A magnetic resonance imaging study of hippocampal, amygdala and subgenual prefrontal cortex volumes in major depression subtypes: Melancholic versus psychotic depression

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Abstract

Background: Volumetric studies examining brain structure in depression subtypes are limited and inconclusive. The aim of the current study was to compare the volumes of brain regions previously implicated in depression among patients with melancholic major depressive disorder (MDD), patients with psychotic MDD and normal controls.

Methods: Twenty two patients with melancholic MDD, 17 with psychotic MDD and 18 normal controls were included in the study. Hippocampal (HV), amygdala (AV), anterior (ASCV) and posterior (PSCV) subgenual cortex volumes were measured on magnetic resonance volumetric images.

Results: There were no volumetric differences between patients with melancholic and psychotic subgroups. We identified larger AVs and smaller left ASCVs in both patient groups compared to controls with medium to large effect sizes. Regression analysis revealed that AVs were predicted by the presence of depression, late depression-onset, insomnia and left hippocampal tail volume in patients, but not in controls. There were no differences in HVs, right ASCVs and PSCVs across the 3 groups.

Limitations: Small sample size, a possible inclusion of paracingulate gyrus in ASCV and PSCV tracings, significant differences in education level and medication status are discussed as limitations.

Conclusions: Diagnostically delineated melancholic and psychotic MDD patients do not differ in medial temporal and cingulate volumes. However, significant volumetric differences were detected between both patient-groups and controls.

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1. Introduction

During the last decade, structural brain imaging has been used extensively to examine structures and associated circuits that have been consistently implicated in the pathophysiology of major depressive disorder (MDD), including hippocampus, amygdala, and prefrontal cortex. Two meta-analyses have shown that patients with MDD have smaller hippocampal volumes than normal controls (Campbell et al., 2004; Videbech and Ravnkilde, 2004), while more recently Bora and colleagues reported no differences (Bora et al., 2011). A great variability in findings is reported in studies examining amygdala volumes (Lange and Irle, 2004; Hastings et al., 2004). Moreover, in a few studies a pattern of smaller hippocampal and larger amygdala volumes has been reported in MDD patients (Bremner et al., 2001; Lange and Irle, 2004). Studies involving the prefrontal cortex have found with greater consistency smaller volumes of orbitofrontal cortex (Bremner et al., 2002) and subgenual cortex (Botteron et al., 2002), although other studies report no differences (Hastings et al., 2004).

A number of explanations for the great variability in findings have been proposed, including differences in imaging protocols and differences in illness characteristics (Campbell et al., 2004). The latter include gender (Hastings et al., 2004; Videbech and Ravnkilde, 2004), age of onset (Lloyd et al., 2004; Campbell et al.,...
comparing psychotic to non-psychotic MDD, found reduced
MDD and normal controls. A secondary aim was to explore
in patients with melancholic MDD, patients with psychotic
metric measures of hippocampus, amygdala, anterior (Broadman
subgenual cortex was identified in psychotic MDD compared to
normal controls, mainly disrupted sleep and short REM latency
both groups are reported in somnographic studies compared to
the two commonest and the most severe (Evans and Nemeroff, 1987)
MDD subgroups. Although questions have been posed regarding
the validity in discriminating melancholic from non-melancholic subjects
(Parker et al., 2010), the features of melancholic MDD include
lack of pleasure, excessive guilt, worsening of depression during
morning hours, early morning awakening, loss of appetite (or weight loss)
and marked psychomotor agitation or retardation. Psychotic
MDD is characterized by the presence of delusions and/or hallucina-
tions, which can co-exist with melancholic features.

Data on biological differences between psychotic and melan-
cholic MDD patients are scarce. Melancholic MDD patients with
psychotic features are reported to have higher HPA axis
dysregulation than their non-psychotic melancholic MDD coun-
terparts as reflected in DST non-response rates (Contreras et al.,
2007). Furthermore, a meta-analysis of DST studies in psychotic
MDD concluded that psychotic depressives had higher DST non-
response rates than melancholic MDD patients (Nelson and Davis,
1997). Psychotic MDD patients exhibited significantly decreased
P300 amplitude than their non-psychotic counterparts (Santosh
et al., 1994; Karaaslan et al., 2003). Similar abnormal profiles of
both groups are reported in somnographic studies compared to
normal controls, mainly disrupted sleep and short REM latency
(Lykouras and Gournelis, 2009; Antonijevic, 2008). Finally, in
female MDD patients, higher HVA excretion in psychotic
MDD compared to non-psychotic MDD has been reported,
but not in melancholic MDD compared to non-melancholic MDD
(Lykouras et al., 1994). Thus, the available data suggest that
HPA axis dysregulation is more prominent in subjects with
psychotic features (Contreras et al., 2007). Brain structures are
susceptible to HPA axis activity, especially hippocampus
(Sapolski, 2001) and posterior subgenual cortex (Diorio et al.,
1993).

To date, few MRI studies in patients with MDD have exam-
ined the volumes of hippocampus, amygdala and subgenual
cingulate cortex according to depression subtypes, while none
have directly compared melancholic to psychotic MDD. One
study has examined the effect of depression subtype on hippo-
campal volume, reporting negative results for melancholic
psychotic features (Contreras et al., 2007). Brain structures are
susceptible to HPA axis activity, especially hippocampus
(Sapolski, 2001) and posterior subgenual cortex (Diorio et al.,
1993).

Given the inconclusive and limited research to date, the
current study aimed to investigate in a comparative way volu-
metric measures of hippocampus, amygdala, anterior (Brodmann
Area (BA) 24 and 32) and posterior (BA 25) subgenual cortices in
patients with melancholic MDD, patients with psychotic
MDD and normal controls. A secondary aim was to explore
possible relationships between clinical and volumetric measures.
Moreover, we investigated volumetric relationships between
hippocampus and amygdala.

2. Methods

2.1. Subjects

Thirty nine patients with a diagnosis of major depression and
18 normal controls were included in the study. Normal subjects
were recruited from the community through word of mouth. All
subjects consented to participate and the study was approved by
the Ethics Committee of Attikon General Hospital and Eginition
Hospital. Inclusion criteria were: 20–65 years of age, a diagnosis
of a single episode of major depression or recurrent major
depression of either the melancholic or the psychotic subtype as
determined by the Structured Clinical Interview for DSM-III-R, no
comorbid Axis I and II psychiatric disorder, no history of neuro-

logistic disorder or any other unregulated medical disorder and
absence of metal parts in the body. Each patient: (i) underwent an
interview with a psychiatrist (K. Vassilopoulou), who conducted
the Structured Clinical Interview for DSM-III-R, the Hamilton
Depression Rating Scale (HDRS) (21 items) and the Mini-mental
State Examination Scale (MMSE) (to exclude patients with
dementia and as a marker of cognitive function); (ii) gave a blood
sample for laboratory tests (to exclude patients with disorders of
renal, liver and thyroid function) and (iii) had a magnetic
resonance imaging (MRI) scan. Two patients were excluded
(initially 41 patients went through the protocol): one was
diagnosed with frontal ischemic leucoencephalopathy and the
other had a poor quality MRI scan. No patient was diagnosed with
dementia or any disorder of renal, liver and thyroid function. All
patients and normals were right-handed and all patients were on
medication.

2.2. MRI scanning

MRI volumetric images were acquired on a 1.5T Philips Inter-
System, using a T1-weighted 3D/FFE gradient-echo sequence with
the following parameters: TR/TE 14.3/3.3 ms, flip angle 30°, field
of view 240, matrix size 256 × 256, slab thickness 3 mm over
contiguous with 1.5 mm spacing and 124–130 partitions with an
inplane resolution of 0.94 × 0.94 mm. All data acquisition was
performed in the standard coronal plane. Analysis of the images
was performed on a View Forum Workstation (Philips Medical
Systems), using the Philips Medical Systems Release 4.1, V1L2
Software. All the volumes were estimated by manual tracing of
the respective area of interest, on each coronal image. The areas of
all slices were added and their sum was multiplied by the
distance between the slices in order to determine the individual
volume for each structure.

2.3. Tracings

All tracings were performed by the same rater (K. Vassilopoulou)
who was trained by an experienced neuroradiologist (M. Papa-
thanasiou). We used the volumes of 10 randomly selected patients
in order to estimate intra-rater reliabilities with a second mea-
urement by the first rater (K. Vassilopoulou) and inter-
rater reliabilities with a second measurement by another one
(M. Papathanasiou). For intracranial volume reliabilities a second
measurement of five patients was used. Raw volumes of all
measurements for the hippocampus, the amygdala and the
subgenual prefrontal cortex were normalized based on
intracranial volume according to a previously described method (Free et al., 1995), in order to control for differences resulting from variability in brain sizes.

2.4. Anatomic boundaries

2.4.1. Hippocampus–amygdala

The dentate gyrus, the subiculum, the hippocampus proper, the alveus and the fimbria were included in the tracings in order to estimate the raw volumes of left and right hippocampus, from the first slice in which the hippocampal head was visualized to the last slice of its tail, as described in previously published methods determining its anatomic boundaries (Pruessner et al., 2000; Malykhin et al., 2007). Furthermore, we estimated the volumes of hippocampal head, body and tail (Fig. 1), using the first slice where the uncal apex was clearly seen as the last slice of hippocampal head and the first slice where the fornix was clearly seen in full profile, or was separated from the wall of the ventricle, whichever came first as the most anterior slice of the hippocampal tail (Malykhin et al., 2007). Hippocampal and amygdala volumes were traced simultaneously in the images where both structures appeared for maximum accuracy of the tracings (Fig. 1), (Malykhin et al., 2007). In the same way, amygdala was traced from the first slice of its anterior pole, at the level corresponding to the section immediately posterior to the section where the optic chiasm first appears as a continuous structure (Convit et al., 1999) to the last slice of its posterior pole, at the point where gray matter first started to appear superior to the alveus and laterally to the hippocampal head (Pruessner et al., 2000). For consistency reasons, the superior border of the amygdala was arbitrarily defined, although small amounts of the medial and central nuclei of the AG were excluded (Pruessner et al., 2000). Tracings were performed with the constant reference to a standard atlas (Mai et al., 2004). Intra-rater and inter-rater reliabilities were 0.96 for the hippocampal volume and 0.85 for the amygdala and inter-rater reliabilities were 0.97 and 0.89 respectively.

2.4.2. Anterior and posterior subgenual cortex

The anterior subgenual cortex was traced (Fig. 2), as described by Drevets et al., beginning from the slice defined by the rostral extreme of the genu of the corpus callosum and finishing at the last slice before the internal capsule was first visualized (Drevets et al., 1997). The first slice where the internal capsule was first visualized was used as the anterior boundary of the posterior subgenual cortex, the inferior border of the corpus callosum was used as the superior border, the medial border of the gyrus rectus as the inferior boundary and the natural limit of the gyrus was used as the posterior boundary (Coryell et al., 2005). Anterior subgenual cortex corresponds to Broadman Area (BA) 24 (anterior cingulate gyrus portion) and BA 32 (paracingulate gyrus portion), while posterior subgenual cortex corresponds to BA 25 (anterior cingulate gyrus portion) (Fornito et al., 2006). Tracings were performed with constant reference to a standard atlas (Mai et al., 2004). Intra-rater reliability was 0.88 for the anterior subgenual volume and 0.94 for the posterior subgenual volume; inter-rater reliabilities were 0.80 and 0.95, respectively.

2.4.3. Intracranial volume

Intracranial volume was estimated by tracing around the dura mater, or the cerebral contour if dura mater was not visualized, excluding the brain stem and the cerebellum, a method used in previously published studies (Brambilla et al., 2002). A strategy of measuring 1-in-10 slices was used, which is estimated to be reliable (Eritaia et al., 2000). Intra-rater and inter-rater reliabilities were very high (0.99 and 0.99 respectively).

2.5. Statistical analysis

Statistical analysis was carried out using SPSS (Version 16.0) for Windows. The following tests were used for the statistical analysis of the data: the Pearson $\chi^2$ test for comparisons of percentages, $t$ test and one-way ANOVA (with Bonferroni correction) for comparison of means of variables with normal distribution, and Mann–Whitney U tests and Kruskal–Wallis non-

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**Fig. 1.** Images of amygdala, hippocampal-amygdala transitional area, and hippocampal body and tail (from top to bottom) before and after the tracings (left and right respectively).
parametric analysis of variance for variables which were not normally distributed. Correlations were tested by the Pearson’s r or the Spearman’s rho coefficient, whether the variables were normally distributed or not. The effect of medication categories on volumetric measurements were examined with regression analysis. Finally, associations between volumetric measures in both patients and controls were investigated by means of Fisher’s z-transformation test.

3. Results

3.1. Demographic and clinical variables

Twenty two patients fulfilled criteria for MDD with melancholic features and 17 for psychotic (without melancholic features) MDD. The sample included patients in active episode and in remission (Hamilton Scale score less than 17 for at least two months). All patients were medicated at the time of scanning (see Table 1).

Both melancholic and psychotic group of patients did not differ significantly compared to controls in age or gender. However, patients had approximately 6 years of education less than normals. Also, there were no significant differences among both groups of patients and controls regarding the presence of treated medical disorders (F=1.109, p=0.337) and the use of other medication (F=1.243, p=0.297).

The two groups of patients did not differ significantly in demographic variables (age, gender, education), clinical variables (age of onset, illness duration, number of episodes, depression status, MMSE score), somatic symptoms of depression (presence of insomnia and appetite disturbance) (see Table 2) and medication use (tricyclic antidepressants, SSRI’s/SNRI’s/Mirtazapine, classic antipsychotics, benzodiazepines, other medication) (see Table 1). The two groups differed on Hamilton score (t=-2.33, p=0.025) (see Table 1) and the use of atypical antipsychotics (see Table 1).

3.2. Between groups comparisons

No between patient-groups differences were detected in any volumetric measures (intracranial, total hippocampal, hippocampal head, body and tail, amygdala, anterior and posterior subgenual cortices).

By contrast, significant differences were found between patient-groups and normal controls regarding both right and left amygdala volumes (see Table 3). More precisely, melancholic patients exhibited larger amygdalae volumes compared to controls (right: 28%, left: 26%) with impressively strong effect sizes (d=1.73 and d=1.95, respectively). Likewise, psychotic patients exhibited larger amygdalae volumes than controls (right: 18%, left:23%) with effect sizes of 1.62 and 1.76, respectively. By contrast, melancholic and psychotic patient groups exhibited smaller volumes of left anterior subgenual cortex volumes than controls by 18% (see Table 3), with medium effect sizes of 0.69 and 0.70, respectively.

Regression analysis indicated that amygdalae volume-differences between patients and controls were not accounted
for by differences in education, age, the presence of medical disorders and medication. Two models were constructed, using left and right amygdala volumes as dependent variables and years of education, age, diagnosis of depression and medication categories as independent variables. Amygdalae volumes were predicted only by the presence of depression (left amygdala: \( B = 1.064, p = 0.039 \), right amygdala: \( B = 1.471, p = 0.004 \)). By contrast, a similar regression analysis using left anterior subgenual volume as dependent variable eliminated the diagnosis of depression from the model (\( B = -0.783, p = 0.188 \)).

### 3.3 Relationships between clinical and volumetric measures

Since no volumetric differences were detected between the two patient-groups, they were merged in further analyses. We examined the relationships between clinical and volumetric measures taking into account multiple testing by regression analysis. A model was constructed for each volumetric measure using clinical variables as independent variables (early/late onset, illness duration, number of episodes, active episode/remission, insomnia, appetite disturbance, HDRS score and MMSE score).

Right and left amygdala volumes were predicted by late illness-onset (Right amygdala: \( B = 0.45, p = 0.016 \), Left amygdala: \( B = 0.35, p = 0.05 \)) and the presence of insomnia (Right amygdala: \( B = 0.48, p = 0.006 \), Left amygdala: \( B = 0.5, p = 0.005 \)). Patients with late onset MDD (\( \geq 45 \) years old) had significantly larger right and left amygdala volumes compared to patients with early onset (\( < 45 \) years old) (Right amygdala: \( t = -2.88, p = 0.006 \), left amygdala: \( t = -2.24, p = 0.031 \)). No other significant associations were detected between clinical and volumetric measures.

### 3.4 Relationship between volumetric measures

Associations between volumetric measures in both patients and controls were investigated by means of Fisher’s transformation. Patients’ right and left amygdala volumes were negatively correlated with volume of left hippocampal tail (Right amygdala: \( r = -0.57, p = 0.001 \), Left amygdala: \( r = -0.51, p = 0.003 \)), but not in controls (Right amygdala: \( r = -0.31, p = 0.25 \), Left amygdala: \( r = -0.03, p = 0.90 \)).

Furthermore, in order to account for multiple testing, a regression analysis was performed with amygdalae volumes as dependent and the remaining volumetric measures as independent variables. Volume of left hippocampal tail was the sole predictor of both right and left amygdala volumes (Right amygdala: \( B = -0.658, p = 0.010 \), Left amygdala: \( B = -0.580, p = 0.033 \)).

### 3.5 Relationships of amygdalae volumes

Finally, all predictors of amygdalae volumes were entered in a single regression analysis as independent variables. All of them remained significant predictors, namely late illness-onset (Right amygdala: \( B = 0.41, p = 0.001 \), Left amygdala: \( B = 0.33, p = 0.017 \)), insomnia (Right amygdala: \( B = 0.26, p = 0.036 \), Left amygdala: \( B = 0.25, p = 0.06 \)) and left hippocampal tail volume (Right amygdala: \( B = -0.47, p < 0.001 \), Left amygdala: \( B = -0.41, p = 0.004 \)).

### 4. Discussion

To our knowledge this is the first study to examine hippocampal, amygdala and subgenual cortex volume differences in patients with melancholic MDD, patients with psychotic MDD and normal controls. No differences were found between the two patient groups for any of the volumetric measures. Compared with normal controls, both patient-groups had larger amygdala volumes bilaterally with very strong effect-sizes, and smaller left anterior subgenual cortex volumes with upper medium effect-sizes. No differences were found for hippocampal and posterior.
subgenual cortex volumes bilaterally, or right anterior subgenual cortex volume.

The lack of differences in amygdala-volumes between melancholic and psychotic MDD-subgroups is congruent with the finding of another study, whereby amygdala volume was not related to psychosis per se (Keller et al., 2008). Further, while Velakoulis et al. found larger amygdala volumes in first-episode psychotic MDD, no such differences were found in schizophrenia-form psychoses (Velakoulis et al., 2006). These findings suggest that increased amygdala size may be a non-specific marker for the presence of a major affective syndrome.

Differences in amygdalae-volume have been connected with illness-characteristics and the use of medication. More precisely, in MDD samples, larger amygdala volumes have been found in first-episode patients compared to patients with recurrent depression (Frodil et al., 2003), in patients in active episode compared to patients in remission (van Eijndhoven et al., 2009) and in medicated patients (Hamilton et al., 2008). On the other hand, reduced amygdala volumes have been found in female, but not in male MDD patients compared to controls (Hastings et al., 2004) and have been positively correlated with age of onset (Keller et al., 2008).

In our study, amygdala volume was significantly larger in patients with late onset MDD compared to their early onset MDD counterparts. This finding would suggest a positive relationship of amygdala volume with chronicity of depression (Keller et al., 2008). However, amygdala volume was not related with number of episodes or illness duration in our study. Additionally, we did not find any differences in amygdala volume regarding gender, depression status (in active episode or in remission), illness-severity or medication-use.

This is the first study to report that amygdala volume correlated inversely with the tail of left hippocampus in patients with MDD, but not in controls. We hypothesize that chronic stress underlies MDD and might account for this finding (Hamen, 2005). Indeed, chronic stress in rats leads to atrophy of hippocampal neurons and hypertrophy of amygdala neurons (Vyas et al., 2002). Furthermore, smaller hippocampal and larger amygdala volumes in humans have been reported in PTSD (Bremner et al., 1995, 1997), but also in MDD (Bremner et al., 2000, 2001). The opposite effects of stress on hippocampal and amygdala structure have been attributed to its differential effects on spine synapse formation (McEwen, 2006). Additionally, posterior hippocampal portions may be more affected in MDD (Neumeister et al., 2005) and reductions in left hippocampal volumes have been consistently reported in MDD patients compared to controls (Campbell et al., 2004).

Finally, amygdala volume was significantly larger in patients with insomnia compared to patients without, irrespective of depression status. Amygdala is known to play an important role in coding the emotional experience of stimuli (Ledoux, 1992), but, also, in sleep and wakefulness control (Pung et al., 2001). An interplay between amygdala and hippocampus is hypothesized to mediate memory consolidation during sleep (Groch et al., 2011). It is of interest that in a functional MRI study, while recollection of negative stimuli in normal individuals after sleep activated hippocampo-neocortical networks, sleep deprivation led to the recruitment of an alternate amygdalo-neocortical network, with increased activation of amygdala (Sterpenich et al., 2007).

Regarding anterior subgenual cortex, no differences between patient-groups were found, but we detected significantly smaller volumes in both MDD groups compared to controls (with upper medium effect-sizes). Smaller anterior subgenual volumes in MDD patients compared to controls are reported consistently (Drevets et al., 1997; Tang et al., 2007), especially in the left hemisphere (Hirayasu et al., 1999; Hastings et al., 2004; Botteron et al., 2002). Our finding is consistent with studies stressing the central role of subgenual cortex in MDD (Skaf et al., 2002; Bora et al., 2011), as well as with those identifying this region as relevant to affective psychoses (Farrow et al., 2005; Koo et al., 2008; Dazzan et al., 2011). In the recent study by Dazzan et al., pre-psychotic individuals at high risk for psychosis showed volume reduction prior to the onset of an affective psychosis compared with other psychoses (Dazzan et al., 2011). These studies show that anterior subgenual volume is related with depressive/affective features rather than psychosis per se. It will be important to examine the relevance of this region to depression per se, as these findings include psychotic patients with bipolar disorder, in which subgenual cingulate is implicated (Fornito et al., 2008, 2009; Bora et al., 2010).

No between groups differences were found in hippocampal volume. Likewise, no differences were detected between patient groups and normal controls, even though mean left and right hippocampal volumes were smaller in both patient groups. Lack of hippocampal volume differences has been reported in a number of studies (Hastings et al., 2004; Keller et al., 2008) as well as in a recent meta-analysis (Bora et al., 2011). Furthermore, our findings are partially concordant with those of two studies in patients with psychotic MDD (Keller et al., 2008; Velakoulis et al., 2006). These negative findings have been attributed to the lower age of the study-samples (Campbell et al., 2004; Hastings et al., 2004), including our own. However, the available evidence suggests that both antidepressants and atypical antipsychotics may exert a neuroprotective influence on brain structures susceptible to HPA actions (Thakore et al., 1997; Cohrs et al., 2004), like the hippocampus (Sapolski, 2001) and posterior subgenual cortex (Diortio et al., 1993). This neuroprotective influence might have led to our negative results regarding their volumes.

We did not find significant differences in posterior subgenual cortex volume in MDD patients compared to controls. Smaller left posterior subgenual cortex volume has been reported in psychotic MDD patients at baseline, but not in subsequent follow-up ranging from 2–8 years compared to controls (Coryell et al., 2005). Additionally, the region has been found smaller in another MDD study, only in patients with more than three episodes of untreated illness (Yucel et al., 2008).

All our patients were taking medication. The issue of medication-effects on amygdala volume is controversial. More precisely, the use of antidepressants has been associated with larger amygdala volumes (Hamilton et al., 2008), although another study failed to replicate this finding (Keller et al., 2008). Larger amygdala volumes have been reported in affective psychoses, but not in schizophrenic patients, both undergoing antipsychotic treatment (Velakoulis et al., 2006). Besides, patient-groups in our study differed significantly with respect to atypical antipsychotics and no between-groups volumetric differences were detected. Though we cannot rule out medication effects on amygdala volumes, they were statistically controlled for by regression analysis without affecting our results. Future studies investigating the impact of medication longitudinally in initially drug-naive patients would help to assess this issue in detail.

Among the remaining limitations of our study, we should stress the small sample size and our inability to conclusively exclude parahippocampal gyrus from anterior and posterior subgenual volume-tracings. Another limitation would be the significant difference in education-years between both patient groups and controls. However, this difference was statistically controlled for by regression analysis without affecting our results. Thus, further studies overcoming the previously mentioned limitations are fully warranted.

This comparative volumetric study in clearly delineated melancholic and psychotic MDD subgroups refines previous investigations. Further examining of the structures involved in MDD is
needed in order to better understand its underlying brain mechanisms. However, there is growing evidence to suggest that there may be different patterns of activation in the various components of the brain circuitry, resulting in various forms of the same syndrome. This hypothesis could explain the inconsistency of findings in structural imaging which is significant for some structures, like the amygdala, but also, lack of differentiation regarding the volume of these structures between the various subtypes of the same disorder, as found in our study.

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Conflict of interest
There are no conflicts of interests regarding this report.

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References

Bremner, J.D., Vythilingam, M., Vermetten, E., Nazeer, A., Adil, J., Khan, S.,


Bremner, J.D., Vythilingam, M., Vermetten, E., Nazeer, A., Adil, J., Khan, S.,


Farrow, T.F., Whitford, T.J., Gomes, L., Harris, A.W., 2005. Diagnosis-related regional gray matter loss over two years in first episode schizophrenia and 65-year-old, Biological Psychiatry 58 (9), 713–723.


