Age-dependent reduction of amygdala volume in bipolar disorder

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Abstract

The amygdala is hypothesized to play a critical role in mood regulation, yet its involvement in bipolar disorder remains unclear. The aim of the present study was to compare measurements of amygdala volumes in a relatively large sample of bipolar disorder patients and healthy controls ranging in age from 18 to 49 years. Subjects comprised 54 adult patients meeting DSM-IV criteria for bipolar disorder and 41 healthy controls matched for age, sex, and education. Magnetic resonance imaging (1.5 T) was performed to obtain volumetric measurements of the amygdala using a manual region-of-interest tracing method with software that allowed simultaneous visualization of the amygdala in three orthogonal planes. The anterior head of the hippocampus was removed in the sagittal plane prior to amygdala volumetry measurement. Multiple regression analysis was computed on amygdala volume measurements as a function of diagnosis, age, sex, and cerebral volume. Bipolar patients showed an age-related reduction of amygdala volume, but controls did not. Among bipolar subjects, amygdala volume was unrelated to medication history. There were no significant hemispheric or sex interactions with the main effects. Results support a role for amygdala dysfunction in bipolar disorder which appears most robustly in older relative to younger adult patients. Differential aging effects in bipolar disorder may compromise amygdala integrity and contribute to mood dysregulation.
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1. Introduction

The amygdala is featured prominently in neurobiological models of mood disorders (Mayberg, 1997; Davidson et al., 2002; Drevets, 2003). Depressed patients tend to show exaggerated amygdala activation to fearful faces, a response that resolves with treatment (Sheline et al., 2001; Fu et al., 2004). Bipolar patients follow this trend, which is particularly found in unmedicated patients (Yurgelun-Todd et al., 2000; Lawrence et al., 2004; Blumberg et al., 2005) and in mania specifically (Altshuler et al., 2005). The amygdala indexes current mood state in healthy adults as well, with increased activation during sad mood, especially when the mood is reinforced by mood-congruent sensory stimuli (Posse et al., 2003; Wang et al., 2006).

While the importance of the amygdala in mood disorders is undisputed, volumetric studies of the amygdala in bipolar disorder have yielded contradictory findings (summarized in Table 1). In an early study of 48 patients, Swayze et al. (1992) found no significant difference in amygdala volume between bipolar patients

Table 1
Studies of amygdala volumetry in bipolar disorder

Study	Sample size (% male)	Bilateral amyg volume (cm^3)	Age	Results	Imaging techniques
Swayze et al. (1992)	48 BP (60% M) 47 C (60% M)	2.19±0.66 BP 2.05±0.66 C	34.0 BP	No significant difference	0.5 T 1-cm coronal
Pearlson et al. (1997)	27 BP (59% M) 60 C (72% M)	1.26±0.21 BP 1.37±0.19 C	34.9±8.6 BP 31.6±8.0 C	L amyg smaller	1.5 T 3-mm coronal
Altshuler et al. (1998)	12 BP (100% M) 18 C (100% M)	1.94±0.36 BP 1.69±0.36 C	50.8±13.3 BP 53.4±11.1 C	Overall amyg larger	1.5 T 1.4-mm coronal
Strakowski et al. (1999)	24 BP (71% M) 22 C (59% M)	3.55±0.55 BP 3.15±0.4 C	27±6 BP 28±6 C	R and L amyg larger	1.5 T 1-mm coronal
Altshuler et al. (2000)	24 BP (100% M) 18 C (100% M)	1.91±0.35 BP 1.69±0.36 C	50.2±12.7 BP 53.4±11.1 C	Overall amyg larger	1.5 T 3D 1.4 mm coronal
Blumberg et al. (2003)	36 BP (44% M) (14 adolescent) 56 C (52% M) (23 adolescent)	1.80±0.05 BP 2.14±0.04 C	31.0±14.1 BP 28.3±13.7 C	Overall amyg smaller	1.5 T 3D 1.2 mm sagittal
Brambilla et al. (2003)	24 BP (63% M) 36 C (61% M)	2.57±0.69 BP 2.17±0.58 C	35±10 BP 37±10 C	L amyg larger	1.5T 3D 1.5 mm coronal
DelBello et al. (2004)	23 BP (61% M) 20 C (55% M) Adolescent	2.5±0.4 BP 2.75±0.3 C	16.3±2.4 BP 17.2±1.9 C	Overall amyg smaller	1.5 T 3D 1 mm axial
Chen et al. (2004)	16 BP (50% M) 21 C (57% M) Adolescent	1.86±0.41 BP 1.99±0.50 C	16±3 BP 17±4 C	Age-dependent reduction of R amyg	1.5 T 3D 1.5 mm coronal
Frazier et al. (2005)	43 BP (54% M) 20 C (60% M) Adolescent	1.67±0.26 BP 1.80±0.30 C	11.3±2.7 BP 11.0±2.6 C	No significant difference	1.5 T 3D 1.5 mm coronal
Chang et al. (2005)	20 BP (80% M) 20 C (80% M) Adolescent	3.93±0.40 BP 4.39±0.45 C	14.6±2.8 BP 14.1±2.8 C	Overall amyg smaller	3 T 3D 1.5 mm coronal

Note: Amyg = amygdala, BP = bipolar disorder, C = control, L = left hemisphere, R = right hemisphere, M = male.

and controls. However, this study had poor spatial resolution, having been conducted at 0.5 T using 1-cm thick slices. Subsequent studies have reported either greater (Altshuler et al., 1998, 2000; Strakowski et al., 1999; Brambilla et al., 2003) or smaller (Pearlson et al., 1997; Blumberg et al., 2003; DelBello et al., 2004; Chen et al., 2004; Chang et al., 2005) amygdala volumes in bipolar disorder. These latter experiments had improved structural imaging techniques relative to those used in the early study of Swayze et al. (1992), but they also had smaller sample sizes and varying anatomic boundary definitions. The majority of studies that reported amygdala enlargement used a method described by Bartzokis et al. (1998), whereas the other studies did not. Across studies, the age of the patients has also varied widely, ranging from adolescents to middle-age adults. The combination of methodology and age of sample likely contributed to the wide range in mean amygdala volumes reported (1.26–4.39 cm^3).

Given the importance of the amygdala to theories of mood regulation, the present study sought to re-evaluate its role in bipolar disorder using a refined method for amygdala volumetry in a larger sample of young and middle-aged adult patients ($N=54$). In addition, the study aimed to examine the impact of age upon amygdala volume, given that results of previous studies may have differed because of age differences in their samples (DelBello et al., 2004; Altshuler et al., 2000).

2. Methods

2.1. Participants

This study used a cross-sectional design. Patients from the psychiatric wards at Duke University Medical Center (Durham, N.C.) and John Umstead Hospital (Butner, N.C.) with bipolar disorder, as well as community-dwelling subjects with a history of bipolar disorder, were recruited to participate in this study. Control subjects were recruited from the community by advertisement and supplemented with additional controls identified in the databases of the Duke MHCRC for the Study of Depression in Late-Life. The study was approved by the Institutional Review Boards of both institutions.

Eligibility for subjects was limited to those under age 50 as part of the NIMH study in Late Life Bipolar Disorder. Exclusion criteria included other major psychiatric illnesses, active substance abuse, neurological illness, evidence of dementia (as suggested by 7 or more errors on the Mini-Mental Status Examination), or inability to undergo an MRI examination. At baseline, all subjects were assessed with the Structured Clinical Interview for DSM and underwent a standardized MRI study (see Section 2.2). Subjects with bipolar disorder met diagnostic criteria as defined by DSM-IV. Controls and patients for these analyses were matched by age and sex. Demographics of the sample are provided in Table 2 and described in Section 3.1.

2.2. MRI scanning protocol

All subjects were screened for the presence of cardiac pacemakers, neurostimulators, metallic implants, metal in the orbit, aneurysm clips or any other condition where MRI was contraindicated. Subjects were imaged under an Institutional Review Board approved protocol with a 1.5 T whole-body MRI system (Signa, GE Medical Systems, Milwaukee, WI) using the standard head (volumetric) radiofrequency coil. Padding was used to immobilize the head without causing discomfort. The scanner alignment light was used to adjust the head tilt and rotation so that the axial plane lights passed across the canthomeatal line and the sagittal lights were aligned with the center of the nose. A rapid sagittal localizer scan was acquired to confirm the alignment.

A dual-echo fast spin-echo (FSE) acquisition was obtained in the axial plane for morphometry of most cerebral structures. An axial IR-prepped 3D series was used for measuring the amygdala. For the FSE series, the pulse sequence parameters were TR=4000 ms,

TE=30 ms, 32 kHz (±16 kHz) full imaging bandwidth, echo train length=16, 256×256 matrix, 3-mm section thickness, 1 Nex and a 20-cm FOV. The images were acquired in two separate acquisitions with a 3-mm gap between sections for each acquisition. The second acquisition was offset by 3 mm from the first so that the resulting data set consisted of contiguous sections. For the 3D series, the pulse sequence parameters were TE= minimum full echo, TI=300 ms, 16 kHz bandwidth, 256×256 matrix, 1.5-mm section thickness, 1 Nex and a 24-cm FOV.

2.3. Whole brain and cerebrum

The NeuroImaging Research Laboratory (NIRL) segmentation protocol has been described previously (Payne et al., 2002). A supervised, semi-automated method used the multiple MR contrasts available to identify different tissue classifications through a seeding process wherein the trained analyst manually selected pixels in each tissue type that was to be identified (gray matter, white matter, cerebrospinal fluid (CSF), background). Once the brain was segmented into tissue types and the non-brain tissue stripped away through a masking procedure, the cerebral hemispheres were measured by tracing.

2.4. Regional tracing procedures

Scans were processed with a semi-automated method for regional measurements using the NIRL software program GRID 6.5. The GRID program allows for viewing and tracing in any of three orthogonal planes, regardless of acquisition plane. Volumes are calculated by multiplying the traced area on each slice by slice thickness, and then summing volumes for all (MacFall

Table 2
Demographics for bipolar patients and healthy controls

Variables	Bipolar patients (*N*=54)	Healthy controls (*N*=41)
Age	30.54±8.98	29.91±7.91
Sex	39 females (72.22%)	28 females (68.29%)
Years of education	14.72±2.84	15.50±2.33
Handedness	44 right-handed (81.48%)	30 right-handed (73.17%)
Age of onset of current condition (self-report)	24.05±9.07	n/a
Age of first symptoms of any mood disorder (self-report)	14.63±7.02	n/a
History of lithium use	38 (70.37%), 7 unclassified (12.96%)	n/a
Lithium at time of scan	16 (29.63%), 4 unclassified (7.41%)	n/a
History of VPA use	35 (64.81%), 12 unclassified (22.22%)	n/a
VPA at time of scan	17 (31.48%), 4 unclassified (7.41%)	n/a
History of neuroleptic use	31 (57.41%), 6 unclassified (11.11%)	n/a
Neuroleptics at time of scan	17 (31.48%), 4 unclassified (7.41%)	n/a

Note: Values indicate mean (±S.D.) in years except for sex and handedness. VPA=valproic acid.

et al., 1994). Using the procedures defined below, a trained image analyst traced the structures of interest (amygdala or temporal lobe) on every slice where each was found. The analysts were blinded to clinical diagnosis and demographic information.

2.5. Amygdala volumetry

In their seminal review, Brierley et al. (2002) rated the method for amygdala volumetry by Convit et al. (1999) with the highest quality score. Convit and colleagues accomplish consistency in defining amygdala borders in several ways. First, the hippocampal–amygdala border is outlined in the sagittal plane, which is then viewed in the coronal plane where the amygdala is traced. Second, the hippocampal–amygdala transition area is identified in the axial plane, and its border is then projected to the coronal plane. Third, the often difficult-to-define anterior amygdaloid area is simplified by using the optic chiasm as a landmark to identify the most anterior slice to be processed. This method created the cornerstone for our volumetry procedure, which included the following steps: realignment in all three orthogonal planes, removal of the anterior hippocampal head in the sagittal view, and tracing of amygdala in the coronal view. Identification of brain structures was assisted by the Duvernoy (1991) brain atlas.

2.5.1. Realignment

For both the axial and coronal planes, a standard realignment procedure was implemented where the rater aligned to a plane that bisected the brain into right and left cerebral hemispheres. This adjusted the scan in cases of incorrect subject placement in the scanner. Realignment in the sagittal plane was customized for the amyg-

dala method (see Section 2.5.3) and used one of the three most medial slices. A line was drawn from the most inferior surface of the orbitofrontal cortex (ISOC) to the most inferior surface of the occipital lobe (ISOL; see Fig. 1). GRID oriented the scan so that this plane was horizontal in the sagittal view.

2.5.2. Defining the hippocampus in the sagittal view

Following Convit et al.'s (1999) delineation of the hippocampal boundaries, our method included removal of the anterior hippocampus by tracing in the sagittal view. The sagittal images contained either a clear white matter boundary (the alveus) or a CSF boundary between the head of the hippocampus and the posterior section of the amygdala. If the white matter boundary was indistinct in one slice of a hemisphere, we utilized GRID's capability to test potential boundaries in the axial plane. If a potential hippocampal boundary fell posterior to or at the level of the temporal horn in the axial plane, then the proposed boundary was used for that sagittal slice (Fig. 2A). This method concentrated on the anterior hippocampal section and resulted in a complete removal of the hippocampus in that portion (Fig. 2Biii). This removal concentrated only on the anterior hippocampal boundary, was used only to prevent the addition of the hippocampal head to the amygdala volume measure, and did not create a hippocampal volume estimate.

2.5.3. Tracing the amygdala

The analyst began processing in the coronal plane at a middle amygdala slice where the angular bundle clearly separated the medial amygdala border from the entorhinal cortex (Fig. 2Bi). Processing continued posteriorly until the end of the amygdala was reached, as signaled by
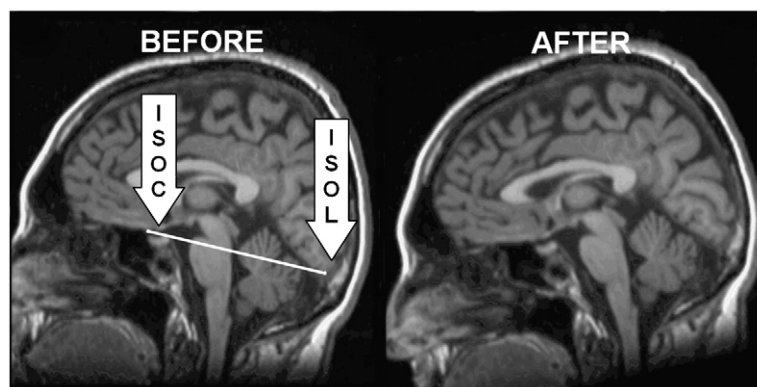

Fig. 1. Realignment of the sagittal plane using the inferior surface of the orbitofrontal cortex (ISOC) and the inferior surface of the occipital lobe (ISOL).
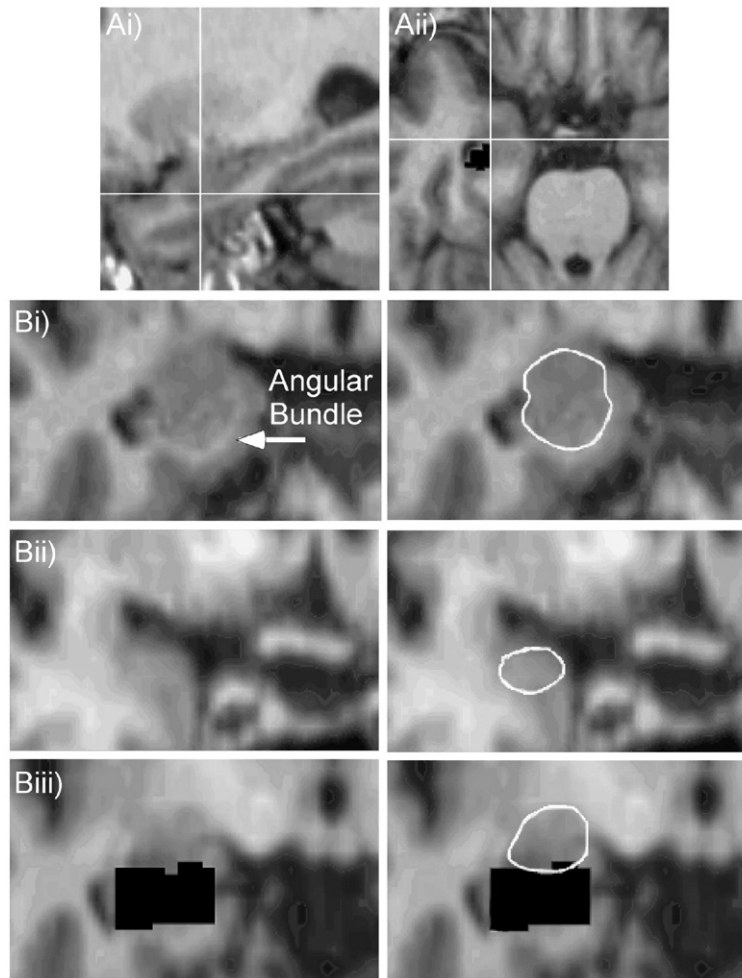
Fig. 2. A. Checking a proposed sagittal hippocampal boundary (Ai) in the axial plane (Aii). The hippocampal head is delineated in the sagittal plane (Ai) prior to measuring the amygdala. When the alveus is indistinct in the sagittal plane and the boundary between the amygdala and the hippocampal head is difficult to discern, our method instructs the rater to consult the axial plane (Aii). Here the black pixels posterior and lateral to the white cross-hairs represent previously removed sections of the anterior hippocampal head. This landmark, combined with the location of the temporal horn of the lateral ventricle, creates a proposed anterolateral border, which can then be identified in the original sagittal image (Ai) to best locate the head of the hippocampus. B. Definition of amygdala borders. (Bi) Start point in middle slice where angular bundle is distinct. (Bii) Anterior amygdala border. This slice is the most anterior image where the optic chiasm appears as one continuous structure. (Biii) Posterior amygdala border at cutout of hippocampus (black pixels).

complete replacement of the amygdala by the cutout of the hippocampus (Fig. 2Bii). Since we did not follow Convit et al.'s (1999) delineation of the hippocampal amygdalar transition area (HATA) in the axial plane due to our inability to reliably identify this region, this area remained in some of our scans. To remedy the potential problem of including this area in our volume measurements, once a brain slice was reached where the amygdala area did not change size significantly in comparison to the amygdala traced in anterior slices, this slice became the posterior boundary of the structure. As the scans were properly realigned to maximize the shape of

the amygdala, the boundary between the amygdala and the HATA was easier to recognize in this final posterior slice. The last coronal slice containing the amygdala was obvious because the next slice posterior jumped to only a sliver of gray matter superior to the hippocampus, which was a clear indication that the volume remaining was the HATA and was confirmed by consulting the axial view. Once the most posterior slice was reached, the analyst returned to the angular bundle segment and processed scans in the anterior direction. Tracing the amygdala was complete anteriorly when the optic chiasm ceased to be a continuous structure. The amygdala was typically

traced on 8–12 slices using 1.88 mm interpolated slice thickness.

In addition to guidelines on which slices to include, our method included clearly defined intra-slice boundaries. For medial and infero-medial boundaries, in the posterior amygdala the angular bundle and projections of the angular bundle created the border, and in the anterior amygdala the CSF became the new medial border as the angular bundle became indistinct (Fig. 2Bii). For these anterior sections, it was important to retain the ovoid shape of the amygdala using the CSF and the lateral and superior-lateral borders (white matter tracts). These white matter tracts served as the superio-lateral border in all slices. However, if these landmarks did not appear distinctly, a border following the ovoid shape created by other distinct landmarks was used. Inferio-lateral boundaries were temporal lobe white matter tracts and extensions of the temporal horn. Most superio-medial boundaries were formed by the CSF, but sometimes this border was created by the white matter tract of the angular bundle. The posterior–inferior border was created by the previously removed hippocampus.

2.6. Reliability

All image analysis technicians received extensive training by experienced analysts. Reliability was established by repeated measurements on multiple (5–10) MR scans before raters were approved to process study data. Intraclass correlation coefficients attained were as follows: total cerebrum=0.997, left amygdala=0.91, right amygdala=0.87.

2.7. Statistical analysis

Amygdala volume estimates were subject to multiple regression analysis using the general linear model. Esti-mates from the left and right hemispheres were entered into a single model as the dependent measures. Independent measures were entered simultaneously into the model and included total cerebral volume, diagnosis (bipolar or control), age, and sex, as well as the inter-action terms diagnosis×age and diagnosis×sex. Sub-sequent analyses explored the effects of medication history within the bipolar group by regressing amygdala volumes with the independent measures age and drug treatment history (lithium, valproic acid and/or neuro-leptics). In these analyses, drug treatments were coded as categorical variables.

3. Results

3.1. Subject characteristics

Scans were obtained from 54 patients (39 female, mean (+S.D.) age=30.54±8.98 years) and 41 healthy controls (28 female, mean (+S.D.) age=29.91±7.91 years) (see Table 2). The mean (+S.D.) self-reported age of illness onset was 24.05 (±9.07) years. Thirty-eight patients had a history of Lithium (Li) treatment, 35 patients had a history of valproic acid (VPA) treatment, and 31 patients had a history of using neuroleptics. Medication data at the time of the scan are presented in Table 2, and six patients (11.11%) were unmedicated at the time of the scan. More detailed information regarding duration and dosage of medication history was unavailable for most patients. The frequency of illness cycles was reported by 13 patients to be 'few' (i.e. less than 15) and 15 patients to be 'frequent' (i.e. more than 15) (26 patients did not provide estimates). Current mood classifications at the time of scanning indicated that 12 patients were stable, 10 patients were depressed, 14 patients were manic, and 1 patient had a mixed episode (17 patients were unclassified).
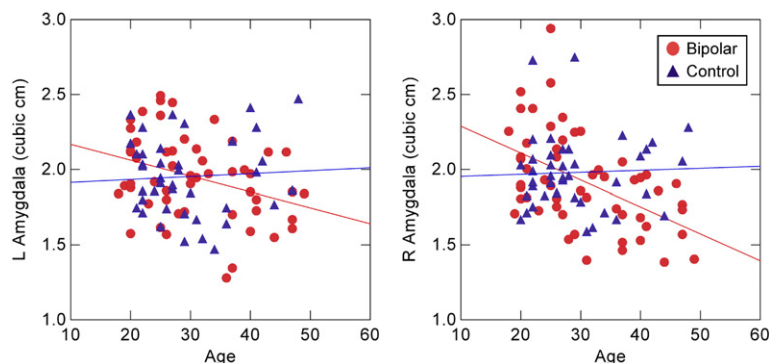

Fig. 3. Amygdala volumes as a function of age in bipolar patients and controls. Regression lines indicate a linear decrease in volume with age in bipolar patients but not in controls. L = left hemisphere, R = right hemisphere.

3.2. Volumetric findings

For bipolar patients, the mean (±S.D.) left amygdala volume was 1.95 (±0.28) cm^3, and the right amygdala volume was 1.92 (±0.32) cm^3. For controls, the mean left amygdala volume was 1.95 (±0.26) cm^3, and the mean right amygdala volume was 1.98 (±0.25) cm^3. Results from the multiple regression analysis revealed significant main effects of cerebral volume, $F(1, 88) = 11.1$, $P < 0.001$, and diagnosis, $F(1, 88) = 7.11$, $P < 0.009$, and a marginal trend of age, $F(1, 88) = 3.44$, $P = 0.067$. However, the diagnosis and age variables interacted with each other, $F(1, 88) = 7.58$, $P < 0.007$. Post-hoc analyses by group showed significant reduction in both left, $r(52) = 0.34$, $P < 0.011$, and right, $r(52) = 0.50$, $P < 0.00012$, amygdala volume with age in bipolar disorder patients, but not in controls (left hemisphere: $r(39) = 0.06$, $P = 0.71$; right hemisphere: $r(39) = 0.04$, $P = 0.80$) (Fig. 3). Subsequent analyses on the age interaction showed that only the oldest bipolar patients in the sample had significant volume decreases in both hemispheres relative to controls, as confirmed by analysis of the data stratified into age tertiles, $F(1, 30) = 4.18$, $P < 0.05$ (Fig. 4). There were no main effects or interactions with the sex or hemisphere variables. In addition, there were no group differences in total cerebral volume. Additional regressions using normalized amygdala volumes produced the same results as the ones reported here (age×diagnosis interaction: $F(1, 89) = 6.14$, $P < 0.015$), as did regressions on left and right hemisphere volumes in separate models.

3.3. Treatment effects

As a secondary analysis, we considered whether the age effects in bipolar disorder were due to history of Li, VPA or neuroleptic treatment or the presence of these medications at the time of scanning. We conducted two follow-up multiple regressions on the bipolar data using left and right amygdala volumes as dependent measures. The first analysis included the independent variables age and self-reported history of Li, VPA and neuroleptic treatment. Patients who were unsure of their history of drug treatment were not included in the analysis (final $N = 35$). Results showed that the main effect of age remained when history of medication treatment was accounted for in the regression model, $F(1, 30) = 6.97$, $P < 0.013$. The second analysis included the independent variables age and presence of Li, VPA or neuroleptics at the time of scanning. This information was obtained from 48 of the 54 patients, who were included in the analysis. Again, the main effect of age remained
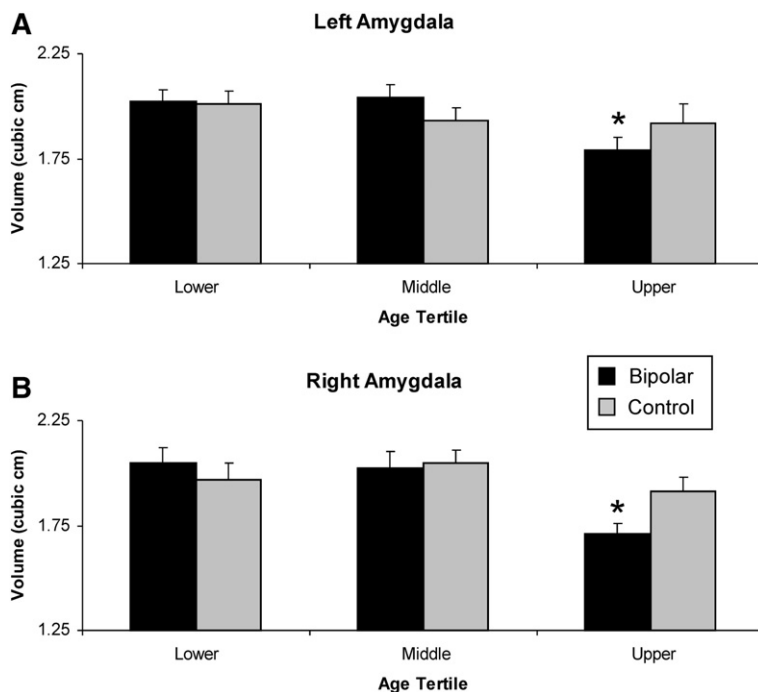

Fig. 4. Amygdala volumes as a function of age tertiles. The bipolar and control samples were divided into the low (age 18–25), middle (age 26–34), and upper thirds (age 36–49) of their respective age distributions. Amygdala volume of bipolar patients in the oldest age cohort is significantly decreased relative to healthy controls in the (A) left and (B) right hemispheres, *$P < 0.05$

even when presence of medications at the time of scanning was accounted for in the regression model, $F(1, 43)=19.61$, $P<0.001$. Similar results were obtained using normalized volumes (age effect controlling for medication history, $F(1, 30)=4.38$, $P<0.045$; age effect controlling for medication at the time of scanning, $F(1, 43)=15.53$, $P<0.001$).

4. Discussion

The principal finding from the present study was an age-dependent reduction of amygdala volume in bipolar disorder. Middle-aged bipolar patients had smaller right and left amygdala volumes relative to younger adult patients and age-matched controls. The aging effects remained even when treatment history and current treatment (Li, VPA, or neuroleptics) were accounted for in the model. There were no effects of sex or hemisphere, and bipolar patients and controls did not differ in cerebral volume estimates.

Previous studies reporting amygdala volumes in bipolar depression patients have yielded conflicting reports (Table 1). Two limitations of existing studies include relatively small sample sizes and mixed volumetry methods. Our finding of age-dependent amygdala atrophy for bipolar depression was calculated from volumes obtained from a larger sample relative to prior studies. This sample size allowed us to model amygdala volumes through the young- to middle-adulthood years. Additionally, these volumes were obtained using a reliable amygdala volumetry procedure which consulted three orthogonal planes to further elucidate potentially indistinguishable boundaries, including the anterior hippocampal head, and therefore avoided including adjacent structures in the volume measurement.

It is unclear why aging in the early-to-middle adult years would impact amygdala volumes in bipolar disorder. One possibility is that overactivation of the amygdala to emotional stimuli, as revealed through functional neuroimaging, is cumulatively excitotoxic. Stress hormones may play a role in this excitotoxicity through a reduction in inhibitory gamma-aminobutyric acid (GABA) transmission, which alters synaptic plasticity in local amygdaloid circuits (Rainnie et al., 2004; Rodriguez Manzanares et al., 2005). Rodent studies have shown that chronic, unpredictable stress has a selective atrophic effect on some neuronal subtypes in the basolateral amygdala (Vyas et al., 2002). Additionally, previous research has concluded that smaller hippocampal volumes as measured in structural MRI scans are associated with depression and might be mediated by high levels of glucocortocoids (Sheline et al., 1996;

McEwen, 1997). Further evidence that cortisol levels mediate atrophy in the hippocampus comes from studies of elderly subjects with elevated cortisol levels (Lupien et al., 1998) and patients with Cushing's disease (Starkman et al., 1999). Both unipolar and bipolar disorder are associated with disruption of the hypothalamic–pituitary–adrenal axis and increased basal cortisol levels, regardless of the current mood state of the patient (Holsboer et al., 1987; Cervantes et al., 2001). It is plausible that the heightened cortisol activity in bipolar depression exerted over time is another mechanism of cumulative excitoxicity in the amygdala. Unipolar depression studies have generally found decreased amygdala volumes, which may serve as more evidence for excitotoxic amygdalar atrophy (reviewed in Campbell and MacQueen, 2006).

Researchers have also suggested that amygdalar glial cells are particularly vulnerable to this atrophy in depression. In a study of post-mortem brains, atrophy of the amygdala was due to glial cell loss in unipolar depression while no change in glial cell loss was found in the medicated bipolar samples (Bowley et al., 2002). Li and VPA have been found to cause an increase in the cell protective agent BCL-2 (Manji et al., 2000), which could explain why drug treatment appears to have prevented glial cell loss. In addition to Li and VPA, neuroleptics may also alter amygdala morphometry, possibly "normalizing" an enlarged amygdala in schizophrenic patients (Tebartz van Elst et al., 2004).

A majority of bipolar disorder patients in the present study had undertaken drug treatment. After conducting a separate analysis of these patients, we found that Li or VPA treatment history had no significant effect on amygdala volumes, regardless of whether age was included as an independent variable in the analysis. One possible explanation for this result is that the patients may have been taking Li or VPA for a limited period of time, and therefore the neuroprotective effects of the drugs may not have manifested themselves. There is good evidence for this scenario as our patients, for the most part, had been recently diagnosed.

Our finding of an age-related reduction in volume is inconsistent with some previous studies of middle-aged bipolar patients which found larger amygdala in bipolar subjects (Table 1). This inconsistency could be accounted for by population differences and/or methodological differences. Our population was predominantly female (72% of bipolar patients, 68% of healthy controls), while almost all studies listed in Table 1 had majority male populations, overwhelmingly so for those studies reporting enlarged amygdala volumes in bipolar populations. Because we report volumes from the

largest adult bipolar sample to date, one cannot rule out amygdala enlargement as a localized effect in a small population that does not generalize to a larger population. Methodologically, we were able to use all three planes and remove the anterior hippocampus in advance to improve upon amygdala volumetry estimates. It is worth noting that all studies reporting enlarged amygdala volumes in bipolar patients used the same volumetry method (Bartzokis et al., 1998), which does not include use of orthogonal views in order to verify boundaries. Co-planar verification is especially helpful to ensure the delineation of the hippocampal head from the amygdala, particularly if there are age- or disorder-related changes that affect the appearance of boundary distinctions. If bipolar specific glial cell loss in the amygdala contributes to white matter degradation as seen in structural MRI scans, without the use of orthogonal views adjacent gray matter structures may accidentally be included in the amygdala measurement. Further improvements can be made in future studies by using co-planar verification at higher field strengths for better signal-to-noise ratios.

The present study is limited by the cross-sectional design employed, particularly given that there may have been cohort differences in treatment history. It is possible that the age-associated atrophy found in our subjects is not a direct result of bipolar disorder alone. Regardless of age, patients had similar self-reported durations of bipolar illness, so differences in illness duration cannot account for the aging effect. However, most patients reported that their first psychiatric symptom occurred before the onset of bipolar illness. Therefore, it is possible that even though older patients had similar durations of bipolar disorder as younger patients, they may have been suffering from comorbid psychiatric disorders (or were misdiagnosed) before they were diagnosed with bipolar disorder. Conclusions from the present study are also limited by not including adolescents or geriatric populations to span a broader age range. Provided that the healthy amygdala gradually declines in volume with senescence and that Li or VPA treatment provides possible long-term neuroprotective effects in bipolar disorder, it is conceivable that the group differences found in the present study would diminish or reverse in old age.

The current study also features a predominantly female population, which potentially limits the scope of the results. When explicitly modeled in the regression analyses using either raw or normalized volumes, there were no gender interactions with the effects reported here. Although a recent large-population study found gender differences only in factors which the current study has controlled for (BP II, comorbid thyroid disease, bulimia, and post-traumatic stress disorder) (Baldassano et al., 2005), future studies will need to more carefully examine the issue of gender and amygdala volumetry in bipolar patients. Finally, our interpretations are limited in that we focus exclusively on the amygdala here and do not include other regions in the analyses.

In conclusion, results from the current study indicate that age may mediate the relationship between bipolar disorder and amygdala volume across the early-to-middle adulthood years. The present study improves upon prior work by investigating a relatively large population of bipolar disorder patients using a precise amygdala volumetry method that was devised to accommodate the quality of structural scans obtained at 1.5 T. Future studies should be careful to include age explicitly in the model and to employ longitudinal designs in order to confirm etiological relationships.

Acknowledgements

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