries see Boes (2008). A neuroanatomist with expertise in amygdala anatomy reviewed the amygdala parcellation technique prior to tracing. A.D.B. established intra-rater reliability on separate set of 20 amygdalae (intra-class *R* coefficient of 0.95 for right and 0.92 for left). The rater was blind to subject identity.

Statistical analyses

We separated boys and girls for the main analysis—i.e. the relationship between amygdala morphology and fearfulness, due to evidence of sex differences present in the amygdala in terms of structure (Tebartz van Elst *et al.*, 1999; Goldstein *et al.*, 2001; Good *et al.*, 2003), development (Giedd *et al.*, 1996) and function (Cahill *et al.*, 2001; Tranel and Bechara, 2009) in this particular structure.

Furthermore, we used subjects' nuclear family history of depression (FHD) as a proxy for genetic liability to determine whether genetic risk changes the relationship between amygdala volume and fearfulness. That is, it was determined whether parent(s) and/or siblings (hence the term 'nuclear') have been diagnosed with depression. Family history for anxiety disorders may have a more obvious relationship to fearfulness; however, anxiety disorders are less frequent than depression (Neale and Kendler, 1995) that was reflected in the absence of family history of anxiety disorders in our sample. This is due to both lower prevalence of family history of anxiety disorders, but also in the structure of the questionnaire. That is, depression is asked about explicitly and anxiety mentioned as a possible 'other' category.

The use of FHD as a proxy for genetic liability for internalizing disorders is further supported by different lines of evidence. First, individuals with a fearful temperament are more likely to develop depression (Hariri *et al.*, 2005; Khan *et al.*, 2005; Pini *et al.*, 1997). Second, anxiety disorders and depression are highly co-morbid (Lewinsohn *et al.*, 1997). Also, anxiety disorders typically precede depression (Neale and Kendler, 1995).

For exploratory purposes, we conducted analyses of variance (ANOVAs) separately in boys and girls to determine whether subjects with a nuclear FHD differed from those without a nuclear FHD in terms of age, SES, IQ, amygdala volume or degree of fearfulness. Variables that showed significant differences between subjects with and without

nuclear FHD were entered as control variables in the partial correlation analysis.

Second, it was determined whether age, SES and IQ correlated significantly with amygdala volume and fearfulness using Pearson product-moment coefficient. Furthermore, we determined whether overall brain size (i.e. ICV) correlated significantly with amygdala volume. If any of the demographic variables or ICV were to correlate significantly with either amygdala volume or fearfulness, they were entered as control variables in subsequent partial correlation analyses.

Partial correlation analyses were then conducted to evaluate the relationship between amygdala volume and fearfulness while controlling for variables that could potentially influence this correlation. The first set of partial correlations was conducted in the entire sample of either boys or girls, thus regardless of nuclear FHD. Hereafter, within each group subjects were compared according to nuclear FHD.

RESULTS

Exploratory analyses

Demographic information is shown in Table 1, whereas Table 2 displays fearfulness ratings and volumetric data (in cubic centimeters) for all groups. Results will first be discussed for girls and then for boys.

The ANOVA for girls showed that those with nuclear FHD did not differ from girls without nuclear FHD in terms of age or IQ (all $P > 0.15$). However, girls with nuclear FHD had a lower social class relative to girls without nuclear FHD (see Table 1), $F(1,54) = 5.35$, $P < 0.05$. We entered SES as a control variable in subsequent analyses.

Furthermore, girls with nuclear FHD did not differ from those without a nuclear family of depression in terms of fearfulness ($P = 0.95$; see Table 2). This implies that depressed parents do not seem to endorse higher ratings of fearfulness in their children. Finally, amygdala volumes for girls with and without nuclear FHD were comparable and did not differ significantly (both, $P > 0.21$; see Table 2).

We then determined whether any of these variables correlated with the amygdala volume or fearfulness. In girls, significant correlations were found for IQ, which correlated significantly with the right amygdala volume ($r = 0.27$, $P < 0.05$), and ICV, which correlated significantly with left ($r = 0.39$) and right ($r = 0.29$) amygdala volume ($P < 0.05$). For subsequent partial correlation analyses in girls, we entered SES, IQ and ICV as control variables.

The analyses of variance in boys revealed that those with nuclear FHD did not differ from those individuals without nuclear FHD in terms of age, IQ or SES (Table 1). In addition, there was no statistical evidence that boys with and without nuclear FHD differed in fearfulness ($P = 0.44$) or amygdala volume ($P = 0.15$; see Table 2).

The exploratory correlation analysis revealed a significant correlation between age and right amygdala volume in boys ($r = 0.36$, $P < 0.05$). Furthermore, ICV correlated significantly with left ($r = 0.29$) and right ($r = 0.34$) amygdala volume (both, $P < 0.05$). Age and ICV were entered as control variables the partial correlation analysis in boys.

Relationship between fearfulness and amygdala volume

For girls there was a significant positive correlation between right amygdala volume and fearfulness, suggesting that larger volume is associated with higher fearfulness, $r = 0.29$, $P = 0.034$ (Table 3). The correlation for the left amygdala was not significant however, $r = 0.16$, $P = 0.26$. Fisher's $R-Z$ transformations showed that the correlation for the left amygdala and fearfulness ($r = 0.16$) was not significantly different from the correlation of the right amygdala ($r = 0.29$) ($z = 0.71$, $P = 0.48$), suggesting that the effect is not unique to right amygdala.

To examine the role of genetic liability, we performed the same partial correlation analysis in girls with and without a nuclear FHD. Results demonstrated significant correlations in both the left and the right amygdala in girls with nuclear FHD ($n = 15$), showing a correlation of $r = 0.58$ ($P = 0.030$, one-tailed) for the right, and $r = 0.63$ ($P = 0.019$, one-tailed)

Table 2 Descriptive behavioral and structural data

Measure	All boys ($n = 60$)	Boys		All girls ($n = 56$)	Girls	
		(−) Nuclear FHD ($n = 44$)	(+) Nuclear FHD ($n = 16$)		(−) Nuclear FHD ($n = 41$)	(+) Nuclear FHD ($n = 15$)
PBS-anx						
Mean (s.d.)	1.62 (1.52)	1.52 (1.53)	1.87 (1.50)	1.71 (1.23)	1.71 (1.21)	1.73 (1.33)
L amygdala ^a						
Mean (s.d.)	1.30 (0.18)	1.31 (0.17)	1.27 (0.22)	1.22 (0.18)	1.21 (0.19)	1.26 (0.17)
R amygdala ^a						
Mean (s.d.)	1.31 (0.19)	1.29 (0.20)	1.34 (0.16)	1.25 (0.17)	1.23 (0.17)	1.29 (0.17)
ICV						
Mean (s.d.)	1469.34 (110.17)	1467.20 (106.37)	1475.24 (123.54)	1352.57 (87.94)	1348.40 (96.19)	1363.98 (61.31)

PBS-anx, Pediatric Behavior Scale anxiety items; s.d., standard deviation; L, left; R, right; ICV, intracranial volume. ^aThe mean values reported here are not corrected for ICV.

Table 3 Structural and behavioral correlations

	Boys (<i>n</i> = 60)	Girls (<i>n</i> = 56)
	Fearfulness symptoms	Fearfulness symptoms
L amygdala	$R = 0.10^a$, $P = 0.236$	$R = 0.16^b$, $P = 0.160$
R amygdala	$R = 0.12^a$, $P = 0.197$	$R = 0.29^b$, $P = 0.017$
(-) nuclear FHD		
L amygdala	$R = 0.08^a$, $P = 0.312$	$R = -0.02^b$, $P = 0.462$
R amygdala	$R = 0.16^a$, $P = 0.157$	$R = 0.21^b$, $P = 0.101$
(+) nuclear FHD		
L amygdala	$R = 0.23^a$, $P = 0.210$	$R = 0.63^b$, $P = 0.019$
R amygdala	$R = 0.02^a$, $P = 0.480$	$R = 0.58^b$, $P = 0.030$

Note: FHD, family history of depression; L, left; R, right; PBS, Pediatric Behavior Scale.
^aIn boys, the correlation is corrected for age and ICV. ^bIn girls, the correlation is corrected for IQ, ICV and SES.

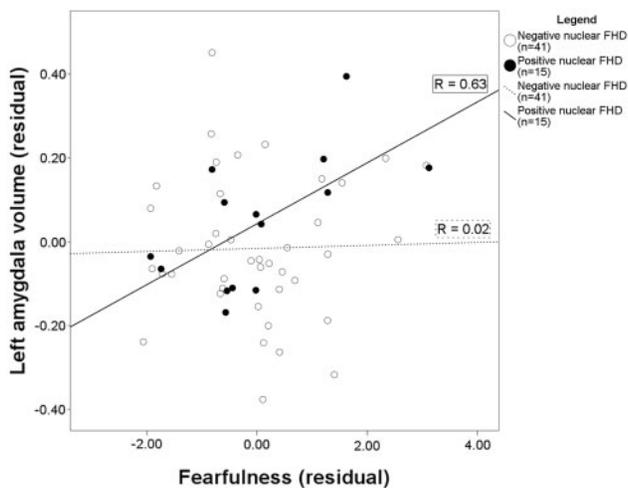


Fig. 1 The relationship between left amygdala volume and fearfulness (while controlling for IQ, SES and ICV) in girls with a positive nuclear family history of depression (black circles, solid line) and girls without a nuclear family history of depression (open circles, dotted line).

for left amygdala volume (see Table 3 and Figures 1 and 2). These correlations did not significantly differ from each other ($z = 0.193$, $P = 0.85$).

For girls without nuclear FHD ($n = 41$), the relationship between right amygdala volume and fearfulness was no longer significant ($r = 0.21$, $P = 0.10$). For the left amygdala, the correlation was low ($r = -0.02$), however, Fisher's $R-Z$ transformations revealed that these two correlations did not differ from that of the right ($z = 1.01$, $P = 0.31$).

Although correlations on both sides are substantially higher in girls with nuclear FHD, Fisher $R-Z$ correlations showed that only the correlation on the left were significantly different between girls with and without nuclear FHD ($r = 0.63$ and -0.02 , respectively; $z = 2.29$, $P = 0.02$).

For boys on the other hand, the partial correlation analysis controlling for age and ICV was not significant, $r = 0.10$ for the left and $r = 0.12$ for the right, both, $P > 0.20$. Even in boys with nuclear FHD, no significant relationship between

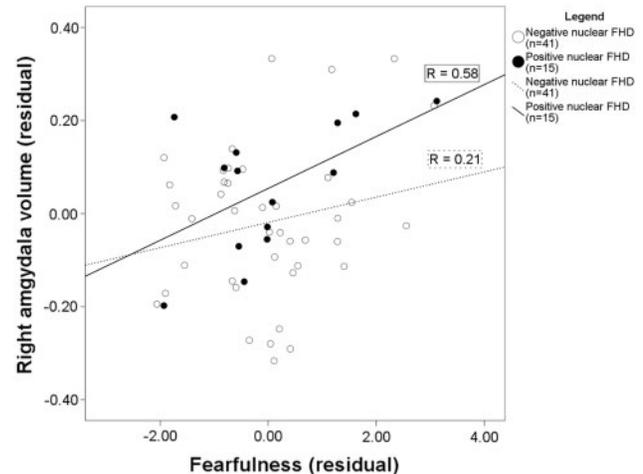


Fig. 2 The relationship between right amygdala volume and fearfulness (while controlling for IQ, SES and ICV) in girls with a positive nuclear family history of depression (black circles, solid line) and girls without a nuclear family history of depression (open circles, dotted line).

fearfulness and amygdala volume was found, $r = 0.23$ for the left and $r = 0.02$ for the right, both, $P > 0.21$.

Our final set of analyses tested the specificity of the significant correlation between fearfulness and amygdala volume in girls. To this end, we correlated total cerebral gray matter volume (all lobes without cerebellum) with fearfulness while controlling for IQ and SES. This relationship was low, $r = -0.05$ and not significant, $P = 0.74$. We then limited our analyses on structures within the medial temporal lobe in close proximity of the amygdala. Gray matter volumes of both the parahippocampal gyrus and the entorhinal cortex were automatically generated using an automated parcellation program called FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>). The relationship between the parahippocampal gyrus and fearfulness while controlling for ICV, IQ and SES was not significant for either the left or the right side, $r = -0.12$ and 0.13 , respectively, both, $P > 0.36$. In addition, there was no evidence for a relationship between the entorhinal cortices and fearfulness, since correlations were low and not significant for the left or the right, $r = -0.04$ and 0.003 , respectively, both, $P > 0.76$.

Furthermore, to examine whether amygdala volume specifically correlates with the fear items and not with other behavioral measures, we correlated left and right amygdala volume with the other main categories of the PBS: depression ($r = 0.07$ for left and $r = 0.19$ for right, both, $P > 0.17$), opposition–aggression ($r = 0.00$ for left and $P = 0.07$ for right, both, $P > 0.63$), hyperactivity–inattention ($r = -0.16$ for left and $r = -0.21$ for right, both, $P > 0.23$) and physical health ($r = -0.04$ for left and $r = 0.01$ for right, both, $P > 0.92$), but none of these correlations were significant. Together, these findings suggest that the relationship between fearfulness and amygdala is specific to the amygdala and not to nearby regions in the medial temporal lobe and that the relationship is relatively specific to fearfulness.

DISCUSSION

In the present study the relationship between fearfulness and amygdala volume in a group of healthy children and adolescents (age 7–17 years) was examined. Our results suggest a positive relationship between fearfulness and right amygdala volume, which was particularly robust, and present bilaterally in girls with a positive nuclear FHD. These findings further underscore the role of the amygdala in fear established earlier by lesion studies (Adolphs *et al.*, 1998) and studies on fear-related psychopathologies (De Bellis *et al.*, 2000). Importantly, the relationship between fearfulness and amygdala morphology is maintained in the absence of pathology. Therefore, structural variability in this region may underlie susceptibility for fear-related pathology, i.e. amygdala volume may be an endophenotype for these pathologies.

More specifically, an endophenotype marks someone's risk for disease susceptibility that is not apparent to the unaided eye and represents an intermediate along the continuum from normalcy to pathology (Gottesman and Gould, 2003). In line with criteria for the identification of endophenotypes (Gottesman and Gould, 2003), amygdala volume is heritable (Munn *et al.*, 2007) and enlargement is associated with depression (van Elst *et al.*, 2000), dysthymia (Tebartz van Elst *et al.*, 1999) and anxiety disorders (De Bellis *et al.*, 2000). Our results provide evidence that enlargement is associated with higher fearfulness even in the absence of illness, suggesting that it is state-independent (Gottesman and Gould, 2003). Variations in the neurobiology of the amygdala possibly underlie temperamental traits characterized by more fearfulness. Prospective longitudinal studies are needed to test the predictive power of increased amygdala volume and fearfulness and increased risk for internalizing disorders.

Interestingly, the relationship between amygdala volume and fearfulness was not significant in boys. Although caution is warranted, our findings are in line with the growing body of evidence suggesting that sex difference may be driven by biological mechanisms, and may manifest in different brain-behavior relationships. First, structural imaging studies suggest that the amygdala is sexually dimorphic with men having relatively larger amygdala volume (Goldstein *et al.*, 2001; Good *et al.*, 2001). In addition, a developmental structural imaging study by Giedd *et al.*, (1997) showed that amygdala volume continues to increase with age more so for boys than for girls (Giedd *et al.* 1997). Indeed, amygdala volume appears to correlate inversely with the number of X chromosomes, i.e. men (46, XY) having larger amygdala volumes than women (46, XX) (Good *et al.*, 2001). Moreover, women with Turner syndrome are monosomic (45, X) and have significantly enlarged amygdala volumes compared to healthy men and women (Good *et al.*, 2003). Furthermore, functional imaging studies (Cahill *et al.*, 2001) and human lesion studies (Tranel and Bechara, 2009) demonstrated evidence for sex-related functional asymmetry in the amygdala as well. More specifically, the right amygdala may be more

important in emotional processing in men, whereas in women, the left amygdala may play a more prominent role in processing emotionally salient stimuli (see Cahill *et al.*, 2001; Tranel and Bechara, 2009). Other evidence comes from studies that used paradigms, which tap into behavioral and physiological manifestations of the amygdala. For example, Campbell and colleagues (2002) showed that the ability to categorize fearful facial expressions was substantially and significantly correlated with facial recognition accuracy in women, but not men (Campbell *et al.*, 2002). Finally, across lifespan women tend to be more reactive to threatening stimuli than men (Bradley *et al.*, 2001; McManis *et al.*, 2001; Gard and Kring, 2007). This is exemplified by higher reported fearfulness (Ollendick *et al.*, 1995; McManis *et al.*, 2001), greater skin conductance change in response to aversive cues, slower progressive decrease in startle response magnitude, greater facial electromyographic (EMG) activity, as well as increased viewing time to aversive cues (McManis *et al.*, 2001; Gard and Kring, 2007; Roy *et al.*, 2008). Altogether, these studies clearly imply the presence of sex differences in the amygdala, with women being more reactive than men. An important next step will be to explore how these sex differences may contribute to psychiatric diseases that show a clear imbalance in the number of men and women affected.

This study suffered from some limitations. First, the assessment of fearfulness is limited to behavioral ratings provided by parents. We relied on parental reports because approximately one-third of our sample was relatively young at the time of testing—i.e. below the age of 11 years, which may limit the use of self-report questionnaires. Specifically, there is evidence that children below the age 11 years show inconsistencies in their answers and have poor understanding of questions comparable to those used in our questionnaire (Edelbrock *et al.*, 1985; Strauss and Broder, 1993; Breton *et al.*, 1995). Adolescents are reliable informants of their behavior (e.g. Verhulst and Vanderende, 1992), but we also relied on parental reports in the older subjects to keep our data as comparable as possible. Unfortunately, this does not rule out the possibility that parents' reports may be biased. On the other hand, parental reports similar to the one used in the current study (e.g., the CBCL) are widely used in research settings under the assumption that parents have sufficient insight in their own child's behavior. Nevertheless, it will be necessary to replicate our results with complementary measures of fearfulness, including psychiatric interviews with parents and participants, self-report data and behavioral tests that tap into fearfulness. The hope is that the use of more sensitive measures of fearfulness results in a more robust relationship than reported here, given that we relied on relatively 'noisier' data.

Concerns could also be raised for the assessment of family history for which we relied on parental ratings of prevalence of depression in the family. Although circumstantial, FHD is a strong predictor of an individual's risk for developing

a certain type of internalizing disorder, with offspring of parents with depression being 40–60% more likely to develop an affective disorder (Beardslee, 1993, 1998).

To conclude, our results show that there is a direct relationship between amygdala volume and fearfulness in healthy girls, which is particularly robust in girls who have direct family members who suffered from depression. Although the amygdala has a well-established role in fear, it is important to consider that the amygdala is only one part of an emotion network that influences mood and potentially mood disorders. Therefore, pathology is probably influenced by a disturbed balance between various brain areas underlying emotional behavior.

Conflict of Interest

None declared.

REFERENCES

- Achenbach, T.M., Edelbrock, C. (1983). *Manual for the Child Behavior Checklist and Revised Child Behavior Profile*. Burlington, VT: University of Vermont.
- Adolphs, R., Tranel, D., Damasio, A.R. (1998). The human amygdala in social judgment. *Nature*, 393(6684), 470–4.
- Adolphs, R., Tranel, D., Damasio, H., Damasio, A.R. (1995). Fear and the human amygdala. *Journal of Neuroscience*, 15(9), 5879–91.
- Beardslee, W.R. (1993). The impact of parental affective disorder on depression in offspring: A longitudinal follow-up in a nonreferred sample. *Journal of the American Academy of Child Adolescent Psychiatry*, 32(4), 723.
- Beardslee, W.R. (1998). Children of affectively ill parents: A review of the past 10 years. *Journal of the American Academy of Child Adolescent Psychiatry*, 37(11), 1134.
- Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., Damasio, A.R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science*, 269(5227), 1115–8.
- Boes, A.D. (2008). *Neuroanatomical Correlates of Externalizing Behavior*. Unpublished Doctor Dissertation, University of Iowa, 2008.
- Bradley, M.M., Codispoti, M., Sabatinelli, D., Lang, P.J. (2001). Emotion and motivation II: sex differences in picture processing. *Emotion*, 1(3), 300.
- Breton, J.J., Bergeron, L., Valla, J.P., Lepine, S., Houde, L., Gaudet, N. (1995). Do children aged 9 through 11 years understand the disc version-2.25 questions. *Journal of the American Academy of Child and Adolescent Psychiatry*, 34(7), 946–54.
- Cahill, L., Haier, R.J., White, N.S., et al. (2001). Sex-related difference in amygdala activity during emotionally influenced memory storage. *Neurobiology of Learning and Memory*, 75(1), 1–9.
- Campbell, R., Elgar, K., Kuntsi, J., et al. (2002). The classification of 'fear' from faces is associated with face recognition skill in women. *Neuropsychologia*, 40(6), 575–84.
- Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual Review of Neuroscience*, 15, 353–75.
- De Bellis, M.D., Casey, B.J., Dahl, R.E., et al. (2000). A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biological Psychiatry*, 48(1), 51–7.
- Edelbrock, C., Costello, A.J., Dulcan, M.K., Kalas, R., Conover, N.C. (1985). Age-differences in the reliability of the psychiatric interview of the child. *Child Development*, 56(1), 265–275.
- Else-Quest, N.M., Hyde, J.S., Goldsmith, H.H., Van Hulle, C.A. (2006). Gender differences in temperament: A meta-analysis. *Psychological Bulletin*, 132(1), 33–72.
- Fales, C.L., Barch, D.M., Rundle, M.A., et al. (2009). Antidepressant treatment normalizes hypoactivity in dorsolateral prefrontal cortex during emotional interference processing in major depression. *Journal of Affective Disorders*, 112(1–3), 206–11.
- Frodl, T., Meisenzahl, E.M., Zetzsche, T., et al. (2003). Larger amygdala volumes in first depressive episode as compared to recurrent major depression and healthy control subjects. *Biological Psychiatry*, 53(4), 338–344.
- Gard, M.G., Kring, A.M. (2007). Sex differences in the time course of emotion. *Emotion*, 7(2), 429.
- Giedd, J.N., Castellanos, F.X., Rajapakse, J.C., Vaituzis, A.C., Rapoport, J.L. (1997). Sexual dimorphism of the developing human brain. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 21(8), 1185–201.
- Giedd, J.N., Snell, J.W., Lange, N., et al. (1996). Quantitative magnetic resonance imaging of human brain development: Ages 4–18. *Cerebral Cortex*, 6(4), 551–60.
- Goldstein, J.M., Seidman, L.J., Horton, N.J., et al. (2001). Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cerebral Cortex*, 11(6), 490–7.
- Good, C.D., Johnsrude, I., Ashburner, J., Henson, R.N.A., Friston, K.J., Frackowiak, R.S.J. (2001). Cerebral asymmetry and the effects of sex and handedness on brain structure: A voxel-based morphometric analysis of 465 normal adult human brains. *NeuroImage*, 14(3), 685–700.
- Good, C.D., Lawrence, K., Thomas, N.S., et al. (2003). Dosage-sensitive X-linked locus influences the development of amygdala and orbitofrontal cortex, and fear recognition in humans. *Brain*, 126, 2431–46.
- Gottesman, I.I., Gould, T.D. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry*, 160(4), 636–45.
- Hariri, A.R., Drabant, E.M., Munoz, K.E., et al. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry*, 62(2), 146–52.
- Hollingshead, A. (1975). *Four Factor Index of Social Skills*. New Haven: Yale.
- Kagan, J., Reznick, J.S., Snidman, N. (1988). Biological bases of childhood shyness. *Science*, 240(4849), 167–71.
- Khan, A.A., Jacobson, K.C., Gardner, C.O., Prescott, C.A., Kendler, K.S. (2005). Personality and comorbidity of common psychiatric disorders. *British Journal of Psychiatry*, 186, 190–6.
- Lanteaume, L., Khalifa, S., Regis, J., Marquis, P., Chauvel, P., Bartolomei, F. (2007). Emotion induction after direct intracerebral stimulations of human amygdala. *Cerebral Cortex*, 17(6), 1307–13.
- Lesch, K.P., Bengel, D., Heils, A., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274(5292), 1527–31.
- Lewinsohn, P.M., Zinbarg, R., Seeley, J.R., Lewinsohn, M., Sack, W.H. (1997). Lifetime comorbidity among anxiety disorders and between anxiety disorders and other mental disorders in adolescents. *Journal of Anxiety Disorders*, 11(4), 377–94.
- Lindgren, S., Koenig, G. (1987). *Assessing Child Behavior Problems in a Medical Setting: Development of the Pediatric Behavior Scale*. Greenwich, CT: JAI Press.
- Machado, C.J., Bachevalier, J. (2008). Behavioral and hormonal reactivity to threat: Effects of selective amygdala, hippocampal or orbital frontal lesions in monkeys. *Psychoneuroendocrinology*, 33(7), 926–41.
- Magnotta, V.A., Harris, G., Andreasen, N.C., O'Leary, D.A., Yuh, W.T.C., Heckel, D. (2002). Structural MR image processing using the BRAIN2 toolbox. *Computerized Medical Imaging and Graphics*, 26(4), 251–64.
- Mai, J.K., Assheuer, J., Paxinos, G. (2007). *Atlas of the Human Brain*, 3rd edn. New York: Academic Press.
- McCarthy, A.M., Lindgren, S., Mengeling, M.A., Tsalikian, E., Engvall, J.C. (2002). Effects of diabetes on learning in children. *Pediatrics*, 109(1), e9 p1–p10.
- McEwen, B.S. (2003). Mood disorders and allostatic load. *Biological Psychiatry*, 54(3), 200–7.

- McManis, M.H., Bradley, M.M., Berg, W.K., Cuthbert, B.N., Lang, P.J. (2001). Emotional reactions in children: Verbal, physiological, and behavioral responses to affective pictures. *Psychophysiology*, 38(2), 222.
- Morris, J.S., Frith, C.D., Perrett, D.I., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*, 383(6603), 812–5.
- Munn, M.A., Alexopoulos, J., Nishino, T., et al. (2007). Amygdala volume analysis in female twins with major depression. *Biological Psychiatry*, 62(5), 415–22.
- Neale, M.C., Kendler, K.S. (1995). Models of comorbidity for multifactorial disorders. *American Journal of Human Genetics*, 57(4), 935–53.
- Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M. (2002). Neurobiology of depression. *Neuron*, 34(1), 13–25.
- Nopoulos, P., Langbehn, D.R., Canady, J., Magnotta, V., Richman, L. (2007). Abnormal brain structure in children with isolated clefts of the lip or palate. *Archives of Pediatrics & Adolescent Medicine*, 161(8), 753–8.
- Ollendick, T.H., Yang, B., Dong, Q., Xia, Y., Lin, L. (1995). Perceptions of fear in other children and adolescents: The role of gender and friendship status. *Journal of Abnormal Child Psychology*, 23(4), 439.
- Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., et al. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience*, 8(6), 828–34.
- Pini, S., Cassano, G.B., Simonini, E., Savino, M., Russo, A., Montgomery, S.A. (1997). Prevalence of anxiety disorders comorbidity in bipolar depression, unipolar depression and dysthymia. *Journal of Affective Disorders*, 42(2–3), 145–53.
- Roy, A.K., Vasa, R.A., Bruck, M., et al. (2008). Attention bias toward threat in pediatric anxiety disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, 47(10), 1189–96.
- Saudou, F., Amara, D.A., Dierich, A., et al. (1994). Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science*, 265(5180), 1875–8.
- Smider, N.A., Essex, M.J., Kalin, N.H., et al. (2002). Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective study. *Child Development*, 73(1), 75–92.
- Strauss, R.P., Broder, H. (1993). Children with cleft-lip palate and mental-retardation – a subpopulation of cleft-craniofacial team patients. *Cleft Palate-Craniofacial Journal*, 30(6), 548–56.
- Tavares, J.V.T., Clark, L., Furey, M.L., Williams, G.B., Sahakian, B.J., Drevets, W.C. (2008). Neural basis of abnormal response to negative feedback in unmedicated mood disorders. *NeuroImage*, 42(3), 1118–26.
- Tebartz van Elst, L., Woermann, F.G., Lemieux, L., Trimble, M.R. (1999). Amygdala enlargement in dysthymia—a volumetric study of patients with temporal lobe epilepsy. *Biological Psychiatry*, 46(12), 1614–23.
- Tranel, D., Bechara, A. (2009). Sex-related functional asymmetry of the amygdala: Preliminary evidence using a case-matched lesion approach. *Neurocase*, 15(3), 217–34.
- Tranel, D., Gullickson, G., Koch, M., Adolphs, R. (2006). Altered experience of emotion following bilateral amygdala damage. *Cognitive Neuropsychiatry*, 11(3), 219–32.
- van Elst, L.T., Woermann, F., Lemieux, L., Trimble, M.R. (2000). Increased amygdala volumes in female and depressed humans. A quantitative magnetic resonance imaging study. *Neuroscience Letters*, 281(2–3), 103–6.
- Verhulst, F.C., Vanderende, J. (1992). Agreement between parents reports and adolescents self-reports of problem behavior. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 33(6), 1011–23.
- Vyas, A., Mitra, R., Chattarji, S. (2003). Enhanced anxiety and hypertrophy in basolateral amygdala neurons mowing chronic stress in rats. *Amygdala in Brain Function: Basic and Clinical Approaches*, 985, 554–5.
- Wechsler, D. (1991). *Wechsler Intelligence Scale for Children*, 3rd edn. San Antonio, TX: The Psychological Corporation.
- Woods, R.P., Cherry, S.R., Mazziotta, J.C. (1992). Rapid automated algorithm for aligning and reslicing pet images. *Journal of Computer Assisted Tomography*, 16(4), 620–33.
- Zahn-Waxler, C., Shirtcliff, E.A., Marceau, K. (2008). Disorders of childhood and adolescence: Gender and psychopathology. *Annual Review of Clinical Psychology*, 4, 275–303.