



Current methodological designs of fMRI studies of panic disorder: Can data be compared?

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Abstract

Panic disorder (PD) is a pluridimensional condition that leads to psychological suffering. Due to advances in neuroimaging techniques, important contributions have been made in the understanding of the neurobiological basis of PD. However, because of diverging research designs and protocols, more conclusive data concerning the neurocircuitry of PD remain difficult to achieve. To address this issue, a bibliographical search was performed using the Institute for Scientific Information Web of Science and Medline/PubMed databases. Fifteen articles were found, and their research methodology including sample, comorbidity, gender, and pharmacological criteria were explored. Although current functional magnetic resonance imaging studies of PD constitute fundamental tools for health sciences, more uniform research protocols must be implemented to provide more consistent and conclusive data concerning the neural substrates of PD. **Keywords:** functional magnetic resonance imaging, panic disorder, methodology, research design criteria.

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Introduction

Panic disorder (PD) is an incapacitating psychiatric condition characterized by recurrent and unexpected panic attacks (PAs), fear of new attacks and their consequences, and important behavioral alterations in an attempt to avoid new PAs (American Psychiatric Association, 2000). Once initiated, the PA is characterized by a growing series of autonomic symptoms such as palpitations, dyspnea, sweating, trembling, suffocation, increased heart rate, and dizziness, often lasting from 10 to 15 min. A strong fear of dying or losing control is often present. Panic disorder has a chronic course and represents a debilitating condition for the patient,

leading to severe social costs as a consequence of absenteeism and medical expenses (Ballenger, 1989).

The biological basis of PD has yet to be totally elucidated, but it continues to be studied and improved. Indeed, the neurobiology of PD has assumed a central position in neuroscience studies and in recent years has been the subject of exhaustive research (Bourin, Baker, & Bradwejn, 1998). The field of neuroscience has received important contributions in the form of new technologies including functional magnetic resonance imaging (fMRI) in the pursuit of elucidating diverse issues related to neural circuits, especially those concerning fear and anxiety (De Carvalho et al., 2010). Functional MRI presents significant methodological advantages. It shows real-time changes in brain functioning during noninvasive cognitive and behavioral tasks (Rocha, Alves, Garridoc, Buchpiguel, Nitrid, & Filho, 2001), allowing researchers to identify brain regions that mediate the studied phenomena at the very moment they occur. Concerning neurobiological research on PD, much attention has been given to elucidating the so-called fear neurocircuitry, a complex neural network that involves different brain structures including deeper subcortical and brainstem structures such as the amygdala (Gorman, Kent, Sullivan, & Coplan, 2000; Massana et al., 2003a), parahippocampal gyrus (Massana et al.,

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2003b), midbrain, pons, and left insula (Gorman et al., 2000; Uchida et al., 2008). Although human cortical complexity has been proposed to be the seat of anxiety disorders (Berkowitz, Coplan, Reddy, & Gorman, 2007), noncortical regions also appear to play major roles in the etiology and development of PD, making fMRI an appropriate neuroimaging approach because it provides anatomical resolution that distinguishes between small and deep structures. Additionally, functional studies allow brain functioning assessment in response to different sensorial stimuli and during cognitive and affective tasks (Amaro & Yamashita, 2001), reasons why this technique has been widely used in recent studies seeking to unravel how the brain works and what characterizes anxious responses in terms of neurobiology.

Although functional neuroimaging studies represent outstanding contributions to the understanding of mental disorders such as PD, they use a wide range of research criteria making data comparisons difficult for researchers and may be one of the reasons why no consensus has been reached regarding more conclusive models of the neurobiological substrates of specific disorders. To further investigate the current research criteria used in fMRI studies of PD, a bibliographic search was performed using the Institute for Scientific Information Web of Science and Medline/PubMed databases. Fifteen articles were selected, and their research methodology components such as laterality, sample, comorbidity, gender, and pharmacological criteria were described.

Methods

A bibliographic search was performed using the Institute for Scientific Information Web of Science and Medline/PubMed databases to collect studies of PD and the functional investigation of its neurocircuitry using fMRI. We used keywords “fMRI” and “panic disorder.” Only original articles written in English and published during the last 10 years were selected. Articles about regional lesions, epilepsy, and panic attack challenges in healthy subjects were excluded. Fifteen articles were selected. Two of these articles refer to fMRI genetic studies of PD.

Results are organized in Table 1 (subjects [*n*], gender, mean age, presence of agoraphobia, other comorbidities, exclusion criteria, pharmacological treatment) and Table 2 (clinical design, type of stimulus, psychological assessment [scales used], objective measures of anxiety, scanner type, image processing, statistical analysis). The following items are described and discussed: sample, gender, laterality criteria, agoraphobia, other comorbidities, stimulus modalities, and psychological evaluation. Considerations of imaging genomics are included at the end of the Results section.

Results

Study samples

Most fMRI studies used a small number of patients in their investigation. Sample sizes of the collected studies varied from 1 (case studies) to 20. Although pioneering and highly relevant, the current studies' samples remain to be considered representative of the population of PD. Because increasingly more studies of PD are being conducted using fMRI, increased sample sizes are expected in future investigations. Notably, sample sizes need to not only be increased but also need to be homogeneous with regard to both demographics (e.g., economic status, ethnicity) and clinical factors (e.g., past and current medical history).

Controlling these variables may be an important step toward more direct comparisons among studies in the pursuit of identifying the neurocircuitry of PD. Conducting cross-cultural fMRI studies of PD might also lead to a deeper understanding of the cultural aspects that contribute to the etiology and development of this disorder.

Gender

As discussed in a recent article, gender may play an important role in the neural activation of emotional processing (Ohrmann et al., 2010). Women display stronger activation of the insula, pre-central gyrus, middle cingulate cortex, left middle temporal gyrus, occipital gyri, and caudate in response to fearful facial stimuli compared with men. In women, significant activation of the bilateral amygdala was observed in response to fearful faces but, in men, no significant activation of the amygdala was observed (Ohrmann et al., 2010). Not all studies selected in the present systematic review balanced the sample with regard to gender.

Interestingly, a recent study demonstrated that women with Social Anxiety Disorder required less emotional intensity to recognize faces that expressed fear, sadness, and happiness on a computer screen, suggesting gender differences with regard to the sensitivity of evaluating threat- and approval-related social cues (Arrais et al., 2010). Because PD is also an anxiety disorder, men and women with PD may also respond differently to anxiogenic stimuli, an issue that must be addressed by future studies.

Laterality criteria

Handedness is one example of behavioral lateralization. Some studies of language, memory, and visuospatial processing demonstrated that handedness may be linked to cerebral dominance (Cuzzocreo, Yassa, Verduzco, Honeycutt, Scott, & Bassett, 2009). Studies have shown marked differences in the neural localization of mental function in the brains of left-

handed compared with right-handed individuals (Cuzzocreo et al., 2009). Some studies have indicated that these mental functions are not necessarily related to handedness and that hemispheric lateralization could vary with material- or task-specific paradigms. Hemispheric specialization can be specific for particular tasks, and a wide variety of mental functions could be successfully processed by both hemispheres depending on their differences in processing efficacy (Gazzaniga, Ivry, & Mangun, 2002).

Considering language as an example, left hemisphere dominance is not strongly related to handedness dominance. Approximately 50% of all left-handed subjects have left hemisphere dominance for language, although they are only 7% to 8% of the general population. Therefore, more than 96% of humans have left hemisphere dominance for language (Gazzaniga et al., 2002).

The decision to choose right-handed subjects to compose the sample for fMRI studies is based on minimizing possible confounds with regard to lateralization of some brain functions and the resulting brain activation, although this is not always true. Specifically, in right-handers, the correlation between cerebral dominance and handedness is much higher.

Importantly, fMRI studies should use objective measures that help determine handedness. Although most studies suggest that a sample inclusion criterion must be that only right-handed subjects are selected, only two articles reported having used an objective measure to assess handedness. The authors used the Edinburgh Handedness Inventory (EHI) to select right-handed individuals for their study.

Agoraphobia

Agoraphobia is a common PD comorbidity. From 25% to 50% of individuals diagnosed with PD in community samples also have agoraphobia, although a much higher rate of agoraphobia is encountered in clinical samples (Weissman et al., 1997). However, a dearth of studies have used agoraphobia groups as experimental samples. Only six of the present studies included agoraphobic patients, but only one selected these subjects to form an experimental group. Future research should consider this issue. As discussed in more detail below, the severity of PD and agoraphobia—and not only the presence or absence of these illnesses—must be considered when interpreting fMRI data because a patient with mild agoraphobia, for example, although meeting clinical criteria, may perceive and interpret stimuli differently from a patient with severe agoraphobia.

Comorbidity

Most of the studies did not provide descriptions of the comorbidities presented by the PD patients. Seven

of the studies described comorbidities of PD but did not use this information to evaluate their results. The results were attributed to the characteristics of brain activation associated with PD. Thus, it is unclear whether other disorders possibly interfered with their findings. Comorbidities reflect clinical complexity and are the rule rather than the exception. For example, in four panic-related subgroups, one or more comorbid conditions were found in 71.9% of the isolated panic-without-agoraphobia subgroup, 83.1% of the PD-without-agoraphobia subgroup, and 100% of the isolated panic-with-agoraphobia and PD-with-agoraphobia subgroups (Kessler, Chiu, Jin, Ruscio, Shear, & Walters, 2006). In this context, studying PD without addressing current and past comorbidities may yield no consistency.

Experimental protocols: stimulus modality

Comparing results of the 15 articles may be misleading because of several methodological differences including different experimental protocols. Divergent neuronal activation patterns can be extracted from different task performances (Davidson, 2002). For example, emotional induction using visual stimuli activated the amygdala in healthy subjects, whereas imagery stimulated the anterior cingulate cortex and insula (Phan, Wager, Taylor, & Liberzon, 2002). As presented in Table 2, PD studies used different modes of stimulus presentation. Eleven articles used visual stimuli in their fMRI paradigms. Three studies used auditory stimuli. One study used motor activation stimuli, and one visual study used electrodermal stimulation to determine “threat” and “safe” conditions. This may impact the brain areas that are activated. The three studies that applied auditory stimuli were very different in their methodological designs. The case report registered a PA during scanning. Despite their differences, two studies showed hyperactivation of the parahippocampal gyrus. Boshuisen, Ter Horst, Paans, Reinders, & den Boer (2002) found increased activity of the parahippocampal gyrus during anticipatory anxiety. This structure is related to memory and context and participates in the visceromotor network (Hasler, Nugent, Carlson, Carson, Geraci, & Drevets, 2008).

This activation may be explained by a compensatory effect attributable to parahippocampal gray matter deficits (Massana et al., 2003b). Lai, Hsu, & Wu (2010) demonstrated a gray matter volume decrease in the parahippocampal gyrus in a drug-naïve sample of PD patients with a first episode of major depressive disorder, which may be associated with abnormalities of interoceptive awareness and emotion modulation.

Important features of the third article that used auditory stimuli were increased activity of the right middle temporal gyrus, left anterior insula, and inferior parietal lobe during the third block of auditory stimulation (Pfleiderer et al., 2010). This last study also

Table 1. Parameters compared among studies with regard to sample characteristics

References	Subjects (n)	M/F	Age (mean \pm SD)	Agoraphobia	Other comorbidities	Exclusion criteria	Pharmacological treatment
Marchand et al., 2008	12 P 18 C	F	27.5 \pm 4.9 26.4 \pm 3.9	_____	GAD (1), GAD and DD (1), SP (1), SP and PTSD (1)	History of head injury, neurological disorder, medical disorder that could impact the central nervous system, any contraindications to fMRI and any psychiatric comorbidity other than another coexistent anxiety disorder or dysthymic disorder	BZD (2; needed basis/wash out 24 h), SSRI (1), SSRI and BZD (3; needed basis)
Pfleiderer et al., 2007	1	F	26	x	_____	Cardiologic, neurological, comorbid psychiatric disorders	SSRI for 3 months prior to the MRI investigation
Pillay et al., 2007	8 P 8 C	4/4 4/4	36 \pm 8.3 25.8 \pm 3.5	_____	_____	History of head injury with loss of consciousness or other medical illness that might affect cognitive function; history of any form of substance abuse or dependence; any other current Axis I disorder	SSRI and BZD (5); SSRI, BZD, and gabapentin (1); BZD (2)
Pillay et al., 2006	8P 8C	4/4 4/4	36 \pm 8.3 31.6 \pm 8.8	_____	_____	History of head injury with loss of consciousness or other medical illness that might affect cognitive function; history of any form of substance abuse or dependence; any other current Axis I disorder.	SSRI and BZD (5); SSRI, BZD, and gabapentin (1); BZD (2)
van der Heuvel et al., 2005	15P (PD) 18P (OCD) 14P (H)	8/7 6/12 10/9	33.7 \pm 2.5 33.4 \pm 2.4 40.6 \pm 3.2	_____	_____	Major internal or neurological illness, other psychiatric disorders, and the use of psychotropic medication	Wash out for at least 4 weeks

Table 1. *Continued*

References	Subjects (n)	M/F	Age (mean ± SD)	Agoraphobia	Other comorbidities	Exclusion criteria	Pharmacological treatment
Maddock et al., 2003	6 P 8 C	2/4 3/5	34 ± ? 35 ± ?	x Only one patient without agoraphobia	_____	_____	_____
Chechko et al., 2009	18 P (remitted PD patients) 18 C	8/10 8/10	34.4 ± 8 30 ± 6.4	_____	_____	Patients with anxiety disorders attributable to a medical or neurological condition, primary unipolar depression or bipolar disorder, major medical illnesses, previous head injury with loss of consciousness, and substance abuse or dependence	All patients were under SSRI medication

analyzed the association between serotonin transporter gene variations and auditory processing and found that altered auditory habituation was associated with the less-active 5-HTTLPR S allele and the less-active 5-HTTLPR/rs25531 haplotype.

Among visual stimuli paradigm studies, paradoxical features can be found such as increased or diminished cingulate cortex and amygdala activity. As discussed by Davidson (2002), some results may reflect different levels of paradigm difficulty or may be influenced by therapy modalities. Most studies did not use physiological measures to determine whether the affective response corresponded to the intended emotion in the paradigm.

Psychological evaluation: the use of scales in fMRI studies of PD

All papers reviewed here used the DSM-IV criteria for diagnosing PD, with the exception of one study that used DSM-III criteria. The fMRI studies selected in this review used the following instruments: Structured Clinical Interview-Axis I, DSM-IV-R (SCID-I), Mini-Mental State Examination (MMSE), Edinburgh Handedness Inventory (EHI), Panic-Associated Symptom Scale (PASS), Hamilton Anxiety Scale (HAM-A), Hamilton Depression Rating Scale (HAM-D), Anxiety Sensitivity Index (ASI), State-Trait Anxiety Inventory – Trait scale (STAI-T), State-Trait Anxiety Inventory-State scale (STAI-S), Anxiety Control Questionnaire (ACQ), Body Sensation Questionnaire (BSQ), Beck Depression Inventory (BDI), Brief Symptom Inventory (BSI),

Clinical Global Impressions (CGI) scale, Differential Emotions Scale (DES), Mobility Inventory (MI), Positive and Negative Affect Schedule (PANAS), State-Trait Anger Expression Inventory (STAXI), and Panic and Agoraphobia Scale (PAS). Different types of scales were used among the studies, and only the following scales were used with more frequency: HAM-A, HAM-D, STAI-T, and EHI. For this reason, these psychometric tools will be briefly reviewed.

Hamilton Anxiety Scale (HAM-A)

The HAM-A (Hamilton, 1959) was one of the first rating scales developed to measure the severity of anxiety symptoms and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (i.e., mental agitation and psychological distress) and somatic anxiety (i.e., physical complaints related to anxiety). Although the HAM-A remains widely used as an outcome measure in clinical trials, it has been criticized for its sometimes poor ability to discriminate between anxiolytic and antidepressant effects and between somatic anxiety and somatic side effects (Maier, Buller, Philipp, & Heuser, 1988). The HAM-A does not provide any standardized probe questions. Despite this, the reported levels of inter-rater reliability for the scale appear to be acceptable. Each item is scored on a scale from 0 (not present) to 4 (severe), with a total score of 0 to 56 in which <17 indicates mild severity, 18-24 indicates mild to moderate, and 25-30 indicates moderate to severe. Six studies used the HAM-A.

Hamilton Depression Rating Scale (HAM-D)

One year after publishing the HAM-A, the depression version of the scale was presented (Hamilton, 1960). The HAM-D has proven useful for many years as a method of determining a patient's level of depression before, during, and after treatment. It should be administered by a clinician experienced in working with psychiatric patients. Although the HAM-D form consists of 21 items, the scoring is based on the first 17 items. It generally takes 15-20 min to complete the interview and score the results. Eight items are scored on a 5-point scale ranging from 0 (not present) to 4 (severe). Nine items are scored from 0 to 2. Since its development, the scale has been widely used in clinical practice and became a standard in pharmaceutical trials. The HAM-D score is the result of summing the scores from the first 17 items, with 0-7 indicating Normal, 8-13 indicating Mild Depression, 14-18 indicating Moderate Depression, 19-22 indicating Severe Depression, and ≥ 23 indicating Very Severe Depression (Hamilton, 1960; Williams, 2001). Several studies evaluated this scale and concluded that the HAM-D exhibited a relatively stable factorial structure based on a large sample of outpatients with unipolar depressive disorders (O'Brien & Glaudin, 1988), but its total score may be a weak index of depressive syndrome severity (Gibbons, Clark, & Kupfer, 1993). Only three studies used the HAM-D; the other two studies used the BDI to assess depressive states.

State-Trait Anxiety Inventory-Trait (STAI-T) scale

The STAI scale, which is appropriate for those who have at least a sixth grade reading level, consists of a four-point Likert scale to assess anxiety. The STAI scale assesses state anxiety (i.e., a transient condition characterized by tension, apprehension, and hyperactivity of the autonomic nervous system) and trait anxiety (i.e., a general tendency that an individual has to respond, with anxiety in response to environmental stimuli). The STAI scale was first published in 1970 (Spielberger, Gorsuch, & Lushene, 1970) with the purpose of measuring anxiety in adults, although currently a version for children is available. It has been widely used by clinicians and researchers, although some suggest that the trait scale may assess both depression and anxiety (Bieling, Antony, & Swinson, 1998; Bados, Gómez-Benito, & Balaguer, 2010). The instrument is divided into two sections, each with 20 questions. The STAI scale has three forms. The current variation is the STAI Form Y, which differentiates between temporary or emotional state anxiety and long-standing personality trait anxiety in adults. The STAI Form X is the first version of the STAI, which is still available. The third form is the STAI for children.

The STAI Form Y serves as an indicator of two types of anxiety: state and trait anxiety. It measures

the severity of the overall level of anxiety and is an administered analysis of reported anxiety symptoms. The first subscale measures state anxiety, and the second subscale measures trait anxiety. The scores range from 20 to 80, with higher scores indicating greater anxiety (Spielberger et al., 1970). Some of the questions are related to the absence of anxiety and are reverse-scored. Results of the STAI scale can be used in the formulation of clinical diagnoses, for psychological and health research, and for the assessment of clinical anxiety in patients in medical, surgical, and psychiatric settings. Four studies from those selected in the present review used this scale to assess anxiety levels among participants.

Edinburgh Handedness Inventory (EHI)

As mentioned above, establishing laterality criteria is an important component of fMRI research designs. The EHI is probably the most widely used scale for assessing this feature (Oldfield, 1971). Briefly, the inventory is composed of 10 items representing daily activities such as drawing, writing, using a spoon, and throwing objects. By reflecting on the way these tasks are performed, the participant is encouraged to check his/her preference in using his/her left or right hand. The inventory includes the possibility that the preference is so strong that the participant would never use the other hand unless absolutely forced to, a situation where the participant may place two checks, resulting in different scoring. Only one study from the selected articles of this systematic review assessed this parameter.

Psychological evaluation and fMRI studies of PD: considerations of the application of scales

An important aspect to be considered concerning the use of scales in fMRI studies is the time during which the scale is applied. Only two studies applied the scales at the time of scanning to quantify the severity of the panic symptoms present at the exact time of the exam, although three other studies reported anxiety symptoms during the fMRI exam. As a measure of state anxiety, one study applied the DES immediately after the fMRI exam to assess the intensity of anxiety during fMRI scanning.

Only 7/15 selected studies reported patients' scores on the scales. This represents important information for correlating the severity of PD and the imaging activation found and should be considered in future studies.

Lack of consensus concerning the choice of scales used, time at which they are applied, and absence of a correlation between severity of the diagnosis and imaging results make it difficult to analyze the data as a whole and to compare them with other studies.

Scales and inventories represent important tools for assessing different dimensions of human adaptive or dysfunctional behavior such as handedness, anxiety, panic and depression levels, and mobility in the case

of agoraphobia. These parameters reveal discrete but important aspects that may show the level of gravity and idiosyncratic specificities of the study's sample. In this sense, these scores should not be neglected when interpreting fMRI data in PD studies.

Imaging genomics

The knowledge about genetics in the pathogenesis of PD has experienced significant advances during the last several years. However, the relationship between PD and genetics is complex. A large number of small-effect genes may contribute to vulnerability to the disease (Rothe et al., 2006).

Only three of the reviewed articles discussed this emerging field of science. All of these studies had small samples. They included both male and female patients, medicated and non-medicated patients, and comorbid psychiatric conditions. Two of the three studies did not utilize a healthy volunteer control group. These may be confounding factors in the discussion of the results. Two of the three studies used neuronal activation in response to visual stimuli as an endophenotype of PD to be associated with polymorphisms. One study analyzed the association between neuronal activation in response to auditory stimulation and the influence of the 5-HTTLPR polymorphism. Similar studies with refined and larger samples are necessary in order to make comparisons.

One of the studies discussed the gene that codes for the enzyme catechol-*O*-methyltransferase (COMT). COMT is an enzyme that metabolizes monoaminergic neurotransmitters including adrenaline, noradrenaline, and dopamine (Weinshilboum, Otterness, & Szumlanski, 1999). The COMT gene is located on chromosome 22q11.2 (Rothe et al., 2006). A nucleotide substitution polymorphism (guanine to adenosine) in codon 158 of the COMT gene results in an amino acid change from valine to methionine, with the valine allele relating to higher COMT activity. The COMT genetic variation may influence limbic and prefrontal brain activation in response to unpleasant stimuli (Domschke et al., 2008). Information processing deficits appear to play a crucial role in the pathogenesis of PD. Domschke et al. (2008) used neuronal activation elicited by emotional stimuli as an endophenotype to study the association with PD and found that patients who carried at least one 158val allele showed increased activation of the amygdala in response to fearful faces and increased activation of the left orbitofrontal cortex (Figure 1). Other alterations were also observed, indicating that activation of the amygdala and prefrontal cortex in response to emotional faces may be influenced by the genetic variation. The COMT variation may alter the neuronal processing of anxiety-related emotional cues.

In a previous study, Domschke et al. (2006) studied serotonergic polymorphisms 1019C/G 5-HT_{1A}

and 5-HTTLPR in PD patients. The serotonergic system appeared to play an important role in the etiology of PD. The G allele of the -1019C/G promoter polymorphism in the gene that codes for the 5-HT_{1A} receptor located on chromosome 5q12.3 was proposed to depress 5-HT_{1A} autoreceptor expression, reducing serotonergic neurotransmission (Domschke

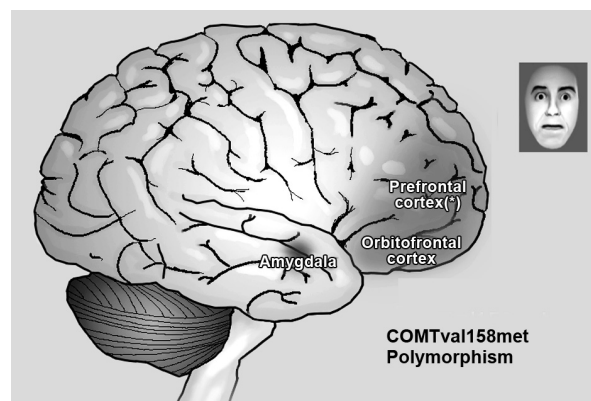


Figure 1. Differential activation of brain areas in PD patients with the COMT polymorphism.

et al., 2006). Another serotonergic polymorphism was the variant site 5HTTLPR (*serotonin-transporter-linked promoter region*). The short "s" allele of the serotonin transporter (5-HTT) is associated with reduced transcription and functional capacity of the serotonin transporter. Perna, Favaron, Di Bella, Bussi, & Bellodi (2005) showed that monozygotes for the short allele (s/s) of 5HTTLPR had a worse response to paroxetine. Domschke et al. (2006) found a significant decrease in the activation of the right ventromedial and orbitofrontal cortex and right anterior cingulate cortex in patients homozygous for the high-risk G allele of the 5-HT_{1A}-1019 C/G promoter polymorphism. They also concluded that both the 5-HT_{1A}-1019 C/G and 5HTTLPR polymorphisms play a role in amygdala activation in response to positive emotional stimuli. This study also had a small sample and no control group with healthy volunteers; additionally, some patients were under treatment and others had psychiatric comorbidities. Despite these limitations, its contribution to the understanding of gene-environment interactions for the PD phenotype is notable.

Discussion

Panic disorder is an incapacitating condition that can elicit long-term negative consequences in patients (Schunck et al., 2006). Neurobiological research on PD represents a crucial tool for a more complete understanding of this condition which, in turn, may significantly contribute to future treatments and better clinical outcomes in terms of both pharmacology

Table 2. Parameters compared among studies with regard to experimental designs and protocols

References	Clinical design	Stimulus (*)= Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Marchand et al., 2008	A motor activation paradigm for the non-dominant hand. The motor task consisted of one 4-min run repeated once for each hand with six blocks of rest and six blocks of activity presented in a pseudorandom order. Subjects alternated pressing buttons with the first and third fingers simultaneously and the middle finger alone. The paradigm was completed for one hand and then repeated for the opposite hand. During the rest blocks, the word “rest” was presented as a visual stimulus.	Motor activation	SCID-I (DSM-IV-R) MMSE EHI PASS	—————		x	SPM2 Marsbar software
Pfleiderer et al., 2007	Three stimulation cycles (A1, A2, and A3) of emotionally neutral, digitally generated pulses of 800 Hz sine wave tones of 2-min duration each (ON) alternating with 1-min rest periods (OFF; R1-A1-R2-A2-R3-A3-R4). The scan time was ~20 min, including localizer and anatomic scans. Association with the 5-HTTLPR gene variation was analyzed.	Auditory	SCID-I (DSM-IV-R) ASI STAI-T 12 h before the MRI scan and after the scan: HAM-A STAI-S ACQ BSQ	Heart rate		x	SPM2

Table 2. Continued

References	Clinical design	Stimulus (*) = Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Pillay et al., 2007	Stimuli consisted of six faces that expressed similar emotional expressions from the Ekman series. The scanning sequence lasted for 150 s, consisting of five alternating 30-s stimulus/rest periods. During the baseline and rest periods, subjects maintained visual fixation on a small white circle located at the center of the screen. Three different face photographs were presented during each of the two stimulation periods for a total of six stimulus expressions. Each facial expression was presented for 9.5 s with a 0.5-s interstimulus interval. The subjects were asked to report the affect of the faces following the conclusion of the scan.	Visual	SCID-I (DSM-IV-R) HAM-A HAM-D STAI-T STAI-S	_____	x		SPM99
Pillay et al., 2006	Stimuli consisted of six faces that expressed fear from the Ekman series. The scanning sequence lasted for 150 s, consisting of five alternating 30 s stimulus/rest periods. During baseline and rest periods, subjects maintained visual fixation on a small white circle located at the center of the screen. Three different face photographs were presented during each of the two stimulation periods for a total of six stimulus expressions. Each facial expression was presented for 9.5 s with a 0.5-s interstimulus interval. Subjects were asked to report the affect of the faces following the conclusion of the scan.	Visual	SCID-I (DSM-IV-R) HAM-A HAM-D STAI-T STAI-S	_____	x		SPM99

Table 2. Continued

References	Clinical design	Stimulus (*) = Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
van der Heuvel et al., 2005	A cognitive and emotional Stroop task consisting of congruent and incongruent color words, obsessive compulsive disorder-related and panic-related negative words, and neutral words. Stimuli were presented in a block design, consisting of 18 randomized blocks (three blocks of each condition), each containing 16 words. Each word was presented for 2 s followed by a 200-ms blank screen. Subjects were asked to respond as fast as possible by pressing the button that corresponded to the color of the ink, regardless of the meaning of the word. After performing the task, subjects were asked to rate subjective distress using a 100-point analog scale.	Visual	SCID-I (DSM-IV-R) BSQ (panic patients)	_____	x		SPM99
Maddock et al., 2003	Stimuli consisted of 10 threat words and 10 emotionally neutral words matched for word length and frequency of usage. Each word was presented once in pseudorandom order in each 16-s block of 10 words of the same type. Sixteen alternating blocks of threat-related and neutral words were presented for 256 s following a 32-s baseline. Subjects were instructed to form a silent judgment of the valence of each word. After scanning, the subjects were questioned about stimulus audibility, task performance, and their emotional state during the scan.	Auditory*	SCD-I (DSM-III-R) HAM-A Panic diaries	_____	x		Medx software BrainMRI software

Table 2. Continued

References	Clinical design	Stimulus (*) = Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Chechko et al., 2009	An emotional conflict paradigm with single trials that consisted of combinations of an emotional face in the background (happy or fearful expression) and the words "HAPPINESS" or "FEAR" in German printed across the face in bold capital red letters. One run consisted of 152 trials in blocks of 38 trials and 30-s breaks. Between the face presentations, a fixation cross was shown. Depending on the congruence between the face expression and word, the trials were classified as congruent (C) or incongruent (I). Participants were instructed to identify the face expression and to answer as quickly and precisely as possible by pressing the right (happy face) or left (fearful face) answer button with their index finger.	Visual	DSM-IV criteria STAI-T STAI-S BDI PAS	_____	x		SPM5
Domschke et al., 2008	Neuronal activation following emotional stimulation. Stimulation was used as an endophenotype and investigated for its association with the COMT val158met polymorphism in panic disorder.	Visual	_____	_____			SPM2
Domschke et al., 2006	Blocks of masked and blocks of unmasked emotional faces (fearful, angry, happy) were presented. Neuronal activation following emotional stimulation was used as an endophenotype and investigated for its association with the -1019C/G 5-HT _{1A} and 5-HTTLPR polymorphism in panic disorder.	Visual	_____	_____			SPM2

Table 2. Continued

References	Clinical design	Stimulus (*) = Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Beutel et al., 2010	Subjects were instructed to perform a right index finger button press immediately after reading a word that appeared in normal font (go trial) and to inhibit this response after reading a word in italicized font (no-go trial). Word lists included words of neutral, negative, and positive valence. Afterward, the participants were instructed to complete a word recognition task based on their respective word list. They then rated the valence of each word on a 7-point Likert scale followed by a short debriefing.	Visual	ICD-10 Diagnosis Checklist ACQ BSQ STAI-S STAI-T	_____	x		SPM
Tuescher et al., 2011	Instructed fear conditioning paradigm. The scanning session consisted of both "Threat" (in which the participants were told that an electrodermal stimulation could occur at any time) and "Safe" (in which participants were told they would receive no stimulation) conditions. Threat and Safe were signified by the presentation of distinguishable colored squares. There were five pseudo-randomly ordered blocks of each color per scanning run and two scanning runs per study session. The participants did not receive any electrodermal stimulation during scanning.	Visual	DSM-IV criteria	_____			SPM

Table 2. Continued

References	Clinical design	Stimulus (*) = Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Ohrmann et al., 2010	Facial stimuli consisted of gray-scale-normalized images that depicted fearful, angry, happy, and neutral expressions of 10 male and female individuals. The subjects were presented with alternating 30-s blocks of one of the four facial expressions or a no-face control stimulus (a gray rectangle). The order of the blocks was counterbalanced across subjects. Each face epoch was preceded by a no-face control epoch and was presented twice, resulting in an overall presentation time of 8 min.	Visual	DSM-IV criteria DES EHI STAI-S STAI-T	_____			SPM2
Wittmann et al., 2010	Twenty-four neutral pictures and 24 agoraphobia pictures were presented in a cued manner. The other remaining 48 neutral and agoraphobia pictures were presented uncued by a random combination of characters. The picture sequence was randomized for each subject. Each picture was presented for 2,000 ms, and cues were presented for 250 ms, separated by the presentation of a fixation cross. The complete presentation duration was ~15 min. The participants were instructed to pay attention to the content of the pictures, experience the presented situation, and pay attention to the cue and its predictive content before picture presentation. They were requested to push a button each time a picture was presented.	Visual	CAP-1-WHO- CIDI HAM-A CGI BSI BDI MI EHI	_____			SPM5

Table 2. Continued

References	Clinical design	Stimulus (*)= Anxiogenic	Scales	Objective measure of anxiety	Scanner type		Image pro- cessing and statistical analysis
					1.5 T	3 T	
Pfleiderer et al., 2010	Three stimulation cycles (A1-A3) of digitally generated pulsed (5 Hz frequency) 800-Hz sine wave tones of 2 min duration, which alternated with rest periods (R1-R3) of 1 min duration. Auditory stimulation was presented binaurally via pneumatic headphones. The hearing threshold was determined individually within the magnet. All subjects had comparable hearing thresholds, and each subject was stimulated with a sound pressure level of 85 dB above their individual hearing threshold.	Auditory	SCD-I (DSM-IV) HAM-A HAM-D	_____			SPM5
Dresler et al., 2011	Participants had to passively view emotional faces. Four different emotions (anxious, happy, sad, and neutral) were used in a block paradigm. Each block contained eight faces (four female, four male) and was presented four times in a random order. Each face was shown for 2 s, directly followed by the next one. Face blocks were alternated with blocks of the same length that showed a white fixation cross. The patients were instructed to attentively look at the faces and empathize with the expression.	Visual	ICD-10 criteria STAI-S STAI-T STAXI PANAS VAS BDI	_____	x		SPM5

and new psychotherapeutic techniques that address specific components of the fear neurocircuitry that underlies PD.

Neuroimaging studies play a crucial role in this scenario because they allow researchers to achieve a deeper understanding of the phenomenology associated with the brain circuits that underlie anxiety disorders (Davidson, 2002). Such knowledge emerges from research designs that allow a better analysis of specific affective and cognitive processing. Additionally, these studies can reveal the heterogeneity of these disorders, which may contribute to descriptions of more accurate subtypes of psychopathologies. Functional neuroimaging

techniques can verify the impact of certain therapeutic interventions on brain function.

Although many aspects of this anxiety disorder have been investigated from different perspectives, much remains to be discovered. The purpose of this review was to highlight the importance of fMRI studies of PD by acknowledging the value of using different available methodological resources in current science and encouraging researchers to address some important points concerning research criteria. In this way, data published by different groups worldwide can be more easily integrated and compared. This may represent an important step towards establishing more conclusive conceptual models of PD.

When considering the use of fMRI in the study of a certain disorder, it would be interesting for researchers to address some of the highlighted points; otherwise, consistency of the data may be compromised or the scientific literature in regard to the issue may be composed of data that cannot be generalized in terms of building consensus and a more uniform delineation of the neurocircuitry of PD. Davidson (2002) suggested that when evaluating treatment-related differences, researchers commonly associate the neural findings with vulnerability to a specific disorder; however, the possibility that the differences are attributable to nonclinical features as a consequence of the pathology should not be ruled out. Additionally, in studies where patients are scanned on two or more occasions, test-retest reliability should be considered, highlighting the importance of associating psychometrics to psychophysiological measures and neuroimaging findings. One of the chosen parameters analyzed among fMRI studies of PD was use of psychological assessment, and consideration should be made of the data obtained from scales and inventories when interpreting neuroimages.

The research criteria that diverged among the selected papers of this present systematic review or that need to be reconsidered in future investigations include the following: (1) samples were small and cannot be considered representative of the PD population and we strongly encourage designing cross-cultural studies in order that a more universal model of the neurocircuitry of PD can be developed; (2) samples must be balanced with regard to gender because men and women display different brain activation patterns; (3) fMRI studies need to use objective measures that help assess handedness, such as the EHI, because handedness can provide evidence of laterality dominance, accounting for differential brain activation; (4) high levels of comorbidity between PD and agoraphobia indicate the need to include agoraphobia in future studies so that the samples are as similar as possible to what is found in the population; (5) comorbidities presented by PD patients should be described and considered in the interpretation of the results because differential activation may be attributable to PD, comorbidities, or their interaction; (6) stimulus modalities varied, which is important with regard to understanding how PD patients process different sensory information; (7) future studies that investigate a given sensory modality should consider applying similar methodologies so that the data can be compared and analyzed across studies rather than just described; (8) the studies used a wide range of scales and inventories to perform psychological evaluations, but these instruments varied considerably among studies both in terms of the phenomena assessed and the psychometric instruments chosen. However, what was most evident was the lack

of consideration of the obtained scores in the analysis of fMRI findings.

Additionally, an important parameter to be assessed in PD patients is agoraphobic avoidance behavior and frequency of panic attacks, which can be consistently addressed with the Mobility Inventory for Agoraphobia (MI; Chambless, Caputo, Jasin, Gracixy, & Williams, 1985). Interestingly, none of the studies mentioned in this present review used this scale as part of the psychological assessment.

Conclusion

Panic disorder leads patients to severe psychological suffering, and fMRI studies constitute important tools for revealing the fear neurocircuitry that underlies this condition. However, the mental health field may benefit from these studies, both in terms of establishing more precise diagnostic criteria for PD based on specific neural activation and designing more accurate pharmacological and psychotherapeutic treatments that may remodulate this circuitry if research designs attempt to follow the complexity of PD and address its multifactorial features. The present systematic review was intended to provide helpful guidelines for future fMRI studies of PD. These include increasing sample sizes, using homogeneous samples, addressing severity and comorbidities issues, and analyzing the psychological evaluations performed because these scores may provide important features of the studied sample and the possibility of correlating them with the neural networks activated by anxiogenic and panicogenic stimuli.

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