A Pilot Study of Amygdala Volumes in Pediatric Generalized Anxiety Disorder

Michael D. De Bellis, B.J. Casey, Ronald E. Dahl, Boris Birmaher, Douglas E. Williamson, Kathleen M. Thomas, David A. Axelson, Karin Frustaci, Amy M. Boring, Julie Hall, and Neal D. Ryan

Background: *The neurodevelopment of childhood anxiety disorders is not well understood. Basic research has implicated the amygdala and circuits related to these nuclei as being central to several aspects of fear and fear-related behaviors in animals.*

Methods: *Magnetic resonance imaging was used to measure amygdala volumes and comparison brain regions in 12 child and adolescent subjects with generalized anxiety disorder and 24 comparison subjects. Groups were matched on age, sex, height, and handedness and were also similar on measures of weight, socioeconomic status, and full scale IQ.*

Results: *Right and total amygdala volumes were significantly larger in generalized anxiety disorder subjects. Intracranial, cerebral, cerebral gray and white matter, temporal lobe, hippocampal, and basal ganglia volumes and measures of the midsagittal area of the corpus callosum did not differ between groups.*

Conclusions: *Although these data are preliminary and from a small sample, the results are consistent with a line of thinking that alterations in the structure and function of the amygdala may be associated with pediatric generalized anxiety disorder.* Biol Psychiatry 2000;48: 51–57 © *2000 Society of Biological Psychiatry*

Key Words: Generalized anxiety disorder, amygdala, neurodevelopment, pediatric anxiety disorders

Introduction

Anxiety disorders are a common form of childhood psychopathology (Kashani and Orvaschel 1990). Pediatric generalized anxiety disorder (GAD) affects approximately 6% of American children (Shaffer et al 1996). The presence of a childhood anxiety disorder increases the risk of adult anxiety disorders (for review see Pine and Grun 1999). Kagan's concept of behavioral inhibition to the unfamiliar is thought to be an extreme inherent temperamental trait (Kagan et al 1988). Behaviorally inhibited children have increased sympathetic tone, increased excretion of urinary catecholamines after completion of cognitive tasks, and higher levels of baseline and laboratory salivary cortisol measures compared to controls (Kagan et al 1988). In prospective investigations, behaviorally inhibited children were shown to be predisposed to generalized social anxiety, but not performance anxiety, separation anxiety, or specific phobias during adolescence (Schwartz et al 1999) and to depression, but not anxiety disorders, during young adulthood (Caspi et al 1996). Other investigations have also suggested that behaviorally inhibited children are at greater risk of developing anxiety disorders in childhood, particularly DSM-III-R overanxious disorder (GAD), social phobia or avoidant disorder, separation anxiety disorder (Biederman et al 1993) and posttraumatic stress disorder (PTSD) in adulthood (Davidson and Fairbank 1993).

One neurobiologic system of interest implicated in anxiety disorders is the amygdala and related nuclei and circuitry (Davis 1997; LeDoux 1998). In clinical studies, electrical stimulation of the amygdaloid region of patients undergoing surgery for temporal lobe epilepsy is associated with complex fear states involving palpitation, mydriasis, pallor, and fear-related thoughts (for review see Gloor 1992). Human imaging studies have implicated an amygdalar contribution to both acquisition and extinction processes during associative emotional learning tasks (LaBar et al 1998). In preclinical studies, electrical stimulation of the amygdaloid region of animals is associated with fearful behaviors, including increases in heart rate, blood pressure, freezing, activation of fear-related facial movements, and increases in plasma corticosteroids (for review see Davis 1992). Amygdala lesions reduce these fearful behaviors and emotional reactivity, and interfere with the acquisition of conditioned fear and the rise in plasma

From the Departments of Psychiatry (MDDB, RED, BB, DEW, DAA, NDR) and Pediatrics (RED), University of Pittsburgh Medical Center, and Developmental Traumatology Neuroimaging Laboratory, Western Psychiatric Institute and Clinic (MDD, KF, AMB, JH), Pittsburgh, Pennsylvania and Sackler Institute for Developmental Psychobiology, Weill Medical College of Cornell University, New York, New York (BJC, KMT).

Address reprint requests to Michael D. De Bellis, M.D., Western Psychiatric Institute & Clinic/UPMC, 3811 O'Hara Street, Pittsburgh PA 15213.

Received November 23, 1999; revised January 19, 2000; accepted January 28, 2000.

0006-3223/00/$20.00
PII S0006-3223(00)00835-0

Table 1. Demographic Characteristics of Children and Adolescents with Generalized Anxiety Disorder (GAD) and Healthy Control Subjects

	GAD	Control	Statistic	*p*
n	12	24	—	—
Age (years)	12.7 ± 2.4	12.5 ± 2.3	$Z(1) = 0.29$	.77
Range in years	(8–16)	(8–16)		
Race				
White/African American/biracial	7/4/1	18/2/4	Fisher exact *t*	.17
Weight (kg)	55.2 ± 21.1	53.5 ± 19.0	$t(34) = 0.25$	.81
Height (cm)	155.1 ± 11.6	154.4 ± 20.4	$Z(1) = 0.50$	.64
SES	41.7 ± 16.4	39.0 ± 10.4	$t(34) = 0.59$	.56
Gender (female/male)	5/7	10/14	$\chi^2 = 0$	ns
Full-scale IQ	123.2 ± 15.1	117.1 ± 19.1	$t(34) = 0.95$	.35

SES, socioeconomic status; *Z*, Wilcoxon/Kruskal–Wallis test.

corticotropin and plasma corticosteroids levels (for review see Davis 1997).

To date, there are few published neuroimaging studies of pediatric anxiety disorders. In this study, we examined amygdala volumes and other comparison brain regions of children and adolescents with generalized anxiety disorder and a matched control group. Among the structures of greatest interest was a planned comparison of amygdala volumes between GAD subjects and control subjects. Global brain morphology (intracranial volume, cerebral gray and white matter, corpus callosum) and comparison regions (temporal lobe and basal ganglia structures) were also measured. We hypothesized that global brain morphology and comparison regions would not differ between groups.

Methods and Materials

Subjects

Children and adolescents with DSM-IV GAD ($n = 12$) and healthy comparison subjects ($n = 24$) were recruited. Because of the high degree of known variability in volume of brain structures (Giedd et al 1996), two controls were case matched for each GAD subject for age (within 6 months), sex, height, and handedness. Groups were similar on weight, socioeconomic status (SES), and full scale IQ. Subjects underwent the Hollingshead four factor index of SES (Hollingshead 1975) for an assessment of SES; the vocabulary, digit span, block design, and object assembly subsets of the Wechsler Intelligence Scale for Children (WISC-R) for an estimate of IQ (Wechsler 1974); and the 12 handedness items from the Revised Physical and Neurological Examination for Subtle Signs (PANESS) Inventory (Denckla 1985), where 8 out of 12 items were defined as right handed. The demographic characteristics of the groups are found in Table 1.

Anxious children were recruited from the Child and Adolescent Anxiety and Depression Program of the Western Psychiatric Institute and Clinic, University of Pittsburgh. Children were evaluated by trained research clinicians blind to the subject's clinical status under the supervision of child psychiatrists (D.A.A., B.B.), and using a modified version of the Schedule for Affective Disorders and Schizophrenia for School-Age, Present Episode (K-SADS-P; Chambers et al 1985) and Lifetime Version (K-SADS-E; Orvaschel and Puig-Antich 1987) interview with both child and parent(s) as informants. Questions concerning traumatic events and PTSD symptoms over the subject's lifetime were incorporated into an expanded assessment of PTSD completed as part of the K-SADS. Additional questions involving the types of interpersonal and non-interpersonal traumas and the nature and circumstances of such traumatic experiences are described (Kaufman et al 1997). A psychiatrist then interviewed the child to confirm the presence of any psychiatric disorder (D.A.A., B.B.). Comorbidity in the GAD group included the following: depressive disorder not otherwise specified ($n = 3$), major depression ($n = 1$), panic disorder ($n = 1$), and social phobia ($n = 1$). The Screen for Child Anxiety Related Emotional Disorders Scale (SCARED; Birmaher et al 1999) is a 41-item parent and child self-report instrument. It consists of five factors that parallel the DSM-IV classification of anxiety disorders: somatic/panic; generalized anxiety; separation anxiety; social phobia; and school phobia (Birmaher et al 1997) and was used as a continuous measure of childhood anxiety symptoms. Total score for child report was 30.5 ± 12.9 and for parent report it was 37.1 ± 14.76. These are above the clinical cutoff of 25 (Birmaher et al 1999). All GAD subjects were psychotropic naive except one who had a history of brief treatment with antidepressants and a stimulant prior to the magnetic resonance imaging (MRI) scan.

Healthy control children were at low familial risk for depression and were required to never have had *any* lifetime psychopathology. Also, they were required to have no first-degree relatives with a lifetime episode of any mood or psychotic disorder, no second-degree relatives with a lifetime history of childhood-onset, recurrent, psychotic, or bipolar depression, schizoaffective, or schizophrenic disorder, and no more than 20% of their second-degree relatives could have a lifetime single episode of major depression.

Exclusionary criteria were the following: 1) the use or presence of medication with central nervous system or hypothalamic–pituitary effects within the past 2 weeks; 2) presence of a significant medical or neurological illness; 3) obesity (weight greater than 150% of ideal body weight) or growth failure (height

or weight under third percentile); 4) IQ lower than 80; 5) anorexia nervosa, autism, or schizophrenia by DSM-IV criteria; 6) inordinate fear of intravenous needles; 7) GAD chronologically secondary to conduct disorder; 8) specific learning disabilities; 9) a current diagnosis of PTSD or severe maltreatment history (with child protective services involvement) in the GAD group; and 10) positive trauma or maltreatment history in subjects in the healthy control group.

This protocol was approved by the University of Pittsburgh Institutional Review Board. Parent(s) or guardian(s) and adolescents 14 years of age and older gave written informed consent. Children assented before participating in this protocol. Subjects received monetary compensation for participation.

MRI Acquisition

Magnetic resonance imaging was performed using a GE 1.5 Tesla Unit (Signa System, General Electric Medical Systems, Milwaukee, WI) running version 5.4 software located at the University of Pittsburgh Medical Center Magnetic Resonance Research Center. A sagittal scout series verified patient position, cooperation, and image quality. A three-dimensional spoiled gradient recalled acquisition in the steady state pulse sequence was used to obtain 124 contiguous images with slice thickness of 1.5 mm in the coronal plane (using time of echo = 5 msec, time of repetition = 25 msec, flip angle = 40°, acquisition matrix = 256 × 192, number of excitations = 1, field of view = 24 cm). Coronal sections were obtained perpendicular to the anterior-commissure–posterior-commissure line to provide a more reproducible guide for image orientation. Axial proton density and T2-weighted images were obtained to enable exclusion of structural abnormalities on MRI. A neuroradiologist reviewed all scans and ruled out clinically significant abnormalities. All subjects tolerated the procedure well. No sedation was used.

Image Analysis

The imaging data were transferred from the MRI unit to a computer workstation (Power Macintosh, Apple Computer, Cupertino, CA) and analyzed using the IMAGE software (version 1.52) developed at the National Institutes of Health (Rasband 1996) that provides valid and reliable volume measurements of specific structures, using a manually operated (hand tracing) approach. All measurements were made by trained and reliable raters who were blind to subject information (JH and AMB or KF and JH). Interrater and intrarater reliabilities for independent designation of regions for all structures described were .91 (putamen) to .99 (intracranial volume) and .97 (putamen, amygdala) to .99 (intracranial volume), respectively. Complete methodological details are provided by De Bellis and colleagues (De Bellis et al 1999) and are only briefly described here.

INTRACRANIAL VOLUMES. Intracranial volumes were calculated by first manually tracing the intracranial volume of each coronal slice after exclusion of the cranium, summing these areas of successive coronal slices, including gray and white matter and cerebral spinal fluid (CSF) volumes, and multiplying by slice thickness. Cerebral volumes were measured after exclusion of cerebellum and brainstem in the same manner and include cortical and subcortical structures (Rosenberg et al 1997b).

CEREBRAL WHITE AND GRAY MATTER VOLUMES. Total cerebral white and gray matter volumes were calculated using a manually operated semiautomated segmentation algorithm. This computerized segmentation technique uses mathematically derived cutoffs for gray matter–white matter–CSF partitions with histograms of signal intensities and includes cortical and subcortical white and gray matter volumes (Rosenberg et al 1997b).

TEMPORAL LOBE STRUCTURES. Measures of temporal lobe, amygdala, and hippocampus were manually traced in the coronal plane as previously described (Giedd et al 1996). The temporal lobe was separated from frontal and parietal lobes by the sylvian fissure. The temporal stem was divided by a line connecting the most inferior point of the insular cisterns to the most lateral point of the hippocampal fissure. The coronal section including the posteriormost aspect of the corpus callosum was arbitrarily designated the temporal lobe posterior boundary (inclusive). The most anterior slice, in which the temporal stem is first visible, was defined as the anterior boundary of the amygdala. The slice showing the most anterior mammillary bodies was used as the amygdala–hippocampus boundary. The ambient cistern defined the medial border of the anterior hippocampus. The superior border of the hippocampus was bounded anteriorly by the temporal horn and posteriorly by the fornix. Our measurement of the hippocampal formation included the cornu ammonis, dentate gyrus, and subiculum. Then the areas were summed for each slice, and multiplied by slice thickness.

BASAL GANGLIA. The caudate (head and body and the nucleus accumbens) and putamen were manually traced in the coronal plane, summed for each slice, and multiplied by slice thickness, delineating the caudate nucleus medially by the lateral ventricle and laterally by white matter tracts from the globus pallidus and putamen.

CORPUS CALLOSUM. The corpus callosum was identified from a single midsagittal section selected as the slice showing full visualization of the cerebral aqueduct and the anterior and posterior commissures. This area was divided into seven regions (rostrum, genu, rostral body, anterior and posterior midbody, isthmus, and splenium) as described earlier (De Bellis et al 1999).

Statistical Methods

All data are presented as means ± SDs. Categorical demographic variables were compared using *t* test, χ^2 with continuity correction, or Fisher's exact tests as appropriate. Test of differences between groups were made using analysis of variance controlling for intracranial volume. Two-way (group-by-side) repeated-measures analyses were used to explore laterality effects. Data distributions were examined for normality using the Shapiro and Wilks W statistic; where significantly nonnormal distributions were found, the data were transformed to normalize the distributions before applying parametric tests. In cases where no

Table 2. Brain Structures of Children and Adolescents with Generalized Anxiety Disorder (GAD) and Healthy Control Subjects

	Unadjusted		Adjusted Least Square[a]			
Structures	GAD	Control	GAD	Control	Statistic[a]	*p*
Global Measures						
Intracranial volume (cm^3)	1525.38 (115.69)	1509.35 (184.67)			$t(34) = 0.27$	.79
Cerebral volume	1278.90 (98.58)	1272.37 (163.07)	1269.61 (24.95)	1277.01 (17.89)	$t(1,33) = 1.01$	.32
Cerebral gray matter	808.67 (83.55)	806.55 (112.81)	803.25 (42.67)	809.26 (68.74)	$t(1,33) = 0.27$	.79
Cerebral white matter	470.23 (45.20)	465.78 (97.00)	466.36 (43.97)	467.71 (64.59)	$t(1,33) = 0.06$	.95
Corpus callosum area (cm^2)	8.14 (1.50)	8.26 (1.09)	8.08 (1.45)	8.29 (0.98)	$t(30) = 0.49$	.63
Temporal lobe structures						
Temporal lobe (total)(cm^3)	190.86 (20.0)	186.92 (25.07)	189.62 (14.33)	187.54 (13.40)	$t(1,33) = 0.42$	.67
Right temporal lobe	100.33 (11.73)	96.25 (12.56)	99.74 (8.28)	96.55 (8.16)	$t(1,33) = 1.08$	.29
Left temporal lobe	90.52 (9.61)	90.61 (13.49)	89.88 (8.17)	90.93 (6.78)	$t(1,33) = 0.41$	.69
Amygdala (total)(cm^3)	5.31 (1.12)	4.61 (0.87)	5.27 (0.95)	4.62 (0.73)	$t(1,33) = 2.24$	.03
Right amygdala	3.00 (0.67)	2.53 (0.54)	2.99 (0.56)	2.54 (0.49)	$t(1,33) = 2.44$	.02
Left amygdala	2.30 (0.61)	2.09 (0.46)	2.29 (0.57)	2.09 (0.38)	$t(1,33) = 1.21$	.24
Hippocampus (total)(cm^3)	8.36 (1.04)	8.30 (1.11)	8.32 (1.12)	8.34 (0.86)	$t(1,33) = .02$	.99
Right hippocampus (total)	4.16 (0.60)	4.19 (0.59)	4.14 (0.65)	4.20 (0.48)	$t(1,33) = 0.29$	.77
Left hippocampus (total)	4.20 (0.53)	4.12 (0.56)	4.18 (0.54)	4.12 (0.44)	$t(1,33) = 0.35$	.73
Basal ganglia structures						
Caudate (total)(cm^3)	10.03 (1.99)	9.34 (1.22)	9.98 (1.63)	9.36 (1.16)	$t(1,33) = 1.30$	.20
Right caudate	5.12 (0.89)	4.80 (0.67)	5.10 (0.70)	4.81 (0.63)	$t(1,33) = 1.24$	.22
Left caudate	4.91 (1.11)	4.54 (0.58)	4.89 (0.95)	4.56 (0.56)	$t(1,33) = 1.30$	.20
Putamen (total)(cm^3)	6.81 (1.78)	7.12 (2.15)	6.79 (1.63)	7.12 (2.17)	$Z(1) = 0.25$	.80
Right putamen	3.01 (0.93)	3.14 (1.09)	3.01 (0.95)	3.14 (1.08)	$Z(1) = 0.18$	.85
Left putamen	3.82 (0.97)	3.93 (1.15)	3.80 (0.82)	3.94 (1.15)	$Z(1) = 0.05$	.96

Values presented are means (± SD). *Z*, Wilcoxon/Kruskal–Wallis test.
[a]Means adjusted for intracranial volume.

transformation normalized the data (age, height, and putamen volumes), nonparametric tests were used. In testing for covariate effects, such as gender and interaction (gender by group), multivariate regression analysis was used. Adjusted least squares brain structural means that differed significantly between the groups (right and total amygdala [adjusted for intracranial volume]) were correlated with clinical data using Spearman correlation coefficients. All significance testing was two tailed with $\alpha = .05$.

Results

Brain Measurements

Compared with control subjects, GAD subjects had larger right and total amygdala volumes than matched controls (Table 2 and Figure 1). The mean ranges for the total amygdala in the GAD group were 3.89–7.13 cm^3 and in control group were 3.118–6.477 cm^3. The mean ranges for the total hippocampus in the GAD group were 6.54–9.55 cm^3 and in the control group were 6.430–10.657 cm^3. Left amygdala, intracranial, and cerebral volumes, cerebral gray and white matter volumes, right and left temporal lobe volumes, right and left hippocampal volumes, right and left caudate and putamen volumes, and corpus callosum area did not differ between groups (Table 2). Corpus callosum regional areas, which reflect axons from specific cortical regions, also did not differ between groups. There were no significant side-by-group or sex-by-group interactions for all structures examined. There were no significant correlations between adjusted right and total amygdala volumes with the child or parent total or the individual five-factor SCARED scores.

Discussion

Children and adolescents with GAD were found to have significantly larger right and total amygdala volumes than matched control subjects. Intracranial, cerebral, cerebral gray and white matter volumes, and right and left temporal lobe, hippocampal, caudate, and putamen volumes, and total corpus callosum area and its comparison regional measures did not differ between groups. There were no significant associations between clinical anxiety ratings and amygdala volumes. To our knowledge, this is the first study to examine the association of amygdaloid dysmorphometry in GAD as well as being the first study to examine these measures in pediatric GAD.

The amygdala consists of several cell groups and many efferent projections involved in fear and anxiety. Direct projections from the central nucleus of the amygdala to a variety of regions (lateral hypothalamus and paraventricular nucleus, parabrachial nucleus, the ventral tegmental area, the locus coeruleus, central gray, nucleus reticularis

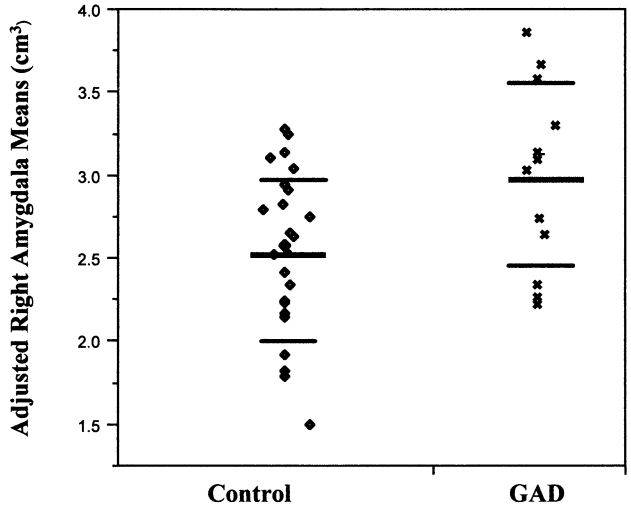

Figure 1. Right [$t(1,33) = 2.44$, $p = .02$] amygdala volume means (cm^3) and standard deviations adjusted for intracranial volume of children and adolescents with generalized anxiety disorder (GAD) and matched comparison subjects.

pontis caudalis, and trigeminal and facial nerve) are associated with many fearful and anxious behaviors (for review see Davis 1997). The functional anatomy of anxiety disorders is complex, however, and is thought to involve many other neural structures and circuits, including the thalamus, entorhinal cortex, orbitofrontal cortex, cortical association areas, and the cingulate gyrus (for review see Charney and Deutch 1996). Some authors have suggested that different fear circuits are responsible for different anxiety disorders (Charney and Deutch 1996; Davis 1998).

For example, results from recent investigations suggest that there are morphometric distinctions between obsessive-compulsive disorder (OCD) and other anxiety disorders. Pediatric OCD is associated with smaller striatum structures (caudate and putamen)(Rosenberg et al 1997b) and a selective deficit in frontostriatal function (Rosenberg et al 1997a) in medication naive subjects. Striatal abnormalities are also seen in adult OCD (Modell et al 1989; Robinson et al 1995). Recently, significantly smaller bilateral orbital frontal and amygdala volumes, as well as a lack of the normal hemispheric asymmetry of the hippocampus–amygdala complex, were found in adult OCD (Szeszko et al 1999). Unlike adult PTSD, pediatric PTSD is associated with global adverse affects (smaller cerebral volumes and corpus callosum areas) and no anatomical changes in limbic structures (De Bellis et al 1999); however, anterior cingulate dysfunction may be associated with both adult (Bremner et al 1999; Hamner et al 1999; Shin et al 1999) and pediatric PTSD (De Bellis et al, in press). Although there are no published studies to date on the psychobiology of panic disorder in children, adult panic disorder is thought to be the result of dysfunction in the locus coeruleus–norepinephrine system (Goddard and Charney 1997).

The core symptoms of GAD are exaggerated worry about several life circumstances and associated autonomic hyperactivity. GAD may be the result of dysfunction of the central amygdala, which may mediate stimulus-bound fears (i.e., specific worries) and the extended amygdala (the bed nucleus of the stria terminalis), which may mediate anxiety-related autonomic hyperactivity (Davis 1998). In support of this idea, children and adults who have a history of temporal lobe epilepsy or status epilepticus, and have damage to the amygdala, also have a greater likelihood of ictal fear (Cendes et al 1994). This suggests that local alterations in inhibitory circuits may contribute to a lowered seizure threshold, and greater excitability within the amygdala, which may led to ictal fear (Pitkanen et al 1998). Additionally, results from a recent positron emission tomography (PET) study showed that during a modified Stroop task, bilateral amygdala activation was significantly greater during color naming of threat words than during color naming of neutral words in healthy subjects (Isenberg et al 1999). Moreover, results from recent functional MRI investigations showed activation in the amygdala during viewing of masked fearful faces (Whalen et al 1998) and in the amygdala/periamygaloid cortex during both conditioned fear acquisition and extinction in healthy subjects (LaBar et al 1998). In the latter study, this activation was right hemisphere dominant.

The finding of larger right amygdala volumes in pediatric GAD is interesting in light of the results of several recent PET studies. Healthy subjects with high-trait anxiety showed greater right/left ratios of cerebral metabolism than low-trait anxiety subjects, particularly during the second PET session (Stapleton et al 1997). Healthy male subjects showed increased connectivity between the right but not the left amygdala and the pulvinar and superior colliculus during presentations of unseen (masked) fear-conditioned faces compared to seen (unmasked) fear-conditioned faces (Morris et al 1999). The glucose metabolic rate of the right but not the left amygdala in healthy subjects, who were viewing emotionally arousing (aversive) film clips, was highly correlated with their long-term memories of these films (Cahill et al 1996). Another PET study has implicated activation of the right amygdala in traumatic autobiographical memories (Rauch et al 1996). Bilateral amygdaloid complex damage in humans is associated with loss of enhanced recall of emotionally aversive memories (Cahill et al 1995). Furthermore, irrespective of generalized brain atrophy and cognitive impairments, the presence of traumatic memories of an earthquake was positively correlated with normalized amygdala volume in

subjects with Alzheimer's disease (Mori et al 1999). Thus, the amygdala may serve to enhance declarative memory for emotionally arousing and fearful events (for review see Cahill and McGaugh 1998).

Our results are also interesting in light of a recent PET study (Abercrombie et al 1998), showing that right amygdalar metabolic rates positively correlated with negative affect in depressed patients. Because behaviorally inhibited children were shown to be predisposed to depression, but not anxiety disorders, during young adulthood (Caspi et al 1996), prospective studies of right amygdalar function in pediatric GAD patients may foster a better understanding of the pathophysiology of adult onset depressive disorders.

In summary, right and total amygdala volumes were significantly larger in GAD subjects compared to control subjects, whereas global and comparison structural measures did not differ between groups. Unlike findings in most neuropsychiatric disorders, the relevant brain region implicated in anxiety disorders and fear-related behaviors is larger in patients than in control subjects. These pilot data suggest that dysmorphometry of the amygdala may represent a vulnerability to childhood GAD; however, small sample and effect sizes may have contributed to these findings. GAD is a complex disorder, involving stimulus-bound fearful cognitions, negative affect, exaggerated focus on memories of emotionally arousing and fearful events, and autonomic hyperactivity. Speculations about the functional significance of larger amygdala volumes include at least two possibilities. Increased amygdala volume may index some genetic trait, such as increased sensitivity to threat cues, which could create a vulnerability for pediatric GAD. Alternatively, the increase in amygdala volume might be the result of increased anticipatory anxiety during development; however, rather than finding an increase or decrease in relevant brain structures, perturbations of the neural networks involved in anxiety symptoms from the healthy state compared to the diseased state is what is most critical in understanding the neurobiology of GAD. For example, results from recent functional MRI investigations of adults who suffer from social phobia revealed that the amygdala may be selectively activated during exposure to neutral faces (Birbaumer et al 1998) and show differing patterns of activation during conditioned aversive stimuli compared to healthy adults (Schneider et al 1999). The etiology, neuropsychological function, and the permanence of our anatomical findings in pediatric GAD need to be examined. Future anatomical and functional MRI brain studies of childhood GAD and of children at risk for anxiety disorders are warranted.

This work was supported mainly by National Institute of Mental Health (NIMH) Grant No. MH 41712, "The Psychobiology of Anxiety and Depression in Children and Adolescents" (Principal Investigator: NDR) and by NIMH Grant No. 5 K08 MHO1324-02 (Principal Investigator: MDDB).

The primary author thanks the research staff—Jennifer Apical, Clayton H. Eccard, and Laura Trubnick—and Jay N. Giedd, M.D., for his technical consultation.

References

Abercrombie HC, Schaefer SM, Larson CL, Oakes TR, Lindgren KA, Holden JE, et al (1998): Metabolic rate in the right amygdala predicts negative affect in depressed patients. *Neuroreport* 9:3301–3307.

Biederman J, Rosenbaum JF, Bolduc-Murphy EA, Faraone SV, Chaloff J, Hirshfeld DR, et al (1993): Behavioral inhibition as a temperamental risk factor for anxiety disorders. *Child Adolesc Psychiatr Clin North Am* 2:667–683.

Birbaumer N, Grodd W, Diedrich O, Klose U, Erb M, Lotze M, et al (1998): FMRI reveals amygdala activation to human faces in social phobics. *Neuroreport* 9:1223–1226.

Birmaher B, Brent DA, Chiappetta L, Bridge J, Monga S, Baugher M (1999): Psychometric properties of the Screen for Child Anxiety Related Emotional Disorders Scale (SCARED): A replication study. *J Am Acad Child Adolesc Psychiatry* 38:1230–1236.

Birmaher B, Khetarpal S, Brent DA, Cully M, Balach L, Kaufman J, et al (1997): The Screen for Child Anxiety Related Emotional Disorders (SCARED): Scale construction and psychometric characteristics. *J Am Acad Child Adolesc Psychiatry* 36:545–553.

Bremner JD, Staib L, Kaloupek D, Southwick SM, Soufer R, Charney DS (1999): Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: A positron emission tomography study. *Biol Psychiatry* 45:806–816.

Cahill L, Babinsky R, Markowitsch HJ, McGaugh JL (1995): The amygdala and emotional memory. *Nature* 377:295–296.

Cahill L, Haier RJ, Fallon J, Alkire M, Tang C, Keator D, et al (1996): Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc Natl Acad Sci U S A* 93:8016–8021.

Cahill L, McGaugh JL (1998): Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci* 21:294–299.

Caspi A, Moffitt TE, Newman DL, Silva PA (1996): Behavioral observations at age 3 years predict adult psychiatric disorders. *Arch Gen Psychiatry* 53:1033–1039.

Cendes F, Andermann F, Gloor P, Gambardella A, Lopes-Cendes I, Watson C, et al (1994): Relationship between atrophy of the amygdala and ictal fear in temporal lobe epilepsy. *Brain* 117:739–746.

Chambers WJ, Puig-Antich J, Hirsch M, Paez P, Ambrosini PJ, Tabrizi MA, et al (1985): The assessment of affective disorders in children and adolescents by semi-structured interview: Test-retest reliability of the schedule for affective disorders and schizophrenia for school-age children, present episode version. *Arch Gen Psychiatry* 42:696–702.

Charney DS, Deutch A (1996): A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. *Crit Rev Neurobiol* 10:419–446.

Davidson JRT, Fairbank JA (1993): The epidemiology of posttraumatic stress disorder. In: Davidson JRT, Foa EB, editors. *Posttraumatic Stress Disorder DSM-IV and Beyond.* Washington, DC: American Psychiatric Press, 147–169.

Davis M (1992): The role of the amygdala in fear and anxiety. *Annu Rev Neurosci* 15:353–375.

Davis M (1997): Neurobiology of fear responses: The role of the amygdala. *J Neuropsychiatry Clin Neurosci* 9:382–402.

Davis M (1998): Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry* 44:1239–1247.

De Bellis MD, Keshavan M, Clark DB, Casey BJ, Giedd J, Boring AM, et al (1999): Developmental traumatology part II: brain development. *Biol Psychiatry* 45:1271–1284.

De Bellis MD, Keshavan MS, Spencer S, Hall J (in press): A pilot study of anterior cingulate N-acetylaspartate concentrations in maltreated children and adolescents with PTSD. *Am J Psychiatry.*

Denckla MB (1985): Revised physical and neurological examination for soft signs. *Psychopharmacol Bull* 21:773–800.

Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, et al (1996): Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4-18. *J Comp Neurol* 366:223–230.

Gloor P (1992): Role of the amygdala in temporal lobe epilepsy. In: Aggleton JP, editor. *The Amygdala: Neurobiological Aspects of Emotion, Memory and Mental Dysfunction.* New York: Wiley-Liss, 339–352.

Goddard AW, Charney DS (1997): Toward an integrated neurobiology of panic disorder. *J Clin Psychiatry* 58(suppl):4–11.

Hamner MB, Lorberbaum JP, George MS (1999): Potential role of the anterior cingulate cortex in PTSD: Review and hypothesis. *Depression Anxiety* 9:1–14.

Hollingshead AB (1975): Four factor index of social status. Unpublished manuscript, Yale University, New Haven, CT.

Isenberg N, Silbersweig D, Engelien A, Emmerich S, Malavade K, Beattie B, et al (1999): Linguistic threat activates the human amygdala. *Proc Natl Acad Sci U S A* 96:10456–10459.

Kagan J, Reznick JS, Gibbons J (1988): Biological basis of childhood shyness. *Science* 240:167–171.

Kashani JH, Orvaschel H (1990): A community study of anxiety in children and adolescents. *Am J Psychiatry* 147:313–318.

Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al (1997): Schedule for affective disorders and schizophrenia for school-age children—present and lifetime version (K-SADS-PL): Initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* 36:980–988.

LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA (1998): Human amygdala activation during conditioned fear acquisition and extinction: A mixed-trial fMRI study. *Neuron* 20:937–945.

LeDoux J (1998): Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry* 44:1229–1238.

Modell JG, Mountz JM, Curtis GC, Greden JF (1989): Neurophysiologic dysfunction in basal ganglia/limbic striatal and thalamocortical circuits as a pathogenic mechanism of obsessive-compulsive disorder. *J Neuropsychiatry* 1:27–36.

Mori E, Ikeda M, Hirono N, Kitagaki H, Imamura T, Shimomura T (1999): Amygdalar volume and emotional memory in Alzheimer's disease. *Am J Psychiatry* 156:216–222.

Morris JS, Ohman A, Dolan RJ (1999): A subcortical pathway to the right amygdala mediating "unseen" fear. *Proc Natl Acad Sci U S A* 96:1680–1685.

Orvaschel H, Puig-Antich J (1987): Schedule for Affective Disorder and Schizophrenia for School-Age Children, Epidemiologic Version (K-SADS-E), 4th version.

Pine DS, Grun J (1999): Childhood anxiety: Integrating developmental psychopathology and affective neuroscience. *J Child Adolesc Psychopharmacol* 9:1–12.

Pitkanen A, Tuunanen J, Kalviainen R, Partanen K, Salmenpera T (1998): Amygdala damage in experimental and human temporal lobe epilepsy. *Epilepsy Res* 32:233–253.

Rasband W (1996): *NIH IMAGE Manual.* Bethesda, MD: National Institutes of Health.

Rauch SL, van der Kolk BA, Fisler RE, Alpert NM, Orr SP, Savage CR, et al (1996): A symptom provocation study of posttraumatic stress disorder using posiyton emission tomography and script-driven imagery. *Arch Gen Psychiatry* 53: 380–387.

Robinson D, Wu H, Munne RE, Ashtari M, Alvir J, Lerner G, et al (1995): Reduced caudate nucleus volume in obsessive-compulsive disorder. *Arch Gen Psychiatry* 52:393–398.

Rosenberg DR, Averbach DH, O'Hearn KM, Seymour AB, Birmaher B, Sweeney JA (1997a): Oculomotor response inhibition abnormalities in pediatric obsessive-compulsive disorder. *Arch Gen Psychiatry* 54:831–838.

Rosenberg DR, Keshavan MS, O'Hearn KM, Dick EL, Bagwell WW, Seymour AB, et al (1997b): Frontostriatal measurement in treatment-naive children with obsessive-compulsive disorder. *Arch Gen Psychiatry* 54:824–830.

Schneider F, Weiss U, Kessler C, Muller-Gartner H-W, Posse S, Salloum J, et al (1999): Subcortical correlates of differential classical conditioning of aversive emotional reactions in social phobia. *Biol Psychiatry* 45:863–871.

Schwartz CE, Snidman N, Kagan J (1999): Adolescent social anxiety as an outcome of inhibited temperament in childhood. *J Am Acad Child Adolesc Psychiatry* 38:1008–1015.

Shaffer D, Fisher P, Dulcan MK, Davies M, Piacentini J, Schwab-Stone ME, et al (1996): The NIMH diagnostic interview schedule for children version 2.3 (DISC-2.3): Description, acceptability, prevalence rates, and performance in the MECA study. *J Am Acad Child Adolesc Psychiatry* 35:865–877.

Shin LM, McNally RJ, Kosslyn SM, Thompson WL, Rauch SL, Alpert NM, et al (1999): Regional cerebral blood flow during script-imagery in childhood sexual abuse-related PTSD: A PET investigation. *Am J Psychiatry* 156:575–584.

Stapleton JM, Morgan MJ, Liu X, Yung BC, Phillips RL, Wong DF, et al (1997): Cerebral glucose utilization is reduced in second test session. *J Cereb Blood Flow Metab* 17:704–712.

Szeszko PR, Robinson D, Alvir J, Bilder R, Lencz T, Ashtari M, et al (1999): Orbital frontal and amygdala volume reductions in obsessive-compulsive disorder. *Arch Gen Psychiatry* 56: 913–919.

Wechsler D (1974): *Manual for the Wechsler Intelligence Scale for Children—Revised.* New York: The Psychological Corp.

Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA (1998): Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J Neurosci* 18:411–418.