REVIEW

Structural magnetic ressonance imaging in anxiety disorders: an update of research findings

Ressonância magnética estrutural em transtornos de ansiedade: atualização dos achados de pesquisa

Maria Cecília Freitas Ferrari,[1] **Geraldo F Busatto,**[2]
Philip K McGuire,[3] **José Alexandre S Crippa**[1]

Abstract

Objective: *The aim of the present report is to present a systematic and critical review of the more recent literature data about structural abnormalities detected by magnetic ressonance in anxiety disorders.* ***Method:*** *A review of the literature in the last five years was conducted by a search of the Medline, Lilacs and SciELO indexing services using the following key words: "anxiety", "panic", "agoraphobia", "social anxiety", "posttraumatic" and "obsessive-compulsive", crossed one by one with "magnetic resonance", "voxel-based", "ROI" and "morphometry".* ***Results:*** *We selected 134 articles and 41 of them were included in our review. Recent studies have shown significant morphological abnormalities in various brain regions of patients with anxiety disorders and healthy controls. Despite some apparently contradictory findings, perhaps reflecting the variability and limitations of the methodologies used, certain brain regions appear to be altered in a consistent and relatively specific manner in some anxiety disorders. These include the hippocampus and the anterior cingulate cortex in posttraumatic stress disorder and the orbitofrontal cortex in obsessive-compulsive disorder.* ***Conclusions:*** *The present review indicates that structural neuroimaging has contributed to a better understanding of the neurobiology of anxiety disorders. Further development of neuroimaging techniques, better sample standardization and the integration of data across neuroimaging modalities may extend progress in this area.*

Descriptors: *Anxiety; Magnetic resonance imaging; Image processing computer-assisted; Cone-beam computed tomography; Models, structural*

Resumo

Objetivo: *Apresentar uma revisão sistemática e crítica dos achados mais recentes da literatura em relação a alterações estruturais avaliados por ressonância magnética nos transtornos de ansiedade.* ***Método:*** *Uma revisão da literatura dos últimos cinco anos foi realizada utilizando uma busca nos indexadores Medline, Lilacs e SciELO utilizando as seguintes palavras-chave: "*anxiety*", "*panic*", "*agoraphobia*", "*social anxiety*", "*posttraumatic*" e "*obsessive-compulsive*" cruzadas uma a uma com "*magnetic ressonance*", "*voxel-based*", "*ROI*" e "*morphometry*".* ***Resultados:*** *Foram selecionados 134 artigos, sendo 41 foram incluídos nesta revisão. Estudos recentes mostram alterações morfológicas significativas entre os pacientes com transtorno de ansiedade e os controles saudáveis em várias regiões cerebrais. Apesar de achados contraditórios, sobretudo devido à variabilidade e às limitações nas metodologias utilizadas, algumas estruturas aparecem alteradas de forma mais consistente e relativamente específica em alguns transtornos de ansiedade, como o hipocampo e o córtex cingulado anterior no transtorno de estresse pós-traumático e o córtex orbitofrontal no transtorno obsessivo-compulsivo.* ***Conclusões:*** *A presente revisão aponta que a neuroimagem estrutural pode ser utilizada na busca de uma maior compreensão da neurobiologia dos transtornos de ansiedade. É possível que o rápido avanço das técnicas de neuroimagem, uma maior padronização das amostras e a associação de dados de diferentes modalidades permitam um maior entendimento deste cenário.*

Descritores: *Ansiedade; Imagem por ressonância magnética; Processamento de Imagem assistida por computador; Tomografia computadorizada volumétrica; Modelos estruturais*

[1] Department of Neurology, Psiqchiatry and Medical Psicology, Medical School of de Ribeirão Preto, Universidade São Paulo (USP), Ribeirão Preto (SP), Brazil
[2] Department of Psichiatry, Medical School, Universidade de São Paulo (USP), São Paulo (SP), Brazil
[3] Department of Psychological Medicine, Institute de Psychiatry, Neuroimaging Section, University of London, London, UK

Correspondence
José Alexandre S. Crippa
Hospital das Clínicas - 3º andar
Av. Bandeirantes, 3900
14048-900 Ribeirão Preto, SP, Brasil
Phone: (+55 16) 3602-2201
Fax: (+55 16) 3602-2544
E-mail: jcrippa@fmrp.usp.br

Submitted: February 27, 2008
Accepted: May 27, 2008

Introduction

The diagnostic classification of anxiety disorders has occurred relatively late within the history of mental disorders. This is mainly due to the fact that the various disorders currently referred to as anxious were not even recognized as belonging to the same entity. In the recent past, this group of disorders was thought to be of a purely psychological nature. However, current studies have raised new hypotheses linking biological components to the etiology and specific symptoms of these disorders.[1]

The framework considered as the most helpful to the understanding of the physiological and behavioral characteristics of anxiety disorders is the fear conditioning model, widely studied in animals over several decades.[2] In a typical fear conditioning paradigm, learned associations generally involve presentation of a neutral stimulus such as a tone and may include lesions or stimulation of different brain regions. It should be noted that not all anxiety disorders in humans necessarily arise as a consequence of learned associations; in fact, by definition, only Posttraumatic Stress Disorder (PTSD) is known to arise in the aftermath of an emotionally traumatic event.[3] Nevertheless, some aspects of the fear conditioning model may indeed be relevant to all forms of pathological anxiety, and much has been learned about the neural circuitry of fear and anxiety from animal models.[4,5]

Based on the fear conditioning framework, anxiety disorders are believed to arise out of some abnormality in cortical/subcortical interactions, resulting in an inappropriate expression of the fear response.[6] Clearly, the amygdala plays a critical role in the functional neurocircuitry of anxiety disorders. This brain area mediates states of increased arousal as well as the fear response, and its central nucleus serves as the hub for the integration of information and for the execution of autonomic and behavioral fear responses. Connections between the amygdala and the sensory thalamus, prefrontal, insular, and somatosensory cortices are relevant in recognizing threat-related information. For instance, the insular cortex seems to be important for subjective feeling states and interoceptive awareness. In addition, other two key brain areas appear to be important to the understanding of anxiety disorders. The hippocampus has been suggested to be involved in processing of contextual information. Dysfunction of this brain area has been related in the pathological anxiety via overgeneralization, as a consequence of deficient appreciation for the contextual specificity of potentially threatening stimuli. Moreover, the medial frontal cortex in general and the anterior cingulate gyrus in particular, are important for cognitive and affective aspects of conflict as well as for the emotional processing and executive control in response to environmental demands. These neural substrates with executive function modulate the activation of the amygdala and the extended limbic system.[3]

Distinctions between the neural circuitry underlying different anxiety disorders have been hypothesized on the basis of animal and pharmacological studies, as well as clinical observation. PTSD, for example, provides perhaps the best example of an anxiety disorder which appears to follow the classical fear conditioning model. The hyper-responsivity of the amygdala to threat-related stimuli is perhaps exacerbated due to inadequate top-down modulation by the ventromedial prefrontal cortex and the hippocampus.[7] Abnormalities in the ventromedial prefrontal cortex may interfere with impaired extinction of the fear response and executive control to threat-related stimuli. Additionally, hippocampal dysfunction may underlie the overgeneralization of fear responding and concurrent impairment of explicit memory.[8] On the contrary, the main theories of Obsessive-Compulsive Disorder (OCD) do not emphasize a major role for the amygdala, since considerable evidence implicates the cortico-striatal circuitry in the pathophysiology of this psychiatric condition. The striatum has been implicated in mediating motor activities and diverse cognitive and affective functions, such as repetitive, stereotyped cognitive processes on an implicit level.[3]

Apart from the above cited animal studies and clinical observations, the research area that has contributed most significantly to bring new insights onto the commonalities and differences between the anxiety disorders and their respective neural circuitries is neuroimaging. Brain imaging techniques allows the *in vivo* evaluation of the human brain, allowing a better understanding of its anatomical, functional and metabolic substrate. Among the various neuroimaging methods used, magnetic resonance imaging (MRI) is one of the most frequently employed mainly because of its high image resolution and the ability in providing distinction between different tissues, in addition to being harmless to the patient. These images can provide diverse qualitative and quantitative information about the cerebral structure of the patient.

One of the well-accepted methods used for the investigation of brain morphometry involves the use of regions of interest (ROI). In its most conventional form, the ROI-based approach requires manual delineation of cerebral regions in sequential MRI slices, and the areas obtained in each slice are summed up to provide a measure of the volume of the brain structure of interest. In order to minimize observer biases, landmarks and rules for manual tracing must be defined *a priori*, and operators must be rigorously trained. The procedure is laborious, thus limiting the number of brain regions analyzed and the sample size, also requiring investigators to have an *a priori* hypothesis regarding specific brain regions.[9] Furthermore, ROI-based studies are limited in their treatment of neocortical morphology because of the inherent difficulties in defining structurally complex and variable regions of the human cortex. The systematic morphometry evaluation of the brain as a whole has recently become possible with the use of automated techniques of voxel-by-voxel analysis.[10] Such voxel-wise methods originate from the automated methods developed in the 1990 decade for the analysis of Positron Emission Tomography (PET) data, most often using a program named Statistical Parametric Mapping (SPM). The application of such methodology to structural MRI, named Voxel-Based Morphometry (VBM), allows the comparison of the concentration/volume of the gray and white matter between groups of interest for each voxel of the cerebral volume after automatic image segmentation, without the need to define ROI margins in advance.[11] Structural MRI scans are spatially normalized to an anatomical template and segmented into gray matter, white matter, and cerebrospinal fluid (CSF) compartments.[11] In optimized versions, the VBM methodology allows the creation of study-specific templates that average MRI scans acquired with the same equipment and imaging parameters.[12] Processing steps have been added to minimize the influence of extra-cerebral voxels on the routines of spatial normalization and segmentation, and to preserve the volumes of brain structures, which may be considerably deformed during normalization.[12] Our previous review,[10] focused on general neuroimaging alterations in anxiety disorders, identified some significant neuroanatomical findings. These included a relevant role attributed to alterations in the orbitofrontal-striatal-thalamic circuits in OCD, abnormalities in the temporal lobe of patients with Panic Disorder (PD) – although not often reproduced –, and

reductions in the volume of the hippocampus, corpus callosum and total brain in patients with PTSD. That review[10] also highlighted the substantial contradictions of findings across different studies, which were attributable, overall, to the variability and limitations in the methodologies adopted. The scarceness of studies on Generalized Anxiety Disorder (GAD), Social Anxiety Disorder (SAD), and specific phobias was also noteworthy.

Since the publication of the above review of neuroimaging findings in anxiety disorders,[10] a large number of important new studies have been published. At that time, many of the comments we offered were tentative, due to an insufficient amount of research studies available for some anxiety disorders. Results of the more recent investigations allow us to comment on structural MRI in such conditions with a greater degree of confidence.

To spare readers the burden of reviewing the earlier manuscript as a prelude to this update (a chapter of a book published in Portuguese and not generally available), we begin each section of the current report by briefly summarizing our earlier observations.

In the present study we reviewed articles published in the last five years, which used MRI for morphometric evaluation of the brain in studies related to anxiety disorders, with emphasis on the structural findings obtained and the different methodologies used, thus expanding our previous review.

Objectives

Neuroimaging can help elucidate the biological processes that occur in brain regions related to psychological experiences manifested in psychiatric disorders. The present report aims to present a systematic qualitative review of the more recent literature findings related to structural abnormalities obtained by MRI for each diagnostic category of anxiety disorders.

We chose this approach rather than using meta-analysis for several reasons: 1) the information needed to compute effect size was not always available and could limit our analysis to a small subset of studies; 2) the methods and extent of detailed information to define ROI varies widely in the studies, preventing accurate comparison; 3) there is a large difference in secondary variables across studies (i.e. gender, medication, co-morbidities); 4) very few MRI studies are available for some disorders, hampering quantitative analyses; 5) meta-analysis has intrinsic limitations in estimating negative findings that do not get published, i.e. the 'file drawer' problem. Therefore, a meta-analytic approach may not be appropriate for a review of this broad scope, which covers more than one specific anxiety disorder.

Finally, it is well known that MRI imaging techniques in animal research can also contribute to the investigation of anxiety disorders.[1] However, we decided to focus on human studies because we believe that the integration of animal and human data would be beyond the scope of the present review.

Method

A search was conducted in the Medline, Lilacs and SciELO indexing services using the following keywords: "anxiety", "panic", "agoraphobia", "social anxiety", "posttraumatic" and "obsessive-compulsive" crossed one by one with "magnetic resonance", "voxel-based", "ROI" and "morphometry". We selected 134 articles that were filtered through an inspection of the abstracts in order to include only publications dealing with human samples and using the case-control experimental design. Thirty-one review studies and 11 case reports were excluded. The final selection included 41 papers published from January 2003 to December 2007, including articles published in the last five years after our previous review. The references of the selected articles were also consulted for additional citations.

Results

Of the 41 articles selected for the present review, 23 dealt with PTSD, 11 with OCD, seven with PD, and one with GAD. Morphological brain abnormalities were evaluated in each study, and only four of them[13-14] have detected no significant differences between the patient and control groups.

1. Posttraumatic stress disorder (PTSD)

PTSD is a psychiatric condition developed by some individuals after exposure to emotionally severe traumatic events such as childhood abuse, war traumas, and natural accidents, among others.[15] The most common symptoms are a persistent re-experience of the traumatic event, blunted general responsiveness, increased excitation, and avoidance of stimuli associated with the traumatic event. In addition to these symptoms, patients with this disorder usually present cognitive dysfunction linked to declarative memory, learning and attention.[16]

Most MRI studies in the literature to date have focused on volumetric differences between groups regarding the hippocampus. This choice is based on the evidence that the hippocampus is critical to mnemonic processing, as well as on the assumption that this brain structure is involved in the pathogenesis of the symptoms of persistent re-experience of the traumatic event.[17-20] Hippocampal abnormalities in association with PTSD may represent pre-trauma vulnerability to the development of the disorder or may be the consequence of exposure to trauma, of PTSD or even of comorbidities associated with the disorder.[21]

The morphometric MRI studies of PTSD discussed in our previous review[10] showed important disagreement in respect to hippocampal volume, having some studies reported volumetric reduction and others indicated absence of alterations in this brain region. Patients with PTSD also presented reduced total brain volume, reduction in the corpus callosum, and ventricular enlargement. Studies focusing on the amygdala, caudate and temporal lobe up until then had not found differences between PTSD patients and controls.

Studies published in the last five years have, in their majority, reinforced our earlier observation[17-20] that there may be significant hippocampal volume reductions in PTSD sufferers compared to controls.[22-27] However, as shown in our previous review,[10] there is also some disagreement about laterality[21,28-30] and even about the presence of this reduction,[13,31-34] in both ROI and VBM-based studies. These contrasting findings, in addition to being due to possible neurobiological abnormalities, may also be due to methodological limitations such as differences in the selection of the hippocampus by manual methods. Bremner et al.,[22] for example, only traced the middle portion of this structure, while other studies measured the hippocampus as a whole.[31,33] Another hypothesis is that changes in the hippocampus may be so subtle that they would not always be detectable by the standard procedure based on MRI.[29]

Other interesting data are related to the effect of treatment on hippocampal volume. While a clinical study using paroxetine demonstrated an increase in hippocampal volume in 23 patients with PTSD and improvement of declarative memory after treatment,[35] another study did not reveal any volumetric changes in a group of PTSD patients investigated before and after effective psychotherapy

treatment.[24] Of note, these contradictory findings may be attributed to differences in the methods of brain volumetric assessment, since the first study[35] used VBM, while the second[24] used the manual, ROI-based approach.

The first morphometric MRI study using automated, voxel-based analysis methods in PTSD[36] detected reduction of the left anterior cingulate cortex, a region not predicted *a priori* in previous studies using manual analysis methods. Several studies later confirmed a volumetric reduction of the anterior cingulate cortex in association with PTSD, although there has been disagreement regarding laterality since differences were detected in the right[37] and left[29] pregenual region[21] and in the anterior cingulated region as a whole.[37] Of particular interest is the fact that the anterior cingulate cortex has close neuroanatomical relations with subcortical components of the "central fear system", including the amygdala and locus coeruleus. If the anterior cingulate cortex actually exerts an inhibitory regulation of the amygdala, then the attenuation of this regulation may explain the multiple symptoms of PTSD.[8,37]

The insula, another region functionally related to the amygdala, has been found to be repeatedly reduced in volume in PTSD patients in recent voxel-based morphometric studies.[21,29,38] There is evidence indicating that the insula is involved both in emotional processing and in cognition, with connections linked to the prefrontal cortex, limbic system and temporal pole.[39] In a meta-analysis of 43 studies using PET and of 12 studies using functional MRI (fMRI), Phan et al. suggested that the anterior cingulate cortex and the insula may be involved in emotional induction in association with cognitive demands.[40] On this basis, these regions may be involved both in the cognitive and emotional processing deficits observed in patients with PTSD. In particular, the insular cortex has been associated with increased bilateral activation in memory processing tests,[41-42] being memory deficits prominent characteristics of PTSD.

PTSD in abused children is a complex scenario, with findings of reduced volume of the brain and of the mediosagittal area of the corpus callosum and of increased lateral ventricle.[43] The same research group, which was the first to examine the cerebellum by a manual method, later detected a reduction of cerebellar volume in children and adolescents with PTSD.[44] In view of previous findings of an increased corticotropin concentration in adults and children with PTSD,[45] a study on a pediatric population focused on the pituitary gland but the authors did not find any significant difference between patients and controls.[46]

In general, the diversity of traumatic events, the chronicity of PTSD and the presence of co-morbidities contribute to the heterogeneity of population samples, impairing the comparability and generalization of the results. Also, some studies used only men, others used only women, whereas still others used equal gender proportions, but without major care in balancing the samples (see Table 1). There is also wide variation in the mean ages of the participants in these studies due to the fact that some studies were conducted on pediatric patients and others on war survivors.

2. Obsessive-compulsive disorder (OCD)

OCD is a psychiatric disorder whose major characteristic is the presence of recurrent and intrusive thoughts (obsessions) and/or voluntary repetitive acts (compulsions).[47]

As is the case for other anxiety disorders, the neurobiology of OCD has not been fully established but, together with PTSD, this disorder has been extensively investigated over the last decades. The dominant neurobiological model postulates that abnormalities in the orbitofrontal-striatal-thalamic circuits may be involved in the pathophysiology of OCD.[48] Dysfunctions in these circuits may be associated with implicit processing deficit and intrusive symptoms.[9] In our previous review,[10] most of the ROI-based morphometric MRI studies were focused on basal ganglia regions, with positive findings in the caudate nucleus, putamen, globus pallidus, and striatal region. In addition, VBM studies analyzing the brain as a whole have suggested that it is possible to identify alterations in regions not restricted to the basal ganglia, giving support to the notion that different portions of the segregated corticostriatal circuits may be distinctly involved in the physiopathology of OCD.

In recent studies using the manual ROI-based approach, the most consistent findings are a reduction in the volume of the orbitofrontal cortex, at times on the left side,[49-50] and at others bilaterally.[51-52] On the other hand, two recent studies that used VBM reported contrasting results. Pujol et al.[53] showed a reduction in volume of the medial orbitofrontal cortex whereas other study[54] observed an increase of the posterior orbitofrontal region. There was also a study using both ROI-based and automatic, voxel-based methods that did not detect significant differences in this cerebral region between patients with OCD and controls.[55]

A factor that may have contributed to the conflicting results is the presence of drug treatment. However, the studies on drug-naïve patients have also obtained contrasting results. In a study on 23 pediatric patients with a diagnosis of OCD but with no previous treatment, a smaller volume of the globus pallidus and a greater volume of the anterior cingulate cortex were detected compared to 26 healthy controls using semi-automated, ROI-based methods.[56] A greater volume of the pituitary gland was also detected using the manual ROI method in untreated pediatric patients with OCD, being more prominent among boys.[57] A recent study using the ROI-based approach on a sample of never-medicated adults detected a reduced volume of the right and left orbitofrontal cortex and a greater volume of the thalamus, also bilaterally.[52] A previous study by the same group had associated these regions with refractoriness to the treatment of OCD.[51]

The efficacy of selective serotonin re-uptake inhibitors (SSRIs) in controlling obsessive-compulsive symptoms strongly suggests the involvement of abnormalities of serotoninergic transmission in the pathophysiology of OCD.[9,58] Szesko et al. monitored 11 children with a diagnosis of OCD before and after treatment with paroxetine and demonstrated that, in addition to these patients having asymmetry of the amygdala before treatment (the left one larger than the right one), their left amygdala volume was reduced after the use of this SSRI in a study using a semi-automated ROI-based method.[59] Serotoninergic transmission in this brain area has been associated with the modulation of fear and of conditioned anxiety, factors that seem to play an important role in OCD.[59]

Other limbic structures have been evaluated in the various morphometric MRI studies of OCD, having been detected inconsistencies in volumetric changes, such as an increase[56] and a reduction of the cingulate cortex,[54,60] an increase of the temporolimbic cortex and of the insula,[54] and a reduction of the insulo-opercular region.[53] Reduction of the white and gray matter in the right parietal cortex was also found,[54,60] an area not previously investigated in structural studies by ROI, but which had already shown lower activity in studies of functional neuroimaging.[61]

As observed above in the studies on PTSD, there was considerable sample heterogeneity regarding sex, age, presence of comorbidities and subtypes of the disorder in the morphometric MRI studies of

Table 1 - Morphologic findings in posttraumatic stress disorder

Reference	Subjects (n)	M / F	Age (mean ± SD)	Approach	Characterization of the sample	Findings	Comments
Bremner et al., 2003	10 patients with PTSD 12 subjects with history of traumatic events without PTSD 11 controls (without history of trauma nor PTSD)	0 / 10 0 / 12 0 / 11	35 ± 6 32 ± 8 38 ± 7	Manual	Comorbidities: past MDD (11), MDD (n = 2); past dysthymia (1); past panic (3); panic (3); past alcohol/substance abuse or dependence (7); past OCD (1), past anorexia (1) Medication: always drug-naive	↓bilateral hippocampus in PTSD patients compared to subjects and controls	PET methods showed failure of hippocampal activation
Bellis et al., 2003	61 patients with PTSD 122 controls	31/30 62/60	11.74 ± 2.63 11.71 ± 2.56	Manual + Semi-automated (Global)	Psychotropic-naive	↓intracranial and cerebral volumes ↓midsagittal area of corpus callosum ↑lateral ventricles and frontal lobe CSF	↓corpus callosum and ↑lateral ventricles in male patients compared to females
Vermetten et al., 2003	23 patients with PTSD before and after treatment	9 / 14	45.3 ± 8.4	Manual	Comorbidities: past MDD (11), MDD (5), dysthymia (1); panic (2); GAD (5); social phobia (3). Treatment in study: Paroxetine 10-50 mg 9-12 months	↑ hippocampus volume after treatment	Treatment with paroxetine resulted in significant improvements in verbal declarative memory
Yamasue et al., 2003	9 patients with PTSD 16 subjects with history of traumatic events without PTSD	5 / 4 10 / 6	44.6 ± 16 44.4 ± 14	Automated (VBM)	Comorbidities: past MDD (1); MDD (1); past panic (1) Treatment: benzodiazepines (2)	↓GM left ACC	Negative correlation between severity of disorder and the left anterior A CC
Pederson et al., 2004	17 patients with PTSD 17 subjects with history of traumatic events without PTSD 17 controls	0 / 17 0 / 17 0 / 17	24.8 ± 5.2 26.8 ± 6.6 23.8 ± 5.6	Manual	Not stated	No difference in hippocampus volume between groups	No differences in memory performance
Thomas et al., 2004	61 patients with PTSD 121 controls	31/30 62/59	11.74 ± 2.6 11.74 ± 2.5	Manual	Psychotropic-naive	No difference in pituitary between groups	Patients had greater differences in pituitary volume with age
Lindauer et al., 2004	14 patients with PTSD 14 subjects with history of traumatic events without PTSD	8 / 6 8 / 6	35.4 ± 11.2 36.9 ± 10.1	Manual	Comorbidities: MDD (4) in first episode	↓bilateral hippocampus	Negative correlation between re-experiencing symptoms and hippocampus volume
Wignall et al., 2004	15 patientswith PTSD 11 controls	9 / 6 9 / 6	43 ± 9 29 ± 10	Manual + Automated (Global)	No psychiatric history No comorbidity	↓right hippocampus ↓global volume	Patients underwent an MRI soon after trauma (mean time = 158 ± 41 days)
Winter et al., 2004	15 patients with PTSD 15 subjects with history of traumatic events without PTSD 15 controls	15 / 0 15 / 0 15 / 0	42 ± 10 41 ± 11 41 ± 17	Manual + Automated (Global)	Psychotropic-naïve	↓bilateral hippocampus in patients and subjects	History of use of NMDA antagonist ketamine to treat burn trauma was related to larger right hippocampal volumes and to stronger PTSD symptoms
Villarreal et al., 2004	12 patients with PTSD 10 controls	2 / 10 2 / 8	43 ± 9.3 44 ± 11.4	Manual + Automated (Global)	Comorbidities: past MDD (6); MDD (6); history of alcohol abuse (1). Treatment: antidepressants (8); benzodiazepines (5); risperidone (1)	↓total corpus callosum	Not stated
Corbo et al., 2005	14 patients with PTSD 14 controls	Not stated	33.36 ± 12.06 33.29 ± 12.31	Automated (VBM) + Semi-automated	Psychotropic-free	↓GM density right ACC ↓GM density left insula	Patients underwent an MRI soon after trauma (no later than six weeks)
Vythilingam et al., 2005	Military groups - 14 patients with PTSD - 23 subjects with history of traumatic events without PTSD - 22 reservists* - 29 controls	8 / 6 15 / 8 9 / 13 9 / 20	35 ± 9 35 ± 7 39 ± 7 34 ± 10	Manual	Comorbidities: past MDD (26); MDD (7); past alcohol abuse (15) and past dependence (13); past (substance abuse 6) and past dependence (2)	↓head of hippocampus in patients compared with controls	↓whole hippocampus in three military groups compared with controls

(To be continued...)

(Continuation)

Reference	Subjects (n)	M / F	Age (mean ± SD)	Approach	Characterization of the sample	Findings	Comments
Lindauer et al., 2005	18 patients with PTSD 14 subjects with history of traumatic events without PTSD	8 / 10 8 / 6	39.6 ± 9 36.9 ± 10.1	Manual	Comorbidities: first-episode MDD (3)	↓bilateral hippocampus ↑parahippocampal gyrus	Smaller hippocampus volumes did not change after effective psychotherapy
Golier et al., 2005	14 patients with PTSD 13 subjects with history of traumatic events without PTSD 20 controls	5 / 9 6 / 7 13 / 7	70.5 ± 5.6 68.5 ± 7.3 71.4 ± 6.4	Manual	Comorbidities: past MDD (13); MDD (9)	No difference in hippocampus volume ↑superior temporal gyrus and lateral temporal lobe in patients and subjects	Patients had poorer memory performance than subjects with history of traumatic events without PTSD and controls
Araki et al., 2005	8 patients with PTSD 13 subjects with history of traumatic events without PTSD	5 / 3 8/ 5	46.6 ± 14.8 47.6 ± 11.5	Automated (VBM)	No comorbidities. Psychotropic-naïve	No difference between groups	Correlation analyses between physiological deficits of controlled attention in patients with ACC
Woodward et al., 2006	Vietnam Cohort - 38 patients with PTSD - 25 subjects with history of traumatic events without PTSD Persian Gulf Cohort - 13 patients with PTSD - 23 subjects with history of traumatic events without PTSD	 38 / 0 25 / 0 10 / 3 19 / 4	 53.5 ± 2.6 56.0 ± 3.5 37.0 ± 5.7 36.7 ± 3.9	Manual	Comorbidities: past MDD (55); MDD (41); past alcohol abuse (43)	↓anterior ACC	The smaller anterior cingulate volume persisted in subjects without history of alcoholism
Freeman et al., 2006	10 patients with PTSD 10 subjects with history of traumatic events without PTSD 6 controls	10 / 0 10 / 0 6 / 0	79.6 ± 3.2 79.8 ± 2.8 80.8 ± 3.5	Manual	Comorbidities: past MDD (7); past or current panic (3); alcohol abuse past (2). Treatment past (4)	No differences between groups	Determinations of hippocampus were made
Lindauer et al., 2006	12 patients with PTSD 12 subjects with history of traumatic events without PTSD	7 / 5 7 / 5	35.1 ± 11.4 36.7 ± 10.1	Manual	Comorbidities: first-episode MDD (3) Psychotropic-naïve	↓bilateral hippocampus	Hippocampus volume did not correlate with memory
Bellis et al., 2006	58 patients with PTSD 13 subjects with GAD 98 controls	30 / 28 8 / 5 50 / 48	12.0 ± 2.4 12.5 ± 2.5 12.0 ± 2.2	Manual	Comorbidities: MDD (34); ODD (25); ADHD (20); separation anxiety (3); panic (1); social phobia (1) Psychotropic-naïve	↓cerebellar volumes compared with GAD and controls	Cerebral volumes positively correlated with age at onset of trauma
Chen et al., 2006	12 patients with PTSD 12 subjects with history of traumatic events without PTSD	8 / 4 8 / 4	34.56 ± 4.91 33.25 ± 5.27	Automated (VBM)	No comorbidities Psychotropic-naïve	↓left hippocampus ↓left ACC ↓bilateral insula	Not stated
Jatzko et al., 2006	15 patients with PTSD 15 controls	13 / 2 13 / 2	48.2 ± 12.2 47.9 ± 12.9	Automated (VBM) + Semi-automated	Just patients with chronic disease. Treatment: past SSRI (8)	No differences between groups	Not stated
Kasai et al., 2007	Twin pairs from patients with PTSD - 18 exposed - 18 unexposed Twin subjects — 23 exposed to traumatic events - 23 unexposed	 18 / 0 18 / 0 23 / 0 23 / 0	 52.8 ± 3.4 52.8 ± 3.4 51.8 ± 2.3 51.8 ± 2.3	Automated (VBM)	Combat-exposed twins with PTSD had more severe alcoholism histories and MDD than the other 3 groups.	↓right hippocampus ↓pregenual ACC ↓bilateral insula in patients twins combated-exposed	Pregenual ACC reduction represents an acquired sign of PTSD
Li et al., 2007	12 patients with PTSD 12 subjects with history of traumatic events without PTSD	4 / 8 4 / 8	34.56 ± 4.91 33.25 ± 5.27	Automated (VBM)	Comorbidities: MDD (2) Psychotropic-naïve	↓left hippocampus	A reduction in the ratio of NAA/Cr in the left hippocampus of patients with recent-onset PTSD indicates a loss of hippocampal neurons

↑, increase; ↓, decrease; N., number of participants; F, female; M, male; SD, standard deviation; PTSD, posttraumatic stress disorder; ADHD, attention deficit hyperactivity disorder; OCD, obsessive compulsive disorder; ODD, oppositional defiant disorder; MDD, major depressive disorder; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; GM, gray matter; VBM, voxel-based morphometry; SSRI, selective serotonin reuptake inhibitors; CSF, cerebrospinal fluid

** number of subjects that served in the military at the time of the Gulf war but were not deployed to the Gulf.*

Table 2 - Morphologic findings in obsessive-compulsive disorder

Reference	Subjects (n)	M / F	Age (mean ± SD)	Approach	Characterization of the sample	Findings	Comments
Szeszko et al., 2004	23 patients with OCD	7 / 16	12.3 ± 2.9	Manual + Semi-automated (Global)	Drug-naïve Comorbidity: anxiety disorders (8); dysthymia (3); ADHD (1); ODD (1); trichotillomania (2)	↓anterior cingulate gyrus ↓globus pallidus	No difference in intracranial volume.
	27 controls	12 / 15	13.6 ± 3.2				
Szeszko et al., 2004b	11 patients with OCD	3 / 8	11.8 ± 3.0	Manual + Semi-automated (Global)	Drug-naïve	Before treatment: left amygdala > right amygdale After treatment: ↓left amygdala	No difference in intracranial volume. Findings before or after treatment with paroxetine for 16 weeks
	11 controls	3 / 8	13.36 ± 2.4				
Kang et al., 2004	36 patients with OCD	28 / 8	26.33 ± 6.18	Manual	Comorbidities: MDD (n = 4); transient tic disorder (n = 5). Medication: drug naïve (11); psychotropic-free for 4 weeks (25)	↓left OFC	Negative correlation between left orbitofrontal cortex and symptom severity (p = 0.017)
	36 controls	28 / 8	26.33 ± 7.58				
Pujol et al., 2004	72 patients with OCD	40 / 32	29.8 ± 10.5	Automated (OVBM)	Comorbidities: anxiety and depression symptoms. Treatment: TMS in frontal lobre12 months before	↓GM in medial frontal gyrus, medial OFC, left insulo-opercular ↑GM bilateral ventral putamen and anterior cerebellum	Correlation between age and relative enlargement in the striatal areas. Correlation between aggressive obsessions and checking compulsions with reduction of amygdala volume
	72 controls	40 / 32	30.1 ± 10.2				
Choi et al., 2004	34 patients with OCD	28 / 6	26.5 ± 7.73	Manual + Semi-automated (Global)	Comorbidities: MDD (4) Treatment: drug naïve (11); psychotropic-free for 4 weeks (23)	↓left anterior OFC	Clinical severity was negatively correlated with putamen volume
	34 controls	28 / 6	26.24 ± 6.28				
Valente et al., 2005	19 patients with OCD	10 / 9	32.7 ± 8.8	Automated (OVBM) + Manual	Comorbidities: MDD (7); past MDD (2); dysthymia (3); specific phobia (5); social phobia (7); GAD (3); panic (1) and lifetime history of tics (5)	↑GM in posterior OFC and parahippocampal regions ↓GM left anterior cingulate cortex	Not stated
	15 controls	7 / 8	32.3 ± 11.8				
Riffkin et al., 2005	18 patients with OCD	8 / 10	36.14 ± 12.99	Automated (OVBM) + Manual	Treatment: drug free (14), medication (4)	↓WM right temporal region	Other results were found when compared to the SCZ group
	18 patients with SCZ	8 / 10	35.92 ± 11.97				
	18 controls	8 / 10	34.55 ± 11.82				
Macmaster et al., 2006	31 patients with OCD	10 / 21	12.79 ± 2.64	Semi-automated	Comorbidities: anxiety disorders (8), trichotillomania (1), dysthymia (1) Treatment: always drug naïve	↓pituitary gland	Smaller pituitary volume was associated with increased compulsive symptom severity
	31 controls	10 / 21	12.89 ± 2.66				
Atmaca et al., 2006	30 patients with OCD	12 / 18	27.1 ± 4.2	Manual	No co-morbidities described	↓left bilateral OFC* ↑bilateral thalamic* volumes	Reduction in OFC and increase in thalamic volumes may be associated with refractoriness
	- 10 Drug-free	4 / 6	26.5 ± 5.2				
	-10 Treatment respondent	4 / 6	27.6 ± 3.7				
	- 10 Treatment refractory	4 / 6	29.0 ± 4.7				
	- 10 controls	4 / 6	29.8 ± 6.4				
Carmona et al., 2007	18 patients with OCD	13 / 5	12.86 ± 2.76	Automated (OVBM)	Comorbidities: tic disorder (11), inattention symptoms (3), anxiety symptoms (6). Treatment: drug naïve(8), SSRI (9), Clomipramine (1)	↓GM bilateral in frontal and cingulate regions ↓WM bilateral frontal and right parietal	Negative correlation between symptom severity and bilateral hippocampal volume and positive correlation between age and GM left caudate volume
	18 controls	13 / 5	13.03 ± 3.04				
Atmaca et al., 2007	12 patients with OCD	6 / 6	26.9 ± 2.64	Manual	Comorbidities: tic disorder (1), MDD (1), panic (1), dysthymia (1). Treatment: always drug naïve	↓left bilateral OFC ↑bilateral thalamic volumes ↑ WM volume	Significant correlations between symptom severity and bilateral OFC and left thalamus volumes
	12 controls	5 / 6	28.7 ± 2.66				

*↑, increase; ↓, decrease; N., number of participants; F, female; M, male; SD, standard deviation; OCD, obsessive-compulsive disorder, ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; MDD, major depressive disorder; ; SCZ, schizophrenia; TMS, transcranial magnetic stimulation; OFC, orbitofrontal cortex; GM, gray matter; WM, white matter; OVBM, optimized voxel-based morphometry; SSRI, selective serotonin reuptake inhibitors. *significant difference between drug-free patients and treatment-responders and controls and between refractory patients and treatment-responder patients*

OCD to date (Table 2). In particular, populations of varied age ranges have been studied. There is evidence of a correlation between greater widening of striatal structures with age,[53] and no structural abnormalities of these regions have been detected in children.[56,60]

3. Panic disorder (PD)

PD is characterized by the occurrence of unexpected panic attacks with consequent anticipatory anxiety about experiencing new episodes. The manifestations of PD include a variety of affective, cognitive, behavioral and physiological symptoms. Due to the nature of the symptoms, subcortical areas such as basal ganglia and limbic system have been suggested to be involved in the pathophysiology of PD.[62-63] In our previous review,[10] abnormalities of the basal ganglia were not reported, whereas a reduced volume of areas of the temporal lobe was described.[64-66] However, functional imaging studies during rest have previously revealed abnormal activity in the hippocampus and in other limbic structures such as the amygdala and cingulated gyrus.[63]

Massana et al. evaluated the amygdala, temporal lobe and hippocampus of 12 patients with PD compared to 12 healthy controls.[67] This was the first study to determine the volume of the amygdala in PD patients, with the observation of a significant bilateral reduction of this region compared to controls. Later, Uchida et al., using the same *a priori* hypothesis, detected a reduction of the left temporal lobe in 11 patients with PD compared to 11 controls.[68]

The absence of abnormalities of the temporal lobe in the study by Massana et al.[67] in contrast to the study by Uchida[68] and to previous literature[66] may be attributed to a highly conservative measurement of the ROI, centered only on the medial segment and excluding the volumes of the hippocampus and amygdala.

In another direction, there is the hypothesis that structures of the septo-hippocampal system may be associated with PD symptoms since this region seems to play a crucial role in the modulation of anxiety.[69] Supporting this notion, another study detected a high frequency of *cavum septi pellucidum* (CSP) in patients with PD

Table 3 - Morphologic findings in panic disorder

Reference	Subjects (n)	M / F	Age (mean ± SD)	Characterization of the sample	Approach	Findings	Comments
Massana et al., 2003	12 patients with PD 12 controls	6 / 6 6 / 6	26 to 43 years (matched)	Comorbidity: agoraphobia (10)	Manual	↓amygdalar volume bilaterally	Not stated
Massana et al., 2003b	18 patients with PD 18 controls	11 / 7 10 / 8	36.8 ± 11.3	Comorbidity: agoraphobia (15)	Automated (VBM)	↓GM left parahippocampal gyrus	Not stated
Uchida et al., 2003	11 patients with PD 11 controls	3 / 8 5 / 6	36.86 ± 11.9 34.27 ± 10.2	Comorbidities: agoraphobia (6); past MDD (n = 5); dysthymia (n = 1). On medication: SSRI (4); Clomipramine (3); benzodiazepine (1)	Manual	↓left temporal lobe	Positive correlation between left hippocampal volume and duration of PD (p = 0.025)
Crippa et al., 2004	21 patients with PD 21 controls	5 / 16 5 / 16	31.1 ± 10.8 38.3 ± 10.0	Comorbidities: agoraphobia (13); past MDD (n = 15); past dysthymia (n = 2). On medication: SSRI (8); tricyclics (7); adjunctive benzodiazepine (5)	Manual	No difference in cavum septum pellucidum	Not stated
Yoo et al., 2005	18 patients with PD 18 controls	9 / 9 11 / 7	33.3 ± 7.1 32.0 ± 5.8	Not stated	Automated (OVBM)	↓GM bilateral putamen	Clinical severity was negatively correlated with putamen volume
Protopopescu et al., 2006	10 patients with PD 23 controls	4 / 6 12 / 13	35.5 ± 9.7 28.7 ± 7.5	Comorbidities: agoraphobia (2); also specific phobia; MDD; social phobia; GAD; past alcohol abuse (1) and post alcohol dependence (1). One with medication	Automated (OVBM)	↑GM in the midbrain and rostral pons of the brainstem ↑ventral hipocampal ↓regional prefrontal cortex	Not stated
Uchida et al., 2008	19 patients with PD 20 controls	3 / 8 5 / 6	37.05 ± 9.7 36.45 ± 9.9	Comorbidities: agoraphobia (14); MDD (n = 3); dysthymia (n = 2). On medication: antidepressants (14); adjunctive benzodiazepine (4); only benzodiazepine (1)	Automated (OVBM)	↑left insula ↑left superior temporal gyrus ↑left parahippocampal gyrus	Not stated

↑, increase; ↓, decrease; N., number of participants; F, female; M, male; SD, standard deviation; GAD, generalized anxiety disorder; MDD, major depressive disorder; GM, gray matter; VBM, voxel-based morphometry; OVBM, optimized voxel-based morphometry; SSRI, selective serotonin reuptake inhibitors

who presented EEG abnormalities.[70] More recently, Crippa et al.[14] investigated the prevalence and size of the CSP in 21 PD patients compared to 21 healthy controls, but no significant differences were detected between groups by manual, ROI-based methods.

The first study on PD patients using VBM-based methods detected a reduction of gray matter in the left parahippocampal gyrus of PD patients.[71] Later, other study,[72] using optimized VBM demonstrated a bilateral reduction of gray matter in the putamen of 18 patients with PD compared to healthy controls. In the cited study, the severity of PD symptoms and the duration of the disorder were negatively correlated with the volume of the putamen. More recently, Protopopescu et al., also using optimized VBM, detected an increased gray mass volume in the brain stem of 10 patients with PD compared to controls, specifically at rostral sites.[73]

More recently, Uchida et al. assessed gray matter volume in 19 PD patients and 20 healthy volunteers using VBM.[74] The authors found relative increase in gray matter volume in the left insula of PD patients as compared to controls. Moreover, it was also observed in the PD group increases in the left superior temporal gyrus as well as in the midbrain and pons. Relative gray matter deficit occurred in the right anterior cingulate cortex. The authors concluded that insula and anterior cingulate abnormalities may be relevant to the evaluation process that ascribes negative emotional meaning to potentially distressing cognitive and interoceptive sensory information in PD and that the abnormalities in brain stem structures may be involved in the generation of panic attacks.

The PD and control groups of the above studies were matched for number, age, years of schooling, socioeconomic level and hand dominance. Small samples were investigated in all of these studies, ranging from 10 to 21 patients with PD. However, the studies mainly differed regarding the use of psychotropic medication and the presence of comorbidities (Table 3).

4. Generalized anxiety disorder (GAD)

GAD is a chronic and recurrent disorder characterized by excessive, pervasive and uncontrollable concern. Associated symptoms include irritability, restlessness and concentration impairment. Somatic symptoms may include muscle tension, sweating, dry mouth, nausea and diarrhea.[75] The prevalence of GAD is considered high in the general population, and the symptoms closely resemble those of other anxiety disorders. This has led some investigators to contest it as a distinct diagnostic category.

Neurobiological studies using different investigative techniques (e.g. neurochemistry, physiology and genetics) in both humans and animals have indicated that, in GAD, there may be abnormalities of some brain regions responsible for emotional processing and social behaviors, such as the amygdala, prefrontal cortex and temporal areas.[76] As previously observed, few studies using structural volumetric MRI are available for this disorder, a fact that prevents definitive conclusions based on volumetric data.[77-78] In our former review[10] the few morphometric MRI studies available up until then supported the hypotheses raised by investigations using other tools, such as functional neuroimaging,[79] which postulated the presence of anatomical abnormalities localized in the amygdala and the temporal lobe, more specifically the superior temporal gyrus, in association with the diagnosis of GAD.[77-78]

One of the recent morphometric MRI studies of GAD used the VBM-based approach to compare children with and without anxiety. The sample of patients with anxiety was heterogeneous, consisting of 9 patients with social anxiety disorder, 3 with separation anxiety, and 13 with a diagnosis of GAD. Reduction of the volume of the left amygdala was demonstrated in the group of patients with anxiety, this being a region commonly implicated in the mediation of emotional responses.[80] However, the heterogeneity of anxiety disorders limits the specificity of the findings.

Discussion

Given that we have only begun to understand the neural circuitry of anxiety, our current diagnostic classification system of the anxiety disorders is arguably not the most effective tool available. Evidence from treatment and neuroimaging studies strongly indicate that the anxiety disorders that are currently classified in separate diagnostic categories may have overlapping pathology, while those that are grouped together within a given category may have very different underlying brain mechanisms. Ongoing efforts in neuroimaging promise to elicit new insights into the commonalities and differences among the anxiety disorders and their respective neural circuitries.[81]

In this updated review of morphometric MRI studies of anxiety disorders, we have once again verified that PTSD remains as the anxiety disorder most extensively investigated by structural neuroimaging over the last five years. In agreement with our previous review,[10] the main morphological feature detected in this disorder is a reduction of the hippocampus,[22-27] although with some degree of disagreement among studies regarding laterality and even the presence of changes.

In some studies using manual ROI-based methods, the investigators looked for associations between clinical data and hippocampal changes, as was the case for the negative correlation detected between re-experiencing the traumatic event and the hippocampal volume.[23] The fact that reduced volume of this structure is already detectable in patients with PTSD examined shortly after the traumatic event[28] supports the hypothesis that reduction of the hippocampus may be a predisposing factor for the development of PTSD (rather than a consequence of PTSD symptoms).

On the other hand, the presence of hippocampal reduction detected in traumatized burn patients without PTSD suggests that this morphological change may be associated with trauma in general, rather than being specifically related to the development of PTSD.[26] In agreement with this possibility, a recent ROI-based MRI study found an increase of the lateral temporal lobe and superior temporal gyrus in survivors of the Holocaust with and without PTSD compared to healthy controls.[33]

Most MRI studies of PTSD to date used ROI-based methods, at times with significant divergence regarding the *a priori* hypotheses across studies, with different findings such as reduction of the *corpus callosum* and cerebellum and increase of the lateral ventricle, superior temporal gyrus and temporal lobe. Since the introduction of automated VBM-based techniques for the investigation of this disorder, findings in brain areas not previously investigated by the ROI-based methods have been reported in several studies, such as bilateral reduction of the insula and of the anterior cingulate cortex.[21,29,30,33,36,37] The anterior cingulate gyrus is considered to have a key role in emotional and cognitive associations in respect to fear and anxiety. Lesions of this brain area have been shown to induce emotional reactions and impair behavioral extinction, seemingly because they disrupt the inhibitory influence of the prefrontal cortex on the amygdala.[3] Therefore, the volumetric reduction of the anterior cingulate cortex is in agreement with the PTSD model

related to the neural circuitry of conditioned fear, being amygdala hyperresponsivity in face of deficits in the cortical components responsible for its regulation.

In general, functional neuroimaging studies conducted on OCD over more than two decades using PET, Single Photon Emition Computed Tomography (SPECT), fMRI and spectroscopy have confirmed the notion that abnormalities of the orbitofrontal-striatal-thalamic circuits are of critical importance for the pathophysiology of this disorder.[9] In the present review, structural abnormalities have also been frequently identified in regions of this circuit such as the orbitofrontal cortex,[49-54] although in disagreement from the laterality and direction of the abnormality. Other significant abnormalities have been reported for the basal ganglia, thalamus, insula, cingulate cortex and amygdala, although the results reported are also discrepant among the various studies.[51-54,56,60] Variations in the nature of the sample or of the volumetric methods may explain such discordances between studies. Regarding OCD, there is also evidence that specific morphological abnormalities may be related to the stage of the disease and to the different clinical subtypes of this disorder, with important implications for the search for new treatment strategies.

Valente et al. using the VBM methodology, obtained different results for the volume of separate portions of the orbitofrontal cortex depending on the severity of OCD symptoms, even after exclusion of depression as a comorbidity.[54] These results are consistent with the idea that the orbitofrontal cortex presents heterogeneous functional subdivisions, each possibly playing a different role in the pathophysiology of the various symptoms of OCD.[82] Additionally, other brain regions may also be associated with different symptomatologic dimensions in OCD. For example, in one study using VBM,[53] patients with aggressive obsessions and compulsions showed reduced amygdala volume in the right hemisphere. Additionally, reduction of the pituitary gland was associated with the severity of compulsive symptoms, but not of obsessive symptoms.[57] These findings partially challenge previous models that associate obsessive symptoms with increased frontal cortex activity, and compulsive symptoms with striatal abnormalities.[3]

Although structures of the corticostriatal circuitry are classically implicated in the pathophysiology of OCD, the observation of increased amygdala volume following treatment described in the present review supports a proposition that this temporolimbic structure may also be of critical relevance to OCD. Amygdala abnormalities have been described in a previous functional study[9] which found a significant correlation between the activation of this brain area and symptom increase in OCD. Given the intimate connections between the amygdala and the striatum, their anatomical proximity and respective roles, it has been suggested that activation of the amygdala during a state of fear or anxiety could promptly induce stereotyped behaviors observed during striatal activation. Furthermore, exposure-based behavioral treatment of OCD can be linked to the process of extinction in the classical fear conditioning paradigm.

In our previous review,[10] the most consistent finding in quantitative neuroimaging studies of PD was the reduced volume of the temporal lobe.[64-66] Thus far, only two studies have been published confirming this reduction.[68] The more recent studies included new anatomical regions, often by means of the VBM technique that allowed the investigation of differences in regional volumes along the whole brain.[83] Thus, reductions of the anterior cingulate cortex, amygdala and hippocampal region have been described, as well as an increase in gray matter in the insula, superior temporal gyrus and the brain stem structures. The insula and anterior cingulate abnormalities may be particularly important to the pathophysiology of PD, since these structures participate in the evaluation process of negative emotional meaning to potentially distressing cognitive and interoceptive sensory information. On the other hand, the abnormal brain stem structures may be involved in the generation of panic attacks.[83]

Recent findings suggestive of a smaller volume of the putamen in patients with PD should also be pointed out, this being an area of the basal ganglia that has also been correlated with the severity of panic symptoms. In general, there is some degree of discrepancy between the findings of the various morphometric MRI studies of PD reported to date. Such variability of findings is possibly due to the different techniques of evaluation, the presence of co-morbidities among the subjects studied and the small number of participants in the majority of investigations. However, another reason for the discrepant results may also be the multiplicity of brain regions that may be involved in the pathophysiology of PD. In contemporary anatomic models of PD, it is proposed that panic attacks may be associated with abnormalities of brain stem regions. In addition, anticipatory anxiety may be related to abnormalities of limbic structures, while phobic avoidance may be related to abnormal activity of the temporal lobe, prefrontal cortical areas, and brain stem.[84]

Structural neuroimaging studies in the GAD are still in an early phase. The only report in the period reviewed in this article involved a highly heterogeneous sample,[80] reflecting the methodological difficulty of conducting studies of this disorder in samples with a precise diagnosis and with no comorbidities. The detection of a reduction of the amygdala is in agreement with general theories that relate this region to the mechanisms of recognition and learning in threatening or dangerous situations.[40,62]

So far, only one structural neuroimaging study[85] has evaluated the volume of brain structures in patients with SAD, as highlighted in our former review.[10] In that ROI-based study, the authors found no differences between patients and healthy controls regarding the measures of the total brain, putamen, caudate, and thalamus.[85] As our earlier observation,[10] the present review notes the scarce number of morphometric MRI studies on SAD and specific phobias with no paper being published in the last five years in such disorders. Once again, it is surprising that SAD, one of the most frequent anxiety disorders in the general population which causes important functional impairments, was not investigated during this period. This is especially intriguing considering the relative easiness of recruiting never-medicated SAD patients and without significant co-morbidities during the initial phases of the disorder.[86]

It is important to point out that the inconsistencies of structural imaging findings on the anxiety disorders reviewed in this paper do not reflect loss of validity of the model of investigation, but may rather reflect confounding factors resulting from the design of the studies. Many studies included subjects with co-morbidities such as depression or other anxiety disorders that might have hampered the specificity of the results. For example, hippocampal reduction, so frequently described in PTSD, is known to be associated with depressive signs and symptoms.[87] Another important point is the inclusion of patients currently or previously taking medications since its use such as paroxetine, among others, has been shown to affect the cerebral morphology of the patients.[35,59] Differences in age and in gender balancing also cause a lack of homogeneity between results. It is preferable to use only one gender or to have a balanced

gender proportion since there are specific gender-linked anatomical abnormalities that may have a negative influence on the results.[88]

An additional confounding factor is the presence of patients with different levels and types of symptoms. Regarding OCD in particular, a previous study suggested that the different symptomatologic dimensions such as contamination/washing and symmetry/ordering may have distinct neural substrates.[89] In agreement with this, several studies reviewed here confirmed correlations between the symptomatologic dimensions of OCD and specific structural abnormalities.[53,54,57]

In respect to the methods of MRI analysis, studies with automated analysis such as VBM hold the promise of capturing larger numbers of cerebral structures, thus reducing, for example, the difficulty of manual studies in delimiting the anatomical margins of the structure of interest and the problems of execution and reproducibility of this task. In the selected studies, analyses by VBM indeed accounted for the observation of changes in other cerebral structures not previously included as *a priori* hypotheses in studies with ROI.[21,29,33,36,37,72,73] However, the diverse findings obtained in studies using this technique, in addition to sample differences, reveal that VBM still is an evolving method. There are limits regarding cerebral normalization and smoothing stages that may cause loss of information and degradation of anatomical details across different brain structures.[90] Thus, it is necessary to determine whether the results obtained with VBM are comparable to those obtained with standard ROI-based morphometry, in order that the findings may be better analyzed in light of these methods, as recently conducted in a study in patients with PD.[83]

Also with respect to MRI methodologies, many studies have been carried out using 1.5 T scanners. However, 3.0 T (or stronger) scanners are increasingly available and may become the standard for research purposes in the next few years. Higher intensity magnets can enhance the signal-to-noise ratio in MRI, improving the distinctions between tissues. The extent to which these differences will impact on research in anxiety disorders should become clearer over the next decade.

Of note, structural neuroimaging findings discussed in the present review are not readily reconcilable with the previous functional imaging literature on the anxiety disorders evaluated. For instance, in the MRI study by Choi et al.[49] there was a reduction of the anterior region of the orbitofrontal cortex, whereas previous functional imaging studies demonstrated an increased activity of this region.[91,92] Moreover, increased activity of the orbitofrontal cortex, especially its anterior subregion, in functional imaging studies may be the result of compensatory hyperactivity of residual tissues secondary to decreased volume of this region.[49] Another study[93] reported increased gray matter density of left orbitofrontal cortex in patients with OCD using a VBM analysis. These observations suggest that increases in regional activity are not necessarily related to volume increases, and that decreases in activity are not necessarily related to volume decreases. In this respect, the structural studies can complement functional ones, especially regarding the delimitation of anatomical changes, the ability to show that increased or reduced tissue volumes are compatible with hypermetabolism due to compensatory mechanisms, and even the suggestion that brain abnormalities may vary progressively during the course of the disorders.[53]

Finally, it is equally important to acknowledge a cautionary note regarding whether these neuroimaging findings can be translated to clinical practice. The majority of neuroimaging studies in psychiatry are research-oriented, and are not designed to have an immediate clinical application. For instance, many neuroimaging findings relate to mean differences in comparisons between groups of patients and controls, and it is difficult to use this information in an individual patient in a clinical setting. Nevertheless, neuroimaging studies are increasingly being designed with the aim of translating findings into psychiatric practice, and neuroimaging is already playing a role in the diagnosis and management of dementia.[94]

Conclusion

The present review indicates that structural neuroimaging methods can be used for a better understanding of the neurobiology of anxiety disorders. Despite a few contradictory findings, mainly due to the variability and limitations of the methodologies used, morphometric abnormalities of some brain structures appear in a more consistent and relatively specific manner in certain anxiety disorders. In particular the hippocampus and anterior cingulate cortex are robustly implicated in PTSD and the orbitofrontal cortex has consistently been found to be the site of abnormalities in OCD. Apparently discrepant results may be due to the different *a priori* hypotheses used in studies with ROI, although the findings may indeed reveal distinct structures involved in the physiopathology of the same disorder. It will be important for future studies to reproduce prior findings and determine which findings are unique to early-onset anxiety disorders, relative to adult-onset illness. Moreover, studies will need to establish the extent to which anxiety disorders may overlap with comorbid disruptive, mood, anxiety, or psychotic disorders. In addition, some diagnoses have been scarcely explored in the neuroimaging field. It is possible that the rapid advancement of neuroimaging techniques, a better sample standardization and greater emphasis on longitudinal studies will allow a clarification of this scenario.

Disclosures

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Spekear's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Maria Cecília Freitas Ferrari	FMRP-USP	FAPESP** CNPq** FAEPA*	---	---	---	---	---
Geraldo F. Busatto	FMUSP	FAPESP** CNPq** WELLCOME-TRUST**	---	---	---	---	---
Philip K. McGuire	Institute of Psychiatry of London	WELLCOME-TRUST**	---	---	---	---	---
José Alexandre S. Crippa	FMRP-USP	FAPESP** CNPq** CAPES** FAEPA*	FAEPA*	---	---	---	FUNPEC* Pfiser* Elli-Lilly* Bristol* THC-Pharm*

** Modest*
*** Significant*
**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*
Note: FMRP-USP = Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo; FMUSP = Faculdade de Medicina da Universidade de São Paulo; FAPESP = Fundação de Amparo à Pesquisa do Estado de São Paulo; CNPq = Conselho Nacional de Desenvolvimento Científico e Tecnológico; FAEPA = Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto; CAPES = Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; FUNPEC = Fundação de Pesquisas Científicas de Ribeirão Preto.
For more information, see Instructions for authors.

References

1. Hetem LAB, Graeff FG. *Transtornos de Ansiedade*. São Paulo (SP): Editora Atheneu; 2004.
2. Delgado MR, Olsson A, Phelps EA. Extending animal models of fear conditioning to humans. *Biol Psychol.* 2006;73(1):39-48.
3. Cannistraro PA, Rauch SL. Neural circuitry of anxiety: evidence from structural and functional neuroimaging studies. *Psychopharmacol Bull*. 2003;37(4):8-25.
4. Graeff FG. Serotonin, the periaqueductal gray and panic. *Neurosci Biobehav Rev*. 2004;28(3):239-59.
5. Pinheiro SH, Zangrossi H Jr, Del-Ben CM, Graeff FG. Elevated mazes as animal models of anxiety: effects of serotonergic agents. *An Acad Bras Cienc*. 2007;79(1):71-85.
6. Cammarota M, Bevilaqua LR, Vianna MR, Medina JH, Izquierdo I. The extinction of conditioned fear: structural and molecular basis and therapeutic use. *Rev Bras Psiquiatr*. 2007;29(1):80-5.
7. Rauch SL, Shin LM, Whalen PJ, Pitman, RK. Neuroimaging and the neuroanatomy of PTSD. *CNS Spectr*. 1998;3(Suppl 2):30-41.
8. Ruiz JE, Barbosa Neto J, Schoedl AF, Mello MF. Psychoneuroendocrinology of posttraumatic stress disorder. *Rev Bras Psiquiatr*. 2007;29(Suppl 1):S7-12.
9. Menzies L, Chamberlain SR, Laird AR, Thelen SM, Sahakian BJ, Bullmore ET. Integrating evidence from neuroimaging and neuropsychological studies ofobsessive-compulsive disorder: the orbitofrontal-striatal model revisited. *Neurosci Biobehav Rev*. 2008;32(3):525-49.
10. Crippa JAS, Busatto G, McGuire PK. Neuroimagem. In: Hetem LAB, Graeff FG. *Transtornos de Ansiedade*. São Paulo (SP): Editora Atheneu; 2004. p. 133-67.
11. Ashburner J, Friston KJ. Voxel-based Morphometry – the methods. *Neuroimage*. 2000;11(6 Pt 1):805-21.
12. Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage*. 2001;14: 21-36.
13. Pederson CL, Maurer SH, Kaminski PL, Zander KA, Peters CM, Stokes-Crowe LA, Osborn RE. Hippocampal volume and memory performance in a community-based sample of women with posttraumatic stress disorder secondary to child abuse. *J Trauma Stress*. 2004;17(1):37-40.
14. Crippa JA, Uchida R, Busatto GF, Guimaraes FS, Del-Ben CM, Zuardi AW, Santos AC, Araujo D, McGuire PK, Graeff FG. The size and prevalence of the cavum septum pellucidum are normal in subjects with panic disorder. *Braz J Med Biol Res*. 2004; 37(3):371-4.
15. Mendlowicz MV, Figueira I. Intergenerational transmission of family violence: the role of post-traumatic stress disorder. *Rev Bras Psiquiatr*. 2007;29(1):88-9.
16. Wignall EL, Dickson JM, Vaughan P, Farrow TF, Wilkinson ID, Hunter MD, Woodruff PW. Smaller hippocampal volume in patients with recent-onset posttraumatic stress disorder. *Biol Psychiatry*. 2004;56(11):832-6.
17. Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB. MRI-based measurement of hipocampal volume in patients with combated-related posttraumatic stress disorder. *Am J Psychiatry*. 1995;152(7):973-81.
18. Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse – a preliminary report. *Biol Psychiatry*. 1997;41(1):23-32.
19. Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med*. 1997;27(4):951-9.
20. Villarreal G, Hamilton DA, Petropoulos H, Driscoll I, Rowland LM, Griego JA, Kodituwakku PW, Hart BL, Escalona R, Brooks WM. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. *Biol Psychiatry*. 2002;52(2):119-25.
21. Kasai K, Yamasue H, Gilbertson MW, Shenton ME, Rauch SL, Pitman RK. Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. *Biol Psychiatry*. 2008;63(6):550-6.
22. Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, Khan S, Vaccarino LV, Soufer R, Garg PK, Ng CK, Staib LH, Duncan JS, Charney DS. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *Am J Psychiatry*. 2003;160(5):924-32.
23. Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, den Heeten GJ, Gersons BP. Smaller hippocampal volume in Dutch police officers with posttraumatic stress disorder. *Biol Psychiatry*. 2004;56(5):356-63.
24. Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP. Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. *Psychol Med*. 2005;35(10):1421-31.
25. Lindauer RJ, Olff M, van Meijel EP, Carlier IV, Gersons BP. Cortisol, learning, memory, and attention in relation to smaller hippocampal volume in police officers with posttraumatic stress disorder. *Biol Psychiatry*. 2006;59(2):171-7.
26. Winter H, Irle E. Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. *Am J Psychiatry*. 2004;161(12):2194-200.

27. Vythilingam M, Luckenbaugh DA, Lam T, Morgan CA 3rd, Lipschitz D, Charney DS, Bremner JD, Southwick SM. Smaller head of the hippocampus in Gulf War-related posttraumatic stress disorder. *Psychiatry Res*. 2005;139(2):89-99.
28. Wignall EL, Dickson JM, Vaughan P, Farrow TF, Wilkinson ID, Hunter MD, Woodruff PW. Smaller hippocampal volume in patients with recent-onset posttraumatic stress disorder. *Biol Psychiatry*. 2004;56(11):832-6.
29. Chen S, Xia W, Li L, Liu J, He Z, Zhang Z, Yan L, Zhang J, Hu D. Gray matter density reduction in the insula in fire survivors with posttraumatic stress disorder: a voxel-based morphometric study. *Psychiatry Res*. 2006;146(1):65-72.
30. Li L, Chen S, Liu J, Zhang J, He Zhong, Lin X. Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy study of deficits in hippocampal structure in fire victims with recent-onset posttraumatic stress disorder. *Can J Psychiatry*. 2006;51(7):431-7.
31. Freeman T, Kimbrell T, Booe L, Myers M, Cardwell D, Lindquist DM, Hart J, Komoroski RA. Evidence of resilience: neuroimaging in former prisoners of war. *Psychiatry Res*. 2006;146(1):59-64.
32. Jatzko A, Rothenhöfer S, Schmitt A, Gaser C, Demirakca T, Weber-Fahr W, Wessa M, Magnotta V, Braus DF. Hippocampal volume in chronic posttraumatic stress disorder (PTSD): MRI study using two different evaluation methods. *J Affect Disord*. 2006;94(1-3):121-6.
33. Golier JA, Yehuda R, De Santi S, Segal S, Dolan S, de Leon MJ. Absence of hippocampal volume differences in survivors of the Nazi Holocaust with and without posttraumatic stress disorder. *Psychiatry Res*. 2005;139(1):53-64.
34. Araki T, Kasai K, Yamasue H, Kato N, Kudo N, Ohtani T, Nakagome K, Kirihara K, Yamada H, Abe O, Iwanami A. Association between lower P300 amplitude and smaller anterior cingulate cortex volume in patients with posttraumatic stress disorder: a study of victims of Tokyo subway sarin attack. *Neuroimage*. 2005;25(1):43-50.
35. Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. *Biol Psychiatry*. 2003;54(7):693-702.
36. Yamasue H, Kasai K, Iwanami A, Ohtani T, Yamada H, Abe O, Kuroki N, Fukuda R, Tochigi M, Furukawa S, Sadamatsu M, Sasaki T, Aoki S, Ohtomo K, Asukai N, Kato N. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *Proc Natl Acad Sci U S A*. 2003;100(15):9039-43.
37. Woodward SH, Kaloupek DG, Streeter CC, Martinez C, Schaer M, Eliez S. Decreased anterior cingulate volume in combat-related PTSD. *Biol Psychiatry*. 2006;59(7):582-7.
38. Corbo V, Clement MH, Armony JL, Pruessner JC, Brunet A. Size versus shape differences: contrasting voxel-based and volumetric analyses of the anterior cingulate cortex in individuals with acute posttraumatic stress disorder. *Biol Psychiatry*. 2005;58(2):119-24.
39. Phillips ML, Drevets WC, Rauch SL, Lane R. Neuobiology of emotion perception I: the neural basis of normal emotion perception. *Biol Psychiatry*. 2003;54(5):504-14.
40. Phan KL, Wagner T, Taylor SF, Liberzon I. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*. 2002;16(2):331-48.
41. Opitz B, Mecklinger A, Friederici AD. Functional asymmetry of human prefrontal cortex: encoding and retrievel of verbally and nonverbally coded information. *Learning and Memory*. 2000;7(2):85-96.
42. Reber PJ, Wong EC, Buxton RB. Encoding activity in the medial temporal lobe examined with anatomically constrained fMRI analysis. *Hippocampus*. 2002;12(3):363-76.
43. De Bellis MD, Keshavan MS. Sex differences in brain maturation in maltreatment-related pediatric posttraumatic stress disorder. *Neurosci Biobehav Rev*. 2003;27(1-2):103-17.
44. De Bellis MD, Kuchibhatla M. Cerebellar volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biol Psychiatry*. 2006;60(7):697-703.
45. De Bellis MD, Hall J, Boring AM, Frustaci K, Moritz G. A pilot longitudinal study of hippocampal volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biol Psychiatry.* 2001;50(4):305-9.
46. Thomas LA, De Bellis MD. Pituitary volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biol Psychiatry*. 2004;55(7):752-8.
47. Potenza MN. Impulsivity and compulsivity in pathological gambling and obsessive-compulsive disorder. *Rev Bras Psiquiatr.* 2007;29(2):105-6.
48. Saxena S, Rauch SL. Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. *Psychiatr Clin North Am*. 2000;23(3):563-86.
49. Choi JS, Kang DH, Kim JJ, Ha TH, Lee JM, Youn T, Kim IY, Kim SI, Kwon JS. Left anterior subregion of orbitofrontal cortex volume reduction and impaired organizational strategies in obsessive-compulsive disorder. *J Psychiatr Res*. 2004;38(2):193-9.
50. Kang DH, Kim JJ, Choi JS, Kim YI, Kim CW, Youn T, Han MH, Chang KH, Kwon JS. Volumetric investigation of the frontal-subcortical circuitry in patients with obsessive-compulsive disorder. *J Neuropsychiatry Clin Neurosci*. 2004;16(3):342-9.
51. Atmaca M, Yildirim H, Ozdemir H, Tezcan E, Poyraz AK. Volumetric MRI study of key brain regions implicated in obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31(1):46-52.
52. Atmaca M, Yildirim BH, Ozdemir BH, Aydin BA, Tezcan AE, Ozler AS. Volumetric MRI assessment of brain regions in patients with refractory obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006;30(6):1051-7.
53. Pujol J, Soriano-Mas C, Alonso P, Cardoner N, Menchon JM, Deus J, Vallejo J. Mapping structural brain alterations in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2004;61(7):720-30.
54. Valente AA Jr, Miguel EC, Castro CC, Amaro E Jr, Duran FL, Buchpiguel CA, Chitnis X, McGuire PK, Busatto GF. Regional gray matter abnormalities in obsessive-compulsive disorder: a voxel-based morphometry study. *Biol Psychiatry.* 2005;58(6):479-87.
55. Riffkin J, Yucel M, Maruff P, Wood SJ, Soulsby B, Olver J, Kyrios M, Velakoulis D, Pantelis C. A manual and automated MRI study of anterior cingulate and orbito-frontal cortices, and caudate nucleus in obsessive-compulsive disorder: comparison with healthy controls and patients with schizophrenia. *Psychiatry Res*. 2005;138(2):99-113.
56. Szeszko PR, MacMillan S, McMeniman M, Lorch E, Madden R, Ivey J, Banerjee SP, Moore GJ, Rosenberg DR. Amygdala volume reductions in pediatric patients with obsessive-compulsive disorder treated with paroxetine: preliminary findings. *Neuropsychopharmacology*. 2004;29(4):826-32.
57. MacMaster FP, Russell A, Mirza Y, Keshavan MS, Banerjee SP, Bhandari R, Boyd C, Lynch M, Rose M, Ivey J, Moore GJ, Rosenberg DR. Pituitary volume in pediatric obsessive-compulsive disorder. *Biol Psychiatry*. 2006;59(3):252-7.
58. Stein DJ, Andersen EW, Overo KF. Response of symptom dimensions in obsessive-compulsive disorder to treatment with citalopram or placebo. *Rev Bras Psiquiatr*. 2007;29(4):303-7.
59. Szeszko PR, MacMillan S, McMeniman M, Chen S, Baribault K, Lim KO, Ivey J, Rose M, Banerjee SP, Bhandari R, Moore GJ, Rosenberg DR. Brain structural abnormalities in psychotropic drug-naive pediatric patients with obsessive-compulsive disorder. *Am J Psychiatry*. 2004;161(6):1049-56.
60. Carmona S, Bassas N, Rovira M, Gispert JD, Soliva JC, Prado M, Tomas J, Bulbena A, Vilarroya O. Pediatric OCD structural brain deficits in conflict monitoring circuits: a voxel-based morphometry study. *Neurosci Lett*. 2007;421(3):218-23.
61. Kwon JS, Kim JJ, Lee DW, Lee JS, Lee DS, Kim MS, Lyoo IK, Cho MJ, Lee MC. Neural correlates of clinical symptoms and cognitive dysfunctions in obsessive-compulsive disorder. *Psychiatry Res*. 2003;122(1):37-47.
62. Graeff FG. Anxiety, panic and the hypothalamic-pituitary-adrenal axis. *Rev Bras Psiquiatr*. 2007;29(Suppl 1):S3-6.
63. Bystritsky A, Pontillo D, Powers M, Sabb FW, Craske MG, Bookheimer SY. Functional MRI changes during panic anticipation and imagery exposure. *Neuroreport*. 2001;12(18):3953-7.
64. Ontiveros A, Fontaine R, Breton G, Elie R, Fontaine S, Déry R. Correlation of severity of panic disorder and neuroanatomical changes on magnetic resonance imaging. *J Neuropsychiatry Clin Neurosci*. 1989;1(4):404-8.

65. Fontaine R, Breton G, Déry R, Fontaine, Elie R. Temporal lobe abnormalities in panic disorder: an MRI study. *Biol Psychiatry*. 1990;27(3):304-10.
66. Vythilingam M, Anderson ER, Goddard A, Woods SW, Staib LH, Charney DS, Bremner JD. Temporal lobe volume in panic disorder - a quantitative magnetic resonance imaging study. *Psychiatry Res*. 2000;99(2):75-82.
67. Massana J, Mercader JM, Gómez B, Tobeña A, Salamero M. Amygdalar atrophy in panic disorder patients detected by volumetric magnetic resonance imaging. *Neuroimage*. 2003;19(1):80-90.
68. Uchida RR, Del-Ben CM, Santos AC, Araújo D, Crippa JA, Guimarães FS, Graeff FG. Decreased left temporal lobe volume of panic patients measured by magnetic resonance imaging. *Braz J Med Biol Res*. 2003;36(7):925-9.
69. Gray JA, McNaughton N. *The Neuropsychology of Anxiety. An Enquiry into the Functions of the Septo-hipocampal System*. 2nd ed. Oxford: Oxford University Press; 2000.
70. Dantendorfer K, Prayer D, Kramer J, Amering M, Baischer W, Berger P, Schoder M, Steinberger K, Windhaber J, Imhof H, Katschnig H. High frequency of EEG and MRI brain abnormalities in panic disorder. *Psychiatry Res*. 1996;68(1):41-53.
71. Massana G, Serra-Grabulosa JM, Salgado-Pineda P, Gastó C, Junqué C, Massana J, Mercader JM. Parahippocampal gray matter density in panic disorder: a voxel-based morphometric study. *Am J Psychiatry*. 2003;160(3):566-8.
72. Yoo HK, Kim MJ, Kim SJ, Sung YH, Sim ME, Lee YS, Song SY, Kee BS, Lyoo IK. Putaminal gray matter volume decrease in panic disorder: an optimized voxel-based morphometry study. *Eur J Neurosci.* 2005;22(8):2089-94.
73. Protopopescu X, Pan H, Tuescher O, Cloitre M, Goldstein M, Engelien A, Yang Y, Gorman J, LeDoux J, Stern E, Silbersweig D. Increased brainstem volume in panic disorder: a voxel-based morphometric study. *Neuroreport*. 2006;17(4):361-3.
74. Uchida RR, Del-Ben CM, Busatto GF, Duran FL, Guimarães FS, Crippa JA, Araújo D, Santos AC, Graeff FG. Regional gray matter abnormalities in panic disorder: A voxel-based morphometry study. *Psychiatry Res.* 2008;163(1):21-9.
75. Allgulander C. What our patients want and need to know about generalized anxiety disorder. *Rev Bras Psiquiatr.* 2007;29(2):172-6.
76. Jetty PV, Charney DS, Goddard AW. Neurobiology of generalized anxiety disorder. *Psychiatr Clin North Am*. 2001;24(1):75-97.
77. De Bellis MD, Casey BJ, Dahl RE, Birmaher B, Williamson DE, Thomas KM, Axelson DA, Frustaci K, Boring AM, Hall J, Ryan ND. A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol Psychiatry.* 2000;48(1):51-7.
78. De Bellis MD, Keshavan MS, Shifflett H, Iyengar S, Dahl RE, Axelson DA, Birmaher B, Hall J, Moritz G, Ryan ND. Superior temporal gyrus volumes in pediatric generalized anxiety disorder. *Biol Psychiatry*. 2002;51(7):553-62.
79. Quirk GJ, Armony JL, LeDoux JE. Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. *Neuron*. 1997;19(3):613-24.
80. Milham MP, Nugent AC, Drevets WC, Dickstein DP, Leibenluft E, Ernst M, Charney D, Pine DS. Selective reduction in amygdala volume in pediatric anxiety disorders: a voxel-based morphometry investigation. *Biol Psychiatry*. 2005;57(9):961-6.
81. Bressan RA, Shih MC, Hoexter MQ, Lacerda AL. Can molecular imaging techniques identify biomarkers for neuropsychiatric disorders? *Rev Bras Psiquiatr*. 2007;29(2):102-4.
82. Zald DH, Kim SW. Anatomy and function of the orbital frontal cortex, I: anatomy, neurocircuitry; and obsessive-compulsive disorder. *J Neuropsychiatry Clin Neurosci*. 1996;8(2):125-38.
83. Uchida RR, Del-Ben CM, Araújo D, Busatto-Filho G, Duran FL, Crippa JA, Graeff FG. Correlation between voxel based morphometry and manual volumetry in magnetic resonance images of the human brain. *An Acad Bras Cienc*. 2008;80(1):149-56.
84. Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. *Am J Psychiatry*. 2000;157(4):493-505.
85. Potts NL, Davidson JR, Krishnan KR, Doraiswamy PM. Magnetic resonance imaging in social phobia. *Psychiatry Res*. 1994;52(1):35-42.
86. Crippa JA, Loureiro SR, Baptista CA, Osório F. Are there differences between early- and late-onset social anxiety disorder? *Rev Bras Psiquiatr*. 2007;29(2):195-6.
87. Caetano SC, Hatch JP, Brambilla P, Sassi RB, Nicoletti M, Mallinger AG, Frank E, Kupfer DJ, Keshavan MS, Soares JC. Anatomical MRI study of hippocampus and amygdala in patients with current and remitted major depression. *Psychiatry Res*. 2004;132(2):141-7.
88. Nopoulos P, Flaum M, O'Leary D, Andreasen NC. Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. *Psychiatry Res*. 2000;98(1):1-13.
89. Mataix-Cols D, Wooderson S, Lawrence N, Brammer MJ, Speckens A, Phillips ML. Distinct neural correlates of washing, checking, and hoarding symptom dimensions in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2004;61(6):564-76.
90. Hulshoff Pol HE, Schnack HG, Mandl RC, van Haren NE, Koning H, Collins DL, Evans AC, Kahn RS. Focal gray matter density changes in schizophrenia. *Arch Gen Psychiatry*. 2001;58(12):1118-25.
91. Baxter LR Jr, Schwartz JM, Bergman KS, Szuba MP, Guze BH, Mazziotta JC, Alazraki A, Selin CE, Ferng HK, Munford P. Caudate glucose metabolic rate changes with both drug and behavior therapy for obsessive-compulsive disorder. *Arch Gen Psychiatry*. 1992;49(9):681-9.
92. Rauch SL, Jenike MA, Alpert NM, Baer L, Breiter HC, Savage CR, Fischman AJ. Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. *Arch Gen Psychiatry*. 1994;51(1):62-70.
93. Kim JJ, Lee MC, Kim J, Kim IY, Kim SI, Han MH, Chang KH, Kwon JS. Grey matter abnormalities in obsessive-compulsive disorder: statistical parametric mapping of segmented magnetic resonance images. *Br J Psychiatry*. 2001;179:330-4.
94. Malhi GS, Lagopoulos J. Making sense of neuroimaging in psychiatry. *Acta Psychiatr Scand*. 2008;117(2):100-17.