STRUCTURAL NEUROIMAGING IN PATIENTS WITH PANIC DISORDER: FINDINGS AND LIMITATIONS OF RECENT STUDIES

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SUMMARY

*   **Background:** Panic disorder, a relatively common anxiety disorder, is often associated to agoraphobia and may be disabling. Its neurobiological underpinnings are unknown, despite the proliferation of models and hypotheses concerning it; investigating its correlates could provide the means for better understanding its pathophysiology. Recent structural neuroimaging techniques may contribute to the identification of possible brain morphological alterations that could be possibly related to the clinical expression of panic disorder.*

*   **Methods:** Through careful major database searches, using terms keen to panic, agoraphobia, structural magnetic neuroimaging and the like, we identified papers published in peer-review journals and reporting data on the brain structure of patients with panic disorder. Included papers were used comparatively to speculate about the nature of reported brain structural alterations.*

*   **Results:** Anxiety, which is the core feature of the disorder, correlates with the function of the amygdala, which showed a smaller volume in patients, as compared to healthy subjects. Data also showed a volumetric decrease of the anterior cingulate along with increased fractional anisotropy, and increase of some brainstem nuclei, particularly of the rostral pons. Other structures with reported volumetric correlates of panic disorder are the hippocampus and the parahippocampal cortices, the insula, the putamen, and the pituitary gland. Volumetric changes in the anterior cingulate, frontal, orbitofrontal, insular, and temporal cortices have also been described in structural neuroimaging studies. Major methodological limitations are considered in context.*

*   **Conclusions:** Several data point to the existence of structural neuroanatomical alterations in panic disorder, consisting in significant volumetric reductions or increases in different brain areas. White matter alterations were shown also in the only diffusion tensor imaging study performed to date. Available data do not allow us to conclude about the possible progression of these alterations.*

*   **Key words:** panic attack disorder – neuroimaging - magnetic resonance imaging - computed tomography - voxel-based morphometry - diffusion tensor imaging*

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INTRODUCTION

The diagnosis of panic disorder (PD) requires the manifestation of recurrent panic attacks, along with any of the following: worrying about possible future attacks, development of phobic avoidance, or other behavioural changes as a consequence of the attacks. Panic attacks are sudden; consist of unexpected, paroxystic, severe anxiety, characterized by fear of dying, going crazy or losing control. The attacks are accompanied by several physical symptoms, often cardiorespiratory, otoneurological, gastrointestinal, or autonomic (APA 2000). Intense fatigue, a frequent feature representing an end-result of the attack, is not part of current diagnostic criteria; nevertheless it accompanies disorders of autonomic arousal and gloomy anticipation of future (catastrophic cognitions). These attacks are very remarkable in their first onset, negatively influence the personal functioning, and may be progressive and disabling, particularly if complicated by agoraphobia (Roy-Byrne et al. 2006).

The estimated lifetime prevalence of PD is about 4.7% (Kessler et al. 2005).

Recent structural neuroimaging techniques enable the analysis of brain morphological alterations involved in the pathophysiology of this disorder.

Due to the intense and comprehensive nature of panic symptoms, different subcortical regions, including the amygdala, the hippocampus and parahippocampal gyri, brainstem nuclei, and other areas, have been suggested as plausible pathophysiological candidate sites for people affected by PD. Other cortical areas, including the orbitofrontal and temporal cortices, can also be involved in the pathophysiology of this disorder. This paper aims to review the major morphological changes of the brain in patients with PD, as reported by specifically focused structural neuroimaging studies.

METHODS

We searched the PubMed/Medline/Index Medicus, PsycInfo and Embase/Excerpta Medica databases using the terms "panic disorder" in combination with "magnetic resonance imaging", "MRI", "computed tomography", "TC", "voxel based morphometry", "VBM", "diffusion tensor imaging", "DTI", and "neuroimaging".

The search yielded 326 papers in PubMed, 154 in PsycInfo, and 470 in Embase, as of November 30, 2012. Further papers were searched in the reference lists of reviews and research papers considered. Included were papers dealing with structural neuroimaging in patients with PD. Papers were considered for inclusion only if they were published in peer-reviewed journals. We included only those satisfying standards for adequate methodology and inclusion criteria. Studies with inadequate methodology (method unspecified and/or inadequately described) were excluded. We also excluded papers that did not include a group of healthy control subjects in their analyses. Most of the included studies were published in the last 10 years. Since different methodologies, procedures and designs were adopted in the various studies, we decided not to carry out a meta-analysis, which would have resulted in the exclusion of a relative large number of papers, so we have performed a critical overview of all included studies.

RESULTS

Volumetric changes of the amygdala and other subcortical structures

Amygdala is central in mediating stress response, and this is relevant to all stress-related disorders, including phobia and anxiety. Gorman et al. (2000) proposed the existence of a 'fear network' in the brain, with the amygdala at its centre, and its reciprocal interactions with the hippocampus, the temporal lobe, and the medial prefrontal cortex.

Using volumetric Magnetic Resonance Imaging (MRI), Massana et al. (2003a) analysed the volume of the amygdala, hippocampus, and temporal lobes in 12 drug-free, symptomatic patients with PD and 12 case-matched healthy controls (HC). Volumetric MRI data were normalized for brain size. Compared to HC, PD patients were found to have smaller bilateral amygdalar volumes, but did not differ for hippocampal and temporal lobe volumes. Although the sample size in this study was relatively small, patients were drug-free and with no other past or current comorbidities; sample homogeneity allowed for conclusions to be more valid. A methodological limitation is in the morphometric analyses, conducted in this study using a manual Regions of Interest (ROI) tracing, which may not offer the precision of other stereological methods based on systematic sampling (Geinisman et al. 1996, Gundersen et al. 1999, Roberts et al. 2000) or other more recent automated methods (Morey et al. 2009). These latter methods can be more accurate and efficient for the volumetric assessment of those regions that do not have clearly defined boundaries, as is the case of the posterior part of the amygdala that partly covers the anterior component of the hippocampal head (Duvernoy 1991). The same group of authors (Massana et al. 2003b) studied grey matter concentration in 18 outpatients with

PD and 18 HC, and compared them by using MRI with voxel-based morphometry (VBM). They found grey matter density of the left parahippocampal gyrus to be significantly lower in patients, without significant differences in other brain regions.

A recent voxel-based meta-analysis by Lai (2011) considered six data-sets of PD patients and HC with the aim to explore deficits of grey matter volume in PD patients compared to the control group, reported in patients decreased regional grey matter volumes in right caudate head and right parahippocampal gyrus. Meta-regression data from this study showed that symptom severity directly correlated with grey matter volume deficits in the right basal ganglion (Lai 2011). This meta-analysis has different limitations. Only six studies were included, and all regarded whole-brain VBM studies; this might limit the interpretations of results. In addition, this meta-analysis did not use raw data, which would have been more accurate, but only summarized data using coordinates (Lai 2011). Other limitations derived from having inherited the same limitations of the enrolled studies.

Hayano et al. (2009), in a VBM MRI study involving 27 patients affected by PD and 30 HC, showed that bilateral volumes of the amygdala, but not of the hippocampus, were decreased in patients. Furthermore, the authors of this study reported a significant inverse correlation between the volume of the left amygdala and the score for trait- and state-anxiety in patients with PD, as compared to HC. The main limitation of this study was in the group of patients that comprised three subjects with a past history of major depressive disorder, which could have affected the reported results.

In addition to the amygdala, hippocampus, and parahippocampal gyrus, other subcortical structures showed volumetric changes in patients with PD, as reported by some studies.

Yoo et al. (2005) found decreased bilateral putaminal grey matter volume comparing 18 patients affected by PD and 18 HC undergoing MRI with optimized voxel-based approach. Limitations of this study may include a relatively small sample size of patients and HC, and a role of prior or current panic medications, which may have influenced the study results.

Uchida et al. (2008), in a VBM study involving 19 PD patients and 20 HC, emphasized the importance of specific subcortical structures in the PD pathophysiology, showing a significant increase in the grey matter volume of the left insula, midbrain and pons in the group of patients. This study failed in detecting amygdalar morphologic alterations. Another MRI study conducted in 17 PD inpatients and 17 matched HC by Sobanski et al. (2010), reported no morphological alterations in the amygdala-hippocampus complex. This study considered the sum of the volumes of the amygdala and hippocampus, and this may have obscured possible differences that could exist in the amygdala and hippocampus if considered separately.

The brainstem, which includes the ascending reticular system and the respiratory and cardiovascular control centres, has been explored by Protopopescu et al. (2006). Subjecting 10 PD patients and 23 HC to VBM MRI, the authors showed that PD patients had a relatively increased grey matter volume in the midbrain and rostral pons of the brainstem, thus partially confirming the results of the previously mentioned study by Uchida and colleagues (2008).

Several neuroendocrinological data indicate that, even if not directly by panic attacks, the hypothalamic-pituitary-adrenal (HPA) axis is activated by anticipatory anxiety (see Graeff & Del-Ben 2008). Kartalci et al. (2011) evaluated pituitary gland volume in 27 patients with PD and 27 age- and gender-matched HC, reporting that PD patients had significantly smaller pituitary volumes. In particular, patients with agoraphobia had a significantly smaller pituitary volume than patients without agoraphobia. Significant associations were detected in the PD group between pituitary volume on one hand, and both symptom severity and illness duration on the other. This study was limited by small sample size; in addition, the authors were unable to determine whether reduced pituitary volume had any functional consequences in patients with PD (i.e., no pituitary hormone measurement, no functional neuroimaging data integration).

Volumetric changes of cortical areas in patients with PD

The pathophysiology of PD is also related to cortical volume abnormalities. The amygdala has several connections with a region of the anterior cingulate cortex (ACC) known as "the affective division" (Devinsky et al. 1995), and recent structural neuro-imaging studies have suggested that this region can play an important role in the pathophysiology of PD (Asami et al. 2008, Han et al. 2008).

The MRI study by Asami et al. (2008), performed on 26 patients with PD and 26 age and gender-matched HC with a combination of ROI and optimized VBM methods, showed significant volume reductions in the right dorsal and rostral ACC in patients. Other changes in the cingulate cortices were reported in the only MRI study with a diffusion tensor imaging (DTI) technique by Han et al. (2008), conducted on patients affected by PD. DTI is a relatively novel MRI-based technique for mapping diffusion properties of water in a constrained compartment, which can be used to measure micro-structural characteristics of the brain. It shows the random distribution of water molecules, which is usually quantified as the mean diffusivity (MD) and fractional anisotropy (FA). The MD is a measure of randomized mean water diffusion (Basser & Pierpaoli 1996).

Table 1a. Structural neuroimaging studies of panic disorder

Study	Technique	Age Group	Participants
Fontaine et al. 1990	MRI	PD: 31.1 y HC: 32.9 y	31 patients with PD and 20 HC
Lai 2011	Voxel-based meta-analysis	PD: 35.5 y HC: 34 y	106 patients with PD and 120 HC
Asami et al. 2008	Regions-of-interest (ROI) and optimized VBM	PD: 37.7 y HC: 38.2 y	26 patients with PD and 26 age and sex-matched HC
Han et al. 2008	Diffusion Tensor Imaging	PD: 31.9 y HC: 30.6 y	24 PD patients with 24 age and sex-matched HC
Hayano et al. 2009	ROI and optimized VBM	38.2 y	27 panic subjects and 30 healthy volunteers
Kartalcy et al. 2011	MRI: 3-D fast field echo (FFE) T1-weighted data set. coronal plane with 1.5 mm contiguous sections	PD: 35.1 y HC: 33.7y	27 patients with PD and 27 age- and gender-matched HC
Massana et al. 2003a	MRI: ROI approach	PD: 35.33 y HC: 37.33 y	12 patients with PD and 12 age- and gender-matched HC
Massana et al. 2003b	VBM	PD: 36.7 y	18 panic disorder outpatients and 18 HC
Protopopescu et al. 2006	VBM	PD: 35.5 y HC: 28.7 y	10 PD patients and 23 HC
Rappongi et al. 2010	3-D high-spatial resolution MRI and VBM	PD: 38.4 y HC: 37.8 y	28 patients with PD and 28 age- and gender-matched HC
Sobanski et al. 2010	VBM	34.9 y	17 patients with PAD and 17 age and sex-matched HC
Uchida et al. 2008	VBM	PD:37.05 y HC:36.45 y	19 PD patients and 20 healthy volunteers
Yoo et al. 2005	optimized VBM	PD: 33.3 y HC: 32.0 y	18 panic subjects and 18 healthy volunteers

Table 1b. Structural neuroimaging studies of panic disorder

Study	General findings	Limitations
Fontaine et al. 1990	PD patients showed focal abnormalities in the right temporal area.	Not mentioned.
Lai 2011	Patients with PD had decreased regional gray matter volumes in right caudate head and right parahippocampal gyrus. Patients with more severe PD showed more gray matter volume deficits in the right basal ganglion	Only six studies were included in this meta-analysis, which comprised only whole-brain VBM studies, thus excluding all studies published using a ROI approach.
Asami et al. 2008	Significant volume reductions in the right dorsal and the rostral ACC in PD patients, compared to HC.	Not mentioned.
Han et al. 2008	Greater FA values in left anterior and right posterior cingulate regions in the patients.	Relatively small sample size. Furthermore, as all PAD subjects were stabilized on medications, this conceivably could have influenced the results. Moreover, associations between duration of treatment, dosage or category of anti-panic medications and FA findings were not assessed. In addition, other confounders, like the clinical and demographic characteristics of the study sample, were not adequately addressed.
Hayano et al. 2009	Showed that bilateral volumes of the amygdala, but not of the hippocampus, were decreased in PD. Significant negative correlation between the volume of the left amygdala and the score for trait and state anxiety in PD.	There were two methodological limitations in this study. First, the sample size for subjects in both groups was not large enough. Second the authors included three subjects with a past history of major depression, therefore, it is possible that this might have affected brain volume.
Kartalcy et al. 2011	PD had significantly smaller pituitary volumes, compared to HC.	Small sample size; furthermore, the authors were unable to determine whether reduced pituitary volume had any functional consequences in patients with PD, because they did not measure pituitary hormones.
Massana et al. 2003a	PD patients were found to have smaller amygdalar volumes than controls, bilaterally. No differences were found in either hippocampus or temporal lobes.	Morphometric analyses were conducted using edge tracing that does not offer the precision that unbiased stereological volumetric determination of volumes allows for. Hence, boundaries of the amygdala, but also of the hippocampus, which were used in volumetric studies, have varied considerably.
Massana et al. 2003b	Matter density of the left parahippocampal gyrus to be significantly lower in patients with panic disorder.	Not mentioned.
Protopopescu et al. 2006	Relatively increased gray matter volume in the midbrain and rostral pons of the brainstem.	Not mentioned.
Rappongi et al. 2010	No significant difference was in POS subtype distribution between the two groups. VBM, however, indicated volume reduction in the right posterior–medial OFC region in PD patients with absent and single POS.	Comorbid depression present in some patients with PD in this study may have affected the anatomical and clinical features. Henceforth, it is necessary to investigate differences between PD patients with and without major depression/dysthymia. VBM studies are needed with larger samples and statistically rigorous threshold Family-Wise Error (FWE)-correction
Sobanski et al. 2010	Bilateral reduction in temporal lobe and in the right frontal lobe volume in patients with PD compared to HC. The Amygdala-Hippocampus Complex showed no between-group differences. Significant gray matter volume reduction in the right middle temporal gyrus and in the medial part of the OFC.	The structures of the amygdala and hippocampus could not be assessed separately and were combined as the Amygdala-Hippocampus Complex.
Uchida et al. 2008	No differences in gray matter volume of the amygdala, hippocampus, thalamus, and hypothalamus. Instead, the authors showed a significant increase in the gray matter volume of the left insula, midbrain and pons in PD patients, compared with HC.	Not mentioned.
Yoo et al. 2005	Decreased bilateral putaminal gray matter volume in patients with panic disorder.	Relatively small sample size and the history of prior or current panic medications that may have influenced the findings

ACC= anterior cingulate cortex; ROI=regions of interest; VBM= voxel based morphometry; PD= Panic attack disease; HC=Healthy controls; OFC=Orbitofrontal cortex; POS=poste

Neurodegenerative processes are often correlated with a progressive failure of the barrier that restricts water molecule distribution in cellular compartments, and DTI sensitively shows degeneration of neural tissue through pathologically elevated MD (Basser & Pierpaoli 1996). In tissues with highly structured organisation, including, for example, the bundles of myelinated fibres, the motion of water molecules is mostly restricted, with a preferential anisotropic diffusion along the axial fibre direction. The fractional anisotropy (FA) is defined as the degree of this motion directionality and is used as a main index of white matter integrity (Pierpaoli et al. 1996). Using DTI with a ROI-based technique to evaluate FA, Han et al. (2008) studied ACC white matter coherence and integrity in PD patients. They compared 24 PD patients with 24 age- and gender-matched HC, reporting greater FA values in left anterior and right posterior cingulate regions in patients. This study was limited by a relatively small sample size. Furthermore, all PD subjects were stabilised on medications, which conceivably could have influenced the study results. The associations between duration of treatment, dosage or category of anti-panic medications and FA findings were not assessed, and other possible confounders, including the clinical and demographic characteristics of the study sample, were not completely addressed.

Another cortical area studied in PD for its reciprocal connections with the amygdala is the orbitofrontal cortex (OFC). Roppongi et al. (2010) focused their attention on the posterior OFC region, exploring the anatomy of sulcogyral pattern formation during neurodevelopment. The authors, using 3-D high-spatial resolution MRI data from 28 patients with PD and 28 age- and gender-matched HC, classified the anatomical pattern of the posterior orbital sulcus (POS) in three categories, according to the presence/absence and number of POS in the OFC: absent, single, or double POS. They reported no significant difference in POS subtype distribution between the two groups. VBM data showed significant volume reduction in the right posterior-medial OFC region in PD patients with absent and single POS, suggesting that these volumetric changes in PD may be neurodevelopment-related. These findings could have been influenced by some comorbidities (agoraphobia, current or past major depression, previous dysthymia) diagnosed in the patient group. The same authors suggested investigating differences between PD patients with and without major depression/dysthymia (Roppongi et al. 2010).

Two studies reported changes in the temporal lobes. The first was a pioneering MRI study by Fontaine et al. (1990), conducted in 31 patients with a diagnosis of PD and 20 HC. This study showed areas of abnormal signal activity, and asymmetric atrophy of the temporal lobe, mostly right-sided. Its main limitation was that all patients received clonazepam and executed the MRI when the panic symptoms and phobic behaviours had significantly improved (Fontaine et al. 1990).

The second was a recent MRI study by Sobanski et al. (2010), which aimed to explore the 'fear network', focusing on the temporal lobe morphology in patients with PD. They subjected 17 PD inpatients and 17 HC matched for age and gender to quantitative VBM MRI to study the temporal and frontal lobes, and the amygdala-hippocampus complex. This study reported bilateral volumetric reduction of the temporal and right frontal lobes in patients with PD compared to HC. Using VBM, the authors showed significant grey matter volume reduction in the right middle temporal gyrus and in the medial part of the OFC in the group of patients.

DISCUSSION

Structural neuroimaging data and neurobiology of panic disorder

Structural neuroimaging data in PD showed changes in limbic structures, in frontal and temporal cortical areas, in the basal ganglia, and in the brainstem structures.

The presence of neuro-mophological alterations in these areas are matched by the presence of neuro-chemical alterations in the same regions. In fact, studies on γ-aminobutyric acid (GABA) showed altered GABA receptor binding in limbic (Hasler et al. 2008) and insular regions (Cameron et al. 2007) in PD patients; spectroscopic studies revealed GABAergic transmission also in fronto-temporal areas (Nikolaus et al. 2010). Alterations in the serotonin 1A receptor (5-HT$_{1A}$) binding capacity were found in ACC and in the basal ganglia (Neumeister et al. 2004); reduced binding properties for the serotonin 5-HT$_{1A}$ receptor were also evident in the amygdala, in the hippocampus, and in the frontal and temporal cortical areas (Nash et al. 2008).

Exploring cerebral metabolism in PD with single photon emission computed tomography (SPECT), some authors found changes in hippocampal (Sakai et al. 2005) and parahippocampal areas (Koh et al. 2010), as well as alterations in amygdalar metabolism (Sakai et al. 2005).

Furthermore, recent studies performed with functional MRI (fMRI) found increased activation in fear system structures like the amygdala, the insula, and the hippocampus (Wittmann et al. 2011, Pfleiderer et al. 2007). As regards the cortical structures, PD patients exhibited increased activation in the right inferior frontal area, the ACC and in the posterior cingulate cortex (PCC) (Bystritsky et al. 2001). These results lend support to the findings of structural studies.

Limitations

The fact that the available studies were methodologically heterogeneous induced us to avoid performing a meta-analysis. An available meta-analysis (Lai 2011) excluded a huge number of otherwise remarkable papers, which was not our intention and which would have led us to correct, but only partial

conclusions. We chose instead to include studies adopting different methodologies, procedures, and designs to weight all possible available evidence. Another problem is to inherit all the limitations of the included studies. Many of them had a relatively small sample size of patients; comorbid depression or other disorders may have affected the results; the role of medications (type of molecule, dosage, drug associations, and duration of treatment) may have influenced the results. Furthermore, in some studies, these issues were not completely addressed. Only one study considered the effective connectivity between brain areas, using the DTI technique, so that the reported DTI data need further confirmation. However, this is a recently introduced technique, so we are confident that in the near future many a study will appear using this technique.

CONCLUSIONS AND PERSPECTIVES

The biological basis of PD remains unknown, despite the fact that a large number of hypotheses have been proposed. In the puzzling task of identifying the exact mechanisms underlying its pathophysiology, one of the main current lines of research focuses on brain circuitry.

This paper underlines several important points about the brain changes underlying PD:

- Anxiety, which is the core feature of PD, is linked to the function of the amygdala, that often showed a smaller volume in patients with PD as compared to HC;
- The hippocampus and particularly the left parahippocampal gyrus can be involved in the pathophysiology of PD, as several data showed volume reductions in patients;
- The brainstem nuclei, which is involved in the autonomic arousal circuitry, can have an increased volume, mainly in the rostral pons;
- The pituitary gland could have a role, as it showed in a study a volume reduction, especially in patients with agoraphobia;
- The main cortical areas involved in the pathophysiology of PD are the ACC, with reported smaller volume and greater FA in patients with PD compared to HC, the OFC, and the temporal and frontal cortices, with reported smaller volume in PD patients.
- The available data do not allow for assessing whether alterations are progressive, nor whether they are amenable to or preventable by treatment. Future studies should focus on relationships between measures like illness duration and time to diagnosis or time to treatment and the extent of alterations.

Future improvements in neuroimaging techniques may help to better understand the neurocircuitry underlying PD and the differences between PD and other anxiety disorders.

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