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Amygdala Volume Analysis in Female Twins with Major Depression

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Abstract

Background—Previous research examining the amygdala volumes in major depressive disorder (MDD) has found conflicting evidence for association. Furthermore, few of these studies have examined differences in individuals with an onset during childhood or adolescence. This study examined amygdala volume and its potential association with early onset major depression.

Methods—A community-based sample of 47 right-handed young adult female monozygotic and dizygotic twin pairs was examined. For 29 twin pairs, one twin per pair had a lifetime history of MDD, while 18 age-matched control twin pairs had no lifetime history of MDD or other Axis I disorder. Core, noncore, and total amygdala volumes were estimated based on a combination of manual tracing, automated segmentation, and expert rater regional boundary definitions.

Results—No significant differences were found in amygdala volumes between depressed, high-risk, or control subjects. However, analyses comparing control monozygotic twins to randomly created control subject pairs suggest that there are familial, perhaps genetic, influences on core and total amygdala volumes.

Conclusions—Findings suggest that although there were no significant differences in amygdala volumes between groups, familial factors influence amygdala volumes. Discrepancies between studies measuring amygdala volume in MDD may be due to differences in amygdala boundary definitions.

Keywords

Amygdala; major depression; methods; MRI; segmentation; twins

Major depressive disorder (MDD) is a syndrome affecting nearly 34 million adults in the United States (Kessler *et al.* 2003) and has been shown to have both environmental and genetic contributions. With the advancement of image technology, recent research has focused on imaging studies to understand both functional and structural changes in the brain related to the disorder (Anand and Shekhar 2003; Botteron 2001; Drevets 2000; Drevets *et al.* 2003; Mayberg 2003; Seminowicz *et al.* 2004; Sheline 2003). By examination of specific regional brain differences in family and twin studies, neuroimaging studies can help in determining both

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genetic and environmental influences on the underlying pathophysiology of MDD. Presently, most neuroimaging studies have focused on adult populations, with little emphasis on childhood or adolescent illness.

A brain region of particular relevance to MDD and imaging studies is the amygdala due to its role in affect regulation. Functional imaging studies in adults have shown that the amygdala has higher activity in response to emotional stimuli in depressed compared with control subjects (Anand *et al.* 2005; Sheline *et al.* 2001; Siegle *et al.* 2003), while girls with MDD showed a blunted amygdala response to fearful faces compared with female control subjects (Thomas *et al.* 2001). Structural imaging studies, using magnetic resonance imaging (MRI), have been less consistent in finding quantitative relationships between amygdala volume and MDD. In adults, some studies have found no relationship between amygdala volume and MDD (Axelson *et al.* 1993; Bremner *et al.* 2000; Caetano *et al.* 2004; Coffey *et al.* 1993; Frodl *et al.* 2004; Mervaala *et al.* 2000), while other studies have found either an increase (Frodl *et al.* 2002, 2003; Lange and Irle 2004) or decrease (Hastings *et al.* 2004; Sheline *et al.* 1998, 1999; Siegle *et al.* 2003) in amygdala volume in MDD subjects compared with healthy control subjects. These studies have not found significant relationships between amygdala volumes and clinical characteristics, such as duration of illness or treatment variables. In childhood and adolescence, one study (Rosso *et al.* 2005) found that depressed youth had smaller left and right amygdala volumes compared with control subjects. Another study (MacMillan *et al.* 2003) found larger amygdala volumes in depressed compared with control youth, yet these results were not significant after controlling for age and intracranial volume. In addition, they found an increase in left and right amygdala: hippocampal volume ratios in depressed children, which was associated with anxiety severity. In sum, studies on amygdala volume in both adults and children present conflicting findings, suggesting that more detailed studies are needed to understand these discrepancies (for a summary of these studies, see Table 1).

Anatomically, the amygdala is composed of a grouping of approximately 12 nuclei, with each nucleus receiving inputs from different sensory modalities and connecting to other brain regions, such as the hippocampus, hypothalamus, and periaqueductal gray matter (Price 2003; Price *et al.* 1987). These nuclei have receptors for different neurotransmitters, including prominent serotonin receptor and transporter distribution (de Olmas 2004). By examining differences in specific clusters of amygdaloid nuclei, we may be able to understand relationships between these nuclei, MDD, and other structures involved in affect regulation. For example, two studies have examined differences in the core (consisting of the lateral, basal, and accessory basal nuclei), noncore (remaining nuclei, which include the central, medial, and periamygdaloid nuclei), and total (all nuclei) amygdala in a sample of elderly depressed female subjects (Sheline *et al.* 1998, 1999). In these studies, the core amygdala volume was smaller in MDD subjects compared with healthy control subjects, while there was no significant difference between groups in either the noncore or total amygdala volumes. Another study (Siegle *et al.* 2003) found a smaller left core amygdala volume in depressed versus control subjects, with no difference in the right core amygdala volume between groups. This group did not report volumes for either the noncore or total amygdala.

This study extends previous work by examining relationships between core, noncore, and total amygdala volumes (following the Sheline *et al.* 1998 definitions of core and noncore amygdala) and MDD in a young adult female twin sample from the community. We hypothesized that there would be differences in amygdala volume between depressed and healthy control twins. Furthermore, given that previous studies have not found significant relationships between depression severity and amygdala volume, we hypothesized that altered amygdala volumes precede the onset of MDD. By comparing volumes between depressed twins and twins who are at high risk (co-twins of MDD subjects) for developing the disorder, we can determine if altered amygdala volumes are a risk factor for developing MDD. By using a twin sample, we

can compare MDD and high-risk subjects, while controlling for other variables, such as age and common environment (Botteron 2001; Goldberg *et al.* 1995; McNeil *et al.* 2000). Finally, given that other brain regions involved in affect regulation show a genetic contribution (Wright *et al.* 2002), we hypothesized that there would be a general genetic contribution to amygdala volume.

Methods and Materials

Participants

Subjects were ascertained from the general population through Missouri birth records (Glowinski *et al.* 2003; Heath *et al.* 1999, 2002). Briefly, this prospective study assessed female-female twin pairs born from 1975 onward. Subjects were initially assessed at 13, 15, 17, and 19 years old, with new cohorts aged 13 years added each year of the study. Zygosity was based on structured parental reports (whenever possible, the biological mother). Compared with genotyping, maternal reports of twin zygosity by a telephone questionnaire yielded 95% accuracy (Eaves *et al.* 1989). In addition, a parent (usually the biological mother) and the twins completed separate structured telephone interviews using a modified version of the Child Semi-Structured Assessment for the Genetics of Alcoholism (C-SSAGA) (Kuperman *et al.* 1999) to assess medical, psychiatric, and first-degree relative family history. Kappa coefficients for the C-SSAGA diagnostic sections were uniformly high (R.D.T.; unpublished data).

In our study, subjects were a subset from this larger sampling population. As part of our ongoing studies of MRI neuromorphometry in twin populations, we have enrolled and imaged 270 twins (90 MDD pairs and 45 control pairs). Once the twin pairs were identified through screening procedures, they were reassessed for inclusion or exclusion in this ongoing study. Inclusion criteria for affected twin pairs in this study were as follows: 1) female twin pair, either monozygotic (MZ) or dizygotic (DZ), with an age at scan of 13 to 25 years; 2) both twins right-handed; 3) at least one twin with a lifetime DSM-IV (American Psychiatric Association 1994) diagnosis of MDD with at least a 4-week duration; 4) age of onset at or before 18 years of age; and 5) a positive family history of DSM-IV lifetime diagnosis of MDD in at least one first-degree relative or at least two second-degree relatives (for subjects less than 15 years old). Control twins met the same inclusion criteria as the affected twin pairs, except that neither twin could have a personal lifetime history of a DSM-IV Axis I diagnosis. In addition, no first-degree relative could have a lifetime history of a mood disorder. Exclusion criteria for both groups were as follows: 1) known mental retardation; 2) autism, schizophrenia, Tourette's disorder, or eating disorder; 3) major medical illness known or hypothesized to affect the central nervous system (e.g., diabetes, muscular dystrophy); 4) significant neurological illness (e.g., epilepsy or other seizure disorder, cerebral palsy); 5) pregnancy (temporary exclusion); 6) history of a serious head injury with loss of consciousness for more than 5 minutes; 7) intraocular metallic objects, cochlear implants, pace-makers, or other electrical, mechanical, or magnetically activated implants; 8) alcohol or drug dependence; or 9) adopted outside of the family. The exclusion criteria were designed to eliminate most known causes of changes in brain structure and conditions contraindicated for MRI.

Here, we examined 37 twin pairs (24 MZ, 13 DZ; 21 affected, 16 control pairs) and 10 individual twins (7 MZ, 3 DZ) aged 15 to 25 years that were part of our ongoing MDD study. Of the 10 individual twins, 5 were MDD subjects, 3 were high-risk subjects, and 2 were control subjects. These twins were selected for the current study to oversample for discordant MZ twin pairs, since discordant MZ pairs provide a powerful design to determine initial estimates of familiarity, in contrast to differences associated with having a disorder and differences that may represent at risk factors to developing the disorder. Furthermore, since structural development is ongoing into adulthood, MZ twins serve as excellent contrasting subjects to examine for regional brain differences related to environmental effects while controlling for

ongoing neurodevelopmental processes (Botteron 2001). Average age of onset of MDD was 15.58 years (range 10 to 18 years). The Washington University School of Medicine Human Studies Committee approved the study. All subjects gave written informed assent (if under 18 years old) or consent (18 years old or older) for participation in the study. Parents of subjects under 18 years of age gave written informed consent.

MRI Acquisition and Processing

Three T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE) images (1.0 mm³ isotropic voxels) were acquired on a Siemens 1.5 Tesla MRI scanner (Siemens AG, Erlangen, Germany) (sagittal acquisition, repetition time [TR] = 10 msec; echo time [TE] = 4 msec; time to inversion [TI]/time delay [TD] = 20/0; flip angle = 10°; slab = 160 mm; 160 partitions; 256 × 256 matrix; field of view [FOV] = 256; 4 signal averages; total scanning time: 26 min 55 sec). The images were coregistered and summed to produce one image, with increased signal-to-noise ratio (Holmes *et al.* 1998). This image was then interpolated to .5 mm isotropic voxels. Figure 1 shows the amygdala using a single MP-RAGE image at 1.0 mm isotropic voxels and the summed image at .5 mm isotropic voxels. (Note: These figures are less clear than what is visible during analysis on the computer console).

The temporal lobe was manually traced out from the rest of the brain to apply a histogram-based expectation-maximization (EM) segmentation algorithm (Ratnanather *et al.* 2004), creating a five-part segmentation (i.e., gray matter, white matter, cerebrospinal fluid [CSF], gray matter/white matter partial volume, and gray matter/CSF partial volume). Both the segmented and unsegmented temporal lobe were then oriented along the long axis of the hippocampus (LAH) for each hemisphere. Using the statistics from the EM segmentation, the entropy of the segmentation was calculated to generate an entropy-based segmentation (EBS). The EBS segmentation is based on the following principles: given an image intensity that is categorized as a particular tissue type by the EM algorithm, the EBS calculated the log ratio of the probability that the intensity is indeed that tissue to the sum of all tissue type probabilities at that intensity. The EBS helped distinguish regions where the EM tissue categorization was made with a smaller margin of the difference in tissue probability. The EBS was used to complement the grayscale image in areas where it was difficult to distinguish between gray matter, white matter, and CSF. The core and noncore amygdala were then manually traced on the grayscale image in coronal sections along the LAH in ANALYZE (Mayo Foundation, Rochester, Minnesota) (Robb 2001) using the EBS to clarify some boundaries which were difficult to discern on the grayscale image alone. Tracings were used to delineate the core and noncore amygdala boundaries to calculate pure gray matter volume and gray matter/white matter partial volume within the temporal lobe based on the EM segmentation. Figure 2 shows both a T1-weighted image and the corresponding EBS at various slices in the amygdala.

Amygdala Boundaries

Core Amygdala

The anterior boundary is the first slice where the amygdala appears in the image. The posterior boundary is the last slice where the amygdala can be distinguished from surrounding gray matter. The superior boundary in anterior sections is defined by white matter. In posterior sections, the superior boundary is defined by a horizontal line extending from the top of the entorhinal cortex to adjacent white matter. The inferior boundary in anterior sections is defined by white matter below the amygdala; however, once the temporal horn is visible, it is used to define the inferior border. In more posterior sections, the inferior boundary is defined by the white matter that divides the amygdala and hippocampus, as well as by the temporal horn. The medial boundary is defined by thin white matter that lies lateral to the semilunar gyrus,

continuing until a notch (sulcus semiannularis) forms that divides the amygdala from the hippocampus. The lateral boundary is defined by white matter.

Noncore Amygdala

The anterior boundary is the first slice where either the cortex becomes less continuously uniform and displays some thickening or the temporal stem first appears— whichever occurs first. The posterior boundary is the last slice where the core amygdala is indistinguishable from the semilunar gyrus and the white matter that serves as the superior boundary of the core amygdala disappears. In both anterior and posterior slices, the superior boundary is defined by a horizontal line extending from the top of the entorhinal cortex to adjacent white matter. In both anterior and posterior sections, the inferior boundary is the same as the core amygdala superior boundary. The medial boundary in all sections is CSF, while the lateral boundary is the white and gray matter junction.

Total Amygdala

The total amygdala is the sum of the core and noncore amygdala. For more detailed anatomical definitions, see Supplement 1.

One rater (M.A.M.), who was blind to subject diagnosis, completed all tracings. Intrarater reliability measurements were based on blind repeated measures for 10 amygdala. Intraclass correlations for the core, noncore, and total amygdala were .89, .60, and .97, respectively. While the noncore amygdala reliability was relatively low, this was the first attempt we are aware of to obtain reliability measurements on the noncore nuclei. We chose to manually trace the amygdala so the tracings could be used for subsequent shape analyses.

Image and Statistical Analysis

The EM segmentation was used to classify voxels within the manually traced area as gray matter, white matter, CSF, gray matter/white matter partial volume, or gray matter/CSF partial volume. All volumes are reported as voxel counts and are the sum of all gray matter plus half of the gray matter/white matter partial volume.

To examine differences between diagnostic groups, two-tailed t tests were used to compare amygdala volumes between MDD and unrelated control twins, as well as between high-risk and unrelated control twins. Paired t tests were used to examine differences some within the discordant twin pairs. For unrelated control twins, one twin was randomly chosen for inclusion in these analyses. The Bonferroni correction was applied for multiple tests. To test whether MZ pairs are more similar than unrelated pairs (i.e., a general genetic contribution) to amygdala volume, Pearson correlations for the volumes were calculated separately for the 12 MZ control twin pairs and 313 randomly created pairs from control subjects. These correlations were then standardized to Z scores and the z -value difference between the two groups was calculated. Additionally, we used a t test to compare the absolute mean percent difference in amygdala volumes for the MZ control twin pairs and randomly created subject pairs. Lastly, we compared the absolute mean percent difference between MZ affected and MZ control twin pairs.

Results

Major depressive disorder, high-risk, and control twins did not differ in age (all p -values $> .98$) and there were no significant differences in total brain volume between MDD, high-risk, and control subjects (all p -values $> .05$). Analyses looking for an interaction between total brain volume and diagnostic group on amygdala volume did not reveal any significant effects (data not shown). Thus, data are presented without covarying for total brain volume.

Mean volumes for the core, noncore, and total amygdala in all three groups are presented in Table 2. Even before correction for multiple testing, no significant differences were found between the left and right core, noncore, and total amygdala volumes in MDD versus control and high-risk versus control groups. No differences were found between MDD and their high-risk co-twins in the paired analysis of discordant twins.

To evaluate whether genetic factors impact amygdala volume in healthy control subjects, we compared MZ control twin pairs ($n = 12$) with randomly created control subject pairs. Because all subjects were already matched on gender and were within a very narrow age range, no additional criteria were used to create random subject pairs. Traditional covariance analysis of within twin pair variance to estimate heritability would include a comparison of MZ and DZ twins; however, there were not sufficient numbers of DZ twins within the current subsample analyzed ($n = 4$) to perform such analysis. When we used Pearson correlations, there was a significant relationship between control twin pairs (twin 1 vs. twin 2) on the left total ($r = .661$, $p = .019$), right core ($r = .750$, $p = .005$), and right total ($r = .713$, $p = .009$) amygdala volumes. There was no significant correlation between randomly created subject pairs and left and right core, noncore, and total amygdala volumes. The z -values of the differences between correlations in MZ control twin pairs and randomly created control subject pairs showed a strong trend toward a significant difference for the right total (z -value = 1.95, $p < .05$) amygdala volume, with the higher correlation in the MZ control twin pairs (data not shown).

Another way to explore genetic influences on amygdala volume was to examine the absolute mean percent difference between the MZ control twin pairs and the randomly created subject pairs ($n = 313$). Using the Satterthwaite method for unequal variances, analyses showed that there was a significant difference for the left total ($t = 4.02$, $p = .001$), right core ($t = 3.75$, $p = .002$), right noncore ($t = 2.16$, $p = .051$), and right total ($t = 4.31$, $p = .001$) amygdala volumes. All of these differences remained significant after correction for multiple comparisons, except for the right noncore amygdala volume ($p = .306$). For all amygdala volumes, there was less variance in the control twin pairs compared with the 313 randomly created subject pairs. When we compared intrapair differences in affected versus control twin pairs, there were no significant percent differences between the groups for the left and right core, noncore, and total amygdala volumes (Table 3).

Discussion

Our study did not find significant differences in left and right core, noncore, and total amygdala volumes between depressed, high-risk, or control twins. These findings are consistent with some previous studies that have not found differences in amygdala volumes between depressed and control subjects (Axelson *et al.* 1993; Bremner *et al.* 2000; Caetano *et al.* 2004; Coffey *et al.* 1993; Frodl *et al.* 2004; Mervaala *et al.* 2000). Additionally, we did not find support for our hypothesis that amygdala volume changes may precede the onset of MDD and represent an at-risk variable in individuals at high risk for MDD (co-twins of MDD subjects).

There could be several factors contributing to the difference in our findings in comparison with some other research groups. First, it may be that the current sample size was too small to detect subtle group differences. It is also possible that since this was a community-based sample and other samples are from clinical settings, our depressed subjects may not include the most severe cases, as are typically seen in clinically based samples. Although severity of illness was not directly reported with amygdala volume, 5 of the 20 patients in the Sheline *et al.* (1998) study had received electroconvulsive therapy (ECT) during their course of treatment, suggesting a more severe illness. In contrast, Glowinski *et al.* (2003) reported that in the larger dataset of Missouri born female twins, only 38% of depressed subjects reported speaking to someone about their depression (counselor, clergy, physician, etc.), 17% were prescribed medications,

and 9% were hospitalized for their depression. In our subsample of this larger study, only nine subjects reported speaking to someone about their depression and two of these subjects were prescribed medication, with only one hospitalized for her depression. Furthermore, only seven of our subjects reported having multiple depressive episodes, and only one of them reported having more than three episodes. Due to the young age of the current sample size, MDD is by definition less chronic. To the extent that volume changes may be related to the length of illness, then the current study would not be expected to demonstrate group differences. However, many studies suggest that early age of onset is a major determinate of future chronicity and severity. Thus, in comparison with other depressed samples looking at amygdala volume, our sample of 26 adolescent-onset depressed female subjects may currently be less severe but would likely display an increasingly recurrent and chronic long-term course.

Differences in measurement techniques may also be contributing to the discrepancy between groups. As a secondary aim of this study, using the advancement of recent imaging technology, we believe we improved the anatomical fidelity and reliability of the measurement of the core and total amygdala volumes. With the use of multiple averaged T1-weighted scans to improve the signal-to-noise ratio (Holmes *et al.* 1998) and by using a probabilistic segmentation technique to supplement the grayscale image, we were better able to discriminate amygdaloid nuclei and thus obtain a more accurate estimate of amygdala volume. We used the long axis of the hippocampus to orient the amygdala, whereas the majority of other studies have used the anterior commissure-posterior commissure (AC-PC) orientation. Although Kates *et al.* (1997) suggested that the AC-PC orientation was slightly more reliable using their methods than the LAH in measuring amygdala volume, the difference was not statistically significant. They suggested that the reason for this difference was because in the AC-PC orientation, amygdala boundaries were more dependent on external rather than internal structures. However, when measuring the core, noncore, and total amygdala volumes, earlier data from our group reveal that using internal structures is more anatomically accurate than using external structures (e.g., temporal stem) in determining the most anterior slice of noncore amygdala. The use of external structures as anatomical landmarks may introduce measurement bias unrelated to characteristics of the region of interest, since nearby regional differences could be related to MDD and subsequently influence the position of this external structure. Thus, using the LAH orientation to measure core, noncore, and total amygdala may be more anatomically accurate than the AC-PC orientation because the LAH orientation relies on internal rather than external structures to determine its boundaries.

Our results add to existing literature by examining whether core, noncore, or total amygdala volumes have a substantial genetic influence. Although the sample is small, data from our MZ control twins provide evidence that there is a general familial, perhaps genetic, influence on amygdala volumes, specifically for the left total, right core, and right total amygdala. Furthermore, the nonsignificant differences in amygdala volumes between MZ affected and MZ control twins suggest that amygdala volume differences within twin pairs may be influenced more by familial effects than by diagnosis. Wright *et al.* (2002) examined genetic contributions to various brain structures and found significant genetic influences on the orbital frontal cortex, hippocampus, and posterior cingulate gyrus, which are other regions thought to be involved in affect regulation. Family or twin studies, including a contrast group of healthy DZ twins, are needed to confirm the familial influences and see if influences on amygdala volume are genetic.

There are a number of strengths associated with this study. First, our sample was community based, allowing us to eliminate referral bias and generalize our results to the general population of young adult women rather than to just a clinical sample. Second, we used a twin design to help disentangle at-risk versus disease-related regional brain effects, i.e., whether or not the amygdala had a significant at-risk or genetic contribution to MDD. Third, the use of a younger

sample aids in determining whether amygdala volume differences are present very early in the course or are the result of the illness. Fourth, we measured the core, noncore, and total amygdala volumes to better account for the relationships between MDD and amygdala structure and function (Sheline *et al.* 1998). Two studies by Sheline *et al.* (1998, 1999) and our study are the only published studies examining differences in these three clusters of amygdaloid nuclei in MDD, while Siegle *et al.* (2003) reported on only one cluster, the core amygdala. Because there are clear differences noted in the function of different clusters of amygdaloid nuclei in animal, including primate, studies (Bonda 2000; Price 2003; Price *et al.* 1987; Sah *et al.* 2003), attempts to differentiate regions within the amygdala may help to localize pathophysiology of disorders of interest. Understanding the relationships between specific nuclei and MDD may influence treatment strategies by suggesting different treatment options that target known receptors associated with involved nuclei or may help to further clarify disease processes in mood disorders. Fifth, our study sought to develop a consistent amygdala measurement that relies on internal structure rather than external landmarks to determine boundary definitions. Previous studies use a variety of amygdala boundary definitions that, for the most part, rely on external structures (e.g., temporal stem). However, we wanted to develop standardized measurement procedures that relied on internal structures for measuring the amygdala to ensure that discrete nuclei are appropriately accounted for. Part of improving the anatomical definition of the amygdala includes using an average of three MP-RAGE images to create one image for analyses to increase our signal-to-noise ratio (Holmes *et al.* 1998). In addition, it is beneficial to use .5 mm isotropic voxels to examine amygdala volume, because this increases our ability to detect subtle changes that occur in the amygdala when progressing from anterior to posterior regions of the brain (Sheline *et al.* 1998). Additionally, smaller voxels minimize volume bias even when image resolution is not improved (Haller *et al.* 1994).

Despite these strengths, there are some limitations that should be noted. First, the sample size was relatively small. However, our sample size was similar to that of previous reports on amygdala volume in MDD (Bremner *et al.* 2000; Hastings *et al.* 2004; Lange and Irlle 2004; Sheline *et al.* 1998). Second, future psychopathology for these subjects is not known. It is noted that a significant fraction of individuals with early-onset MDD later develop bipolar disorder (Geller *et al.* 2001). Most studies of subjects with bipolar affective disorder have found smaller amygdala volumes in comparison with control subjects, both in adolescents and adults (Blumberg *et al.* 2003, 2005; Chen *et al.* 2004; Pearlson *et al.* 1997). In addition, although control subjects had a negative family history for MDD, there is a chance that they could later develop the disorder. Third, as assessed by the C-SSAGA, depressed subjects included in this study only expressed mild to moderate MDD prior to inclusion in this study. Thus, our results may not generalize to individuals with more severe MDD.

We believe this study should encourage other research groups to take advantage of the advancements in imaging techniques to more accurately assess differences in core, noncore, and total amygdala volumes in subjects with MDD and other psychiatric disorders. Expansion of the current sample size would help to more clearly establish the potential relationship between amygdala structure and early-onset MDD, as well as to determine if amygdala volumes present an at-risk variable for MDD. Lastly, evidence is needed from both longitudinal and genetic studies to see if differences in amygdala volume between depressed and control subjects develop over time and if longitudinal changes are related to clinical characteristics, treatment variables, or are influenced by other environmental or genetic factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Comparison of amygdala using standard methods versus newer image averaged methods. (A) Standard method to measure amygdala = one T1-weighted MR image at 1.0 mm³. (B) Newer method to measure amygdala = three summed T1-weighted MR images at .5 mm³.

Figure 2. Anterior (top) to posterior (bottom) slices illustrating the core, noncore, and total amygdala. Note: grayscale MR images (left) with corresponding entropy-based segmentation (right).

Table 1

Studies Measuring Amygdala Volume in Major Depressive Disorder

Author (Year)	Population	Depressed Subjects (n), Mean Age (SD)	Control Subjects (n), Mean Age (SD)	Region	Slice Thickness	Orientation	Results
Axelson <i>et al.</i> (1993)	MDD	19, 46.7 yrs (20.4)	30, 56.6 yrs (19.1)	AHC	5.0 mm	Not mentioned	NS
Coffey <i>et al.</i> (1993)	Both	48, 62.4 yrs (16.4)	76, 61.6 yrs (15.9)	AHC	5.0 mm	Not mentioned	NS
Sheline <i>et al.</i> (1998)	MDD	20, 53.0 yrs (17)	20, 54.0 yrs (18)	C, NC, and T Amygdala	.5 mm	AC-PC	L and R C: MDD < Con. NC and T: NS
Sheline <i>et al.</i> (1999)	MDD	24, 52.8 yrs (18.4)	24, 52.8 yrs (17.8)	C, NC, and T Amygdala	.5 mm	AC-PC	L and R C: MDD < Con. NC and T: NS
Bremner <i>et al.</i> (2000)	MDD	16, 43.0 yrs (8)	16, 45.0 yrs (10)	Amygdala	3.0 mm	LAH	NS when controlled for TBV
Mervaala <i>et al.</i> (2000)	Both	34, 42.2 yrs (12.2)	17, 42.1 yrs (14.6)	Amygdala	3.0 mm	LAH	NS MDD: R < L
Frodl <i>et al.</i> (2002)	MDD	30, 40.3 yrs (12.6)	30, 40.6 yrs (12.5)	Amygdala	1.5 mm	AC-PC	L and R: MDD > Con.
Frodl <i>et al.</i> (2003)	MDD (first-episode [F] and recurrent [R])	F: 30, 40.3 yrs (12.6); R: 27, 49.1 yrs (10.5)	F: 30, 40.6 yrs (12.5); R: 27, 46.3 yrs (11.3)	Amygdala	1.5 mm	Not mentioned	L and R: F > R L and R: F gt; Con.
MacMillan <i>et al.</i> (2003)	MDD	23, Range 8–17 yrs	23, Range 8–17 yrs	AHC	1.5 mm	AC-PC	L and R: MDD > Con.
Siegle <i>et al.</i> (2003)	MDD	7, Range 24–46 yrs	8, Range 21–47 yrs	C Amygdala	Not mentioned	AC-PC	L C: MDD < Con.
Caetano <i>et al.</i> (2004)	MDD	31, 39.2 yrs (11.9)	31, 36.7 yrs (10.7)	Amygdala	1.5 mm	Not mentioned	Trend for L: MDD < Con.
Frodl <i>et al.</i> (2004)	MDD ^a	30, 48.4 yrs (13.4)	30, 45.7 yrs (12.9)	Amygdala	1.0 mm	AC-PC	NS; No change at 1 year FU
Hastings <i>et al.</i> (2004)	MDD	18, 38.9 yrs (11.4)	18, 34.8 yrs (13.6)	Amygdala	1.5 mm	AC-PC	R: MDD < Con. in female subjects
Lange and Irie (2004)	MDD	17, 34.0 yrs (10)	17, 32.0 yrs (6)	Amygdala	1.0 mm	Not mentioned	L and R: MDD > Con.
Rosso <i>et al.</i> (2005)	MDD	20, 15.35 yrs (.34)	24, 14.08 yrs (.31)	Amygdala	1.5 mm	Not mentioned	L and R: MDD < Con.

MDD, major depressive disorder; Both, MDD and bipolar disorder; F, first episode; R, recurrent; AHC, amygdala-hippocampal complex; C, core; NC, noncore; T, total; AC-PC, anterior commissure-posterior commissure; LAH, long axis of the hippocampus; NS, no significant differences between diagnostic groups; L, left amygdala; R, right amygdala; Con., control subjects; SD, standard deviation; TBV, total brain volume; FU, follow-up.

^aStudied at baseline and 1 year follow-up (FU).

Table 2
Demographic and Amygdala Volumetric Values in Depressed, High-Risk, and Control Twins

Population	Age in Years		Left Mean (SD)			Right Mean (SD)		
	Mean (SD)		Core	Noncore	Total	Core	Noncore	Total
MDD (<i>n</i> = 26)	20.54 (1.75)		9483.78 (905.95)	5032.94 (965.08)	14516.72 (1653.22)	9380.76 (1168.41)	5046.12 (983.39)	14426.88 (1956.37)
HR (<i>n</i> = 24)	20.64 (1.68)		9433.68 (1030.46)	4889.19 (888.71)	14322.87 (1760.83)	9374.21 (987.80)	5190.59 (907.48)	14564.80 (1621.44)
C (<i>n</i> = 18)	20.73 (2.07)		9377.18 (1051.60)	4937.73 (970.67)	14314.91 (1807.46)	9480.07 (1177.38)	4782.89 (788.06)	14262.96 (1747.51)
<i>p</i> -Values								
MDD vs. HR ^a	1.00		.99	.50	.69	.63	.55	.53
MDD vs. C	.99		.72	.75	.70	.78	.35	.78
HR vs. C	1.00		.86	.87	.99	.75	.14	.57

Values are presented in voxels (.5 mm × .5 mm × .5 mm).

MDD, major depressive disorder; HR, high-risk; C, control; SD, standard deviation.

^aFor the paired *t*-test comparing MDD versus HR, only the 21 complete twin pairs were used; thus, mean scores for all amygdala volumes are slightly different but nonsignificant between the two groups.

Table 3

Absolute Mean Percent Differences in Amygdala Volumes in Monozygotic (MZ) Control Twin Pairs ($n = 12$) Versus Randomly Created Control Subject Pairs ($n = 313$) and MZ Affected ($n = 12$) Versus MZ Control Twin Pairs

	Left Mean (SD)			Right Mean (SD)		
	Core	Noncore	Total	Core	Noncore	Total
% Difference						
Control Pairs	.07 (.03)	.14 (.11)	.06 (.03)	.06 (.05)	.13 (.13)	.08 (.04)
Random Subject Pairs	.09 (.07)	.18 (.13)	.10 (.08)	.12 (.09)	.21 (.14)	.14 (.10)
Affected Pairs	.08 (.07)	.17 (.11)	.07 (.07)	.06 (.05)	.13 (.08)	.05 (.05)
<i>p</i> -Values						
Control Pairs Versus Random Subject Pairs	.09	.28	.00 ^a	.00 ^a	.05	.00 ^a
Control Pairs Versus Affected Pairs	.86	.46	.59	.98	.92	.24

MZ, monozygotic; SD, standard deviation.

^aSignificant at $p < .01$.