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ORIGINAL ARTICLE

A meta-analytic review of neuroimaging studies of specific phobia to small animals

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Abstract
Introduction: Neuroimaging techniques have been used to identify the neurological bases of phobias.
Objective: This meta-review examines functional magnetic resonance imaging studies of individuals with specific animal phobia compared to healthy controls.
Method: Searches on Medline, Psycinfo, Academic Search Complete, PubMed, PsycARTICLES, Redalyc, Scopus, and Cochrane databases were conducted. Twenty high quality studies were selected. The effect size estimation was calculated.
Results: The random-effects model showed a high overall effect size for both limbic and frontal sites. Data analyses showed greater brain activity in the left amygdala and insular cortex in phobic individuals. We also observed an activation of the fusiform gyrus, the dorsolateral prefrontal cortex left, and the left cingulate cortex, although these areas were less frequently involved. Healthy controls showed high heterogeneity in the brain areas activated by phobic stimuli.
Conclusions: These findings suggest the possible existence of a double processing pathway in phobic stimuli: a rapid processing pathway involving limbic areas and a slow pathway involving both limbic and frontal areas.

Introduction

The use of neuroimaging techniques has contributed to a better understanding of the neural circuitry involved in mental illness. Neuroimaging scans have delimited not only the anatomical and functional brain structures of many psychopathological disorders but also the regional metabolism of such disorders. As a consequence, neuroimaging has helped to increase our knowledge about the processes that underlie psychopathological disorders. This knowledge has had practical clinical implications, facilitating the diagnosis of mental disorders and the development of new treatments, especially those derived from psychiatric drugs.[1]

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Phobias, a special case of anxiety disorders, are one of those mental disorders. Phobia disorder refers to a high persistent anxiety response that people usually (but not always) consider excessive or irrational to the presence or anticipation of a threatening object or situation. There are three groups of phobias: agoraphobia, social phobia, and specific phobias.[2] Phobias are one of the most frequent mental disorders, with specific phobias (SP) reaching the highest prevalence rates (from 7.7% to 12.1%).[3,4]

The etiology of phobias involves both environmental and constitutional/biological factors.[5] Environmental factors refer to learning/instructional processes in the acquisition of an anxiety response. Biological factors justify the concept of preparedness: some people are biologically vulnerable to developing phobia disorders. Yet, although there is some consensus about the learning/instructional processes associated to phobia acquisition,[6] no agreement has been reached so far on the neurological bases of phobias, including biochemical markers.[7]

Functional neuroimaging studies have been conducted with the aim of providing evidence of those neurological bases. Most of these studies have dealt with specific phobias (insects, spiders, blood-injury). Systematic reviews have found a group of similar brain areas and circuits related to brain responses to phobic stimuli.[8,9] The areas most often found are those associated with limbic and paralimbic structures (insula, amygdala, thalamus). The cingulate gyrus, the medial prefrontal cortex, and the orbitofrontal cortex have also been found to be activated by those stimuli. However, several other areas have been found in various studies. These disparities hamper research on the specific biological bases of phobias.

Because of the many methodological differences between studies (i.e., age, gender, type of phobia, absence of a control group), a more restrictive review and meta-analysis were carried out in an attempt to select more combinable and comparable studies.[10] In 10 of the 13 studies selected, the amygdala (especially the left amygdala) was the brain structure found to be most closely associated with specific phobia, followed by the globus pallidus, the pulvinar thalamus, the left insula, and the right cerebellum. The left insula was found to have the highest activation level (effect size data were not provided). Yet, depending on the study, several other areas (e.g., the anterior cingulate cortex, the hippocampus, the cingulate gyrus) were also found to be associated with phobias. Given that methodological disparities did not disappear (there were descriptive studies and experimental/treatment studies, designs with and without a control/healthy group, different subtypes of specific phobias), differences between studies may also be due to those discrepancies.

Considering the above, the aim of this meta-analysis was to provide an update of the neurological bases of specific phobias by applying more refined inclusion criteria in order to find more combinable studies. As regards techniques, we only included studies in which functional magnetic resonance imaging (fMRI) was used because the variability found in the brain activation areas may be mediated by the analysis technique used. There are few studies on specific phobias to small animals that used other neuroimaging techniques than fMRI. Techniques as Loreta and EEG as PET are not incorporated in our analysis because while LORETA provides high resolution temporal EEG in comparison with fMRI, it is worse in 3D, especially in subcortical brain spatial resolution. Recently published studies combining both techniques.[11] In addition, PET implies some advantages in eliminating artifacts due to the mobility of participants during data recording and facilitates the study of brain biochemistry with several isotopes. But, it also provides both less spatial resolution and statistical significance results comparatively to fMRI.[12,13] Given these arguments it was decided to include studies with a similar level of comparison. However, studies tested with these different techniques showed similar functional results to fMRI in specific phobias.[14–16] As regards methodologies, we only considered descriptive studies with a control/healthy group and a group of individuals with animal phobia because results suggest that there are partially different neurobiological substrates between animal and blood-injection-injury phobic subtypes.[17,18]

Methods

This study was performed according to the PRISMA statement on the information that should be included in a systematic review.[19]

Identification and selection of studies

We identified eligible studies by searching the Medline, Psycinfo, Academic Search Complete, PubMed, PsycARTICLES, Redalyc, Scopus, and Cochrane databases. We included studies published from inception of the database until September 2015 using combinations of database-specific index and free-text terms to identify studies of subjects diagnosed with specific phobia who were scanned using functional magnetic resonance imaging. 'Specific phobia,' OR 'simple phobia,' OR 'phobia' AND 'imaging,' OR 'neuroimaging,' OR 'functional magnetic resonance', OR 'fMRI' were used as syntax. No search period was specified, as this is a recent area of clinical psychology.

Inclusion criteria

The scientific papers included in this review were studies dealing with functional magnetic resonance imaging in specific phobia that were published in peer-reviewed journals. To be eligible for inclusion, studies had to include at least two groups: one in which participants had been diagnosed with specific phobia to small animals according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V)[2] or the *International Statistical Classification and Related Health Problems of Diseases* (ICD-10),[20] and a control group.

Inclusion criteria for the articles used in the current study were: complete and original articles, in English, with samples of patients whose main diagnosis was specific phobia and who had undergone fMRI scanning.

Exclusion criteria

We excluded studies about participants with blood-injection-injury specific phobia and functional magnetic

resonance studies of cognitive behavioral therapy. Meta-analytic and review studies were also excluded.

Articles were excluded if they failed to clearly specify the method for diagnosing SP or if SP was not the main diagnosis and also if imaging methods were unspecified or inadequately described. We also excluded secondary studies, studies with no control/comparison group, and those that did not provide data to calculate the effect size.

Selection process

Two reviewers carried out the study selection process individually. Reviewers used the following blind and structured hierarchical strategy: first, reading titles and abstracts; second, reading the selected articles in full; and third, selecting articles that met the specific inclusion criteria. In case of discrepancies, a third reviewer verified the selection criteria.

Methodological quality of the studies

We conducted an assessment of methodological quality using the CONSORT Statement,[21] with a detailed explanation and justification for each of the 22 proposed items. The CONSORT Statement is structured into five sections: title and abstract, introduction, method, results, and discussion. Two reviewers assessed 25% of the papers selected. The Kappa coefficient reached (.80) showed a considerable degree of agreement between raters. After that, we assessed the remaining studies.

Data extraction

The same researchers who selected the studies also extracted data independently. Any disagreements were resolved by consensus. The following information was extracted from each of the selected articles: age and gender of the population, study procedure, main results, effects of exposition to stimuli on brain activity, and effect sizes and confidence intervals.

We calculated estimates of effect size (standardized mean differences or Cohen's *d*) for each of the scores reported in each study according to the proposed protocol.[22] We verified whether the use of a meta-analysis was supported by the absence of heterogeneity between studies. Statistical analyses were conducted with the metafor package: a meta-analysis package for R.

Quantitative analysis

We calculated effect sizes of the differences between phobic and control groups using 95% confidence intervals.[22] Results were classified as small ($0.20 \leq d \leq 0.40$), moderate ($0.50 \leq d \leq 0.70$), or large ($0.80 \leq d$).

Heterogeneity was evaluated using DerSimonian and Laird's *Q* statistic and the I^2 index, both with a 95% confidence interval. A random effects method was applied. Finally, mean effect size was calculated for the different brain areas following a previous study[8]: cortex sites, which included prefrontal, orbitofrontal, insular, cingulated and visual association cortices; and limbic sites, which included the limbic system, the thalamus and amygdala and other structures such as the cerebellum.

Official, ethical approval was not requested in view of the nature of this study

Results

Identified studies

Using the search strategy described above, a total of 227 references were identified. After eliminating 203 duplicates, non-fMRI studies, and studies that did not deal with patients with phobia to small animals, 24 references were selected by title and abstract. Fig. 1 shows the search and selection process used to identify references.

Included and excluded studies

Out of 24 studies selected and read in full, 20 were included (see Table 1). Four references were excluded because of the unavailability of comparison data or because they did not include a clear comparison group.

Methodological quality

The methodological quality of the studies was high (mean = 0.79), ranging from .53 to .92. The items with the lowest scores were ''random sampling'' and ''side/adverse effects.''

Characteristics of included studies

Studies showed a heterogeneity that may be due to differences in the areas studied ($I^2 = 25.62\%$, 95% CI = 15.18–53.60%; $Q(117) = 171.96$; $p < 0.001$). Given the heterogeneity of the samples, we used a random effects model to measure effect sizes.

Based on the data extraction sheet, the main characteristics obtained for each study are shown in Table 1. The technique characteristics of the scanners were also included.

Most of the studies compared the brain activity correlates of stimulus exposure between patients with SP and healthy controls, using BOLD fMRI and region of interest (ROI) analysis. Calculating percentages in studies analyzed, differences in brain activity in subjects with SP compared to healthy controls were most frequently found in the left amygdala and bilateral insula (40%), the right amygdala and bilateral fusiform gyrus (25%), the left dorsolateral prefrontal cortex (DLPFC) (15%), and the left cingulated cortex and left thalamus (10%); the remaining differences occurred less frequently (5%).

The random-effects model of the studies showed a high overall effect size for both limbic and frontal sites (see Fig. 2).

In phobic participants, the brain activity areas with the highest mean effect sizes were the amygdala (0.45), the insula (0.31), other limbic sites (0.18), the cingulate and orbitofrontal cortex (0.12), the prefrontal cortex and

Table 1 Summary of studies included in the review.

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Dilger et al. (2003)[23]	10 female phobics (*M*/*S.D.* age = 25/2.3 years) and 10 female controls (*M*/*S.D.* age = 21.3/0.6 years).	Exposure to phobia and non-phobia-related pictures.	The between-group comparison for spider pictures showed stronger activation in phobics in the right and left insula, the right orbitofrontal cortex, the left uncus, the right and left fusiform gyrus and the posterior cingulate cortex. Between-group comparisons for the other stimulus types (snakes, mushrooms) revealed clusters of higher activation in the left and right fusiform gyrus and the left uncus in phobics, but also one cluster of higher activation in the left fusiform gyrus in control subjects.	Phobics > controls Spider Amygdala L 4.41 Posterior cingulate 5.98 Gyrus insula R 6.87 Insula R 6.60 Orbitofrontal cortex L 7.03 Fusiform gyrus R 8.82 Fusiform gyrus L 6.45 Uncus L 5.84 Snake Fusiform gyrus R 6.01 Mushroom Fusiform gyrus L 5.58 Uncus L 5.58 Control > phobics Mushroom Fusiform gyrus L 5.21	 2.07 (0.95–3.16) 2.82 (1.53–4.07) 3.24 (1.85–4.59) 3.1 (1.75–4.41) 3.31 (1.91–4.68) 4.16 (2.53–5.75) 3.04 (1.70–4.34) 2.75 (1.48–3.98) 2.83 (1.54–4.08) 2.63 (1.39–3.83) 2.63 (1.39–3.83) 2.46 (1.26–3.63)
Wright et al. (2003)[24]	10 (6 female; *M*/*S.D.* age = 29.8/6.8 years) phobics and 10 controls (6 female; *M*/*S.D.* age = 28.7/11.1 years). All were right-handed, medication free and were matched for age, gender, and years of education.	Viewed Ekman emotional faces.	Results revealed a significantly greater response to the fearful versus neutral faces in the right insular cortex of the specific phobia group than in the control group. Amygdala hyper-responsivity to emotional faces was not observed in subjects with small animal specific phobia.	Posterior insular cortex R: $F = 9.4$	1.45 (0.44–2.43)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Martis et al. (2004)[25]	10 phobics and 10 controls matched for age (*M*/*S.D.* = 29.8/6.8 years, versus *M*/*S.D.* = 26.7/6.7), gender (6 women), right-handed (8), and educational level (*M*/*S.D.* = 17.4/2.5 years, versus *M*/*S.D.* = 17.0/2.2).	Serial reaction time task paradigm.	Brain activation in the striatum of subjects with specific phobia does not significantly differ from that of normal comparison subjects during implicit sequence learning.	No significant differences between patients and control groups were found.	NA
Straube et al. (2004)[26]	11 Female phobics (*M* age = 20.8) and 11 female controls (*M* age = 22.4). All were right-handed.	Exposure to phobia-related or unrelated words.	Phobia-related versus phobia-unrelated words elicited increased activation in prefrontal cortex, insula, and posterior cingulate cortex in spider phobics, while these effects were absent in controls.	Phobics > controls DLPFC L 5.35 IFG L 3.62 IFG R 3.83 Insula L 3.67 Insula R 3.83 PCC L 3.96	 2.39 (1.26–3.49) 1.62 (0.63–2.58) 1.71 (0.71–2.68) 1.64 (0.65–2.60) 1.71 (0.71–2.68) 1.77 (0.76–2.75)
Schienle et al. (2005)[27]	10 female phobics (*M*/*S.D.* age = 22.5/2.2 years) and 13 female controls (*M*/*S.D.* age = 23.9/6.8 years). All subjects were medication-naïve and right-handed.	Exposure to blocks of phobia-relevant, generally fear-inducing, disgust-inducing and affectively neutral pictures.	The patient group showed greater activation of the visual association cortex, the amygdalae, the right dorsolateral prefrontal cortex and the right hippocampus. The patients also showed greater amygdala activation during the presentation of generally disgust- and fear-inducing pictures.	Phobics > controls Phobia > neutral Amygdala L 3.8 Amygdala R 3.4 DLPFC R 4.7 Hippocampus R 4.4 Fusiform gyrus L 5.3 Fusiform gyrus R 4.7 Disgust > neutral Amygdala L 3.5 Fear > neutral Amygdala L 5.9 Amygdala R 3.8 Insula R 4.9 Hippocampus L 5.6 Fusiform gyrus L 4.8	 1.66 (0.68–2.61) 1.48 (0.53–2.40) 2.05 (1.00–3.06) 1.9 (0.88–2.89) 2.3 (1.21–3.36) 2.05 (1.00–3.06) 1.5 (0.55–2.43) 2.59 (1.44–3.71) 1.66 (0.68–2.61) 2.13 (1.07–3.16) 2.44 (1.32–3.53) 2.09 (1.04–3.11)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Larson et al. (2006)[28]	27 female (13 phobics: *M*/*S.D.* age = 18.46/52 years and 14 controls: *M*/*S.D.* age = 19.21/1.05 years)	Exposure to phobia-relevant pictures.	BOLD responses in the amygdala early in picture processing consistently differentiated between phobic and nonphobic. Phobic responses were characterized by strong but brief amygdala responses, whereas nonphobic responses were weaker and more sustained.	Phobics: Faster onset for Amygdalae L 3.28 Amygdalae R 2.67	 1.31 (0.46–2.14) 1.07 (0.25–1.87)
Straube et al. (2006)[29]	28 female spider phobics (*M*/*S.D.* age = 22.5/2.90 years) and 14 female controls (*M*/*S.D.* age = 22.3/2.84 years).	Exposure to spider videos.	Activation was greater in the left and right anterior insula, and in the ACC in phobics compared to nonphobic subjects, while controls showed increased activation in the left amygdala and bilaterally in the parahippocampal gyrus. Phobics as compared to nonphobic control subjects exhibited greater responses in the left extrastriate visual cortex (lingual gyrus), while control subjects showed increased activation in the pre- and postcentral gyri.	Phobics > controls ACC 4.51 Insula R 4.03 Insula L 3.39 Lingual gyrus 4.97 Controls > phobics Amygdala L 3.71 Parahippocampal R 4.08 Parahippocampal L 3.91 Precentral gyrus R 5.03 Postcentral gyrus R 4.48	 1.43 (0.71–2.14) 1.27 (0.56–1.96) 1.07 (0.38–1.75) 1.57 (0.83–2.29) 1.17 (0.47–1.85) 1.29 (0.58–1.98) 1.24 (0.54–1.93) 1.59 (0.85–2.31) 1.42 (0.7–2.12)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Straube et al. (2006)[30]	11 right-handed female phobics (*M*/*S.D.* age = 20.9/2.3 years) and 12 right-handed female controls (*M*/*S.D.* age = 21.3/6 years)	Exposure to pictures of spiders and mushrooms. Subjects had to indicate object (identification task) or line orientation (distraction task).	Phobics showed significant greater responses to spiders versus mushrooms in the left amygdala, left insula, left anterior cingulate gyrus (ACC), and left dorsomedial prefrontal cortex (DMPFC) during the identification task and in the left and right amygdala during the distraction task compared to control subjects.	Phobics > controls Identification task DMPFC L 3.3 ACC L 3.5 Insula L 3.4 Amygdala L 4.6 Distraction task Amygdala L 4.8 Amygdala R $t(21) = 3.4$	 1.44 (0.50–2.35) 1.53 (0.58–2.45) 1.48 (0.53–2.40) 2.00 (0.97–3.00) 2.09 (1.04–3.11) 1.48 (0.53–2.40)
Goossens et al. (2007)[31,a]	13 female and 2 male phobics (*M*/*S.D.* age = 24/2 years) and 12 female and 2 male control subjects (*M*/*S.D.* age = 23/1 years).	Exposure to phobia relevant, potentially fear-relevant, and neutral stimuli.	The involvement of the amygdala and implication of the pulvinar nucleus of the thalamus in the process of phobic fear was replicated.	Phobics > controls Amygdala L 3.71 Thalamus, pulvinar R 7.18 Thalamus, pulvinar L 3.53	 1.43 (0.53–2.30) 2.8 (1.66–3.91) 1.36 (0.47–2.22)
Hermann et al. (2007)[32]	9 phobic (*M*/*S.D.* age = 22.9/4.7 years) and 10 non-phobic (*M*/*S.D.* age = 27.6/10.7 years).	Exposure to phobic, disgust, fear and neutral pictures and rating them as disgust, fear, valence and arousal.	Patients compared to controls for phobia-relevant inducing pictures diminished in medial prefrontal cortex (MPFC) activity. The comparison between both groups showed more pronounced activation in the left SMA for Phobia > Neutral pictures in patients relative to controls.	Phobics > controls Phobia > neutral SMA L 4.51 Disgust > neutral DLPFC L 4.87 Controls > phobics Phobia > neutral DMPFC L 4.79 DMPFC R 6.56 VMPFC L 4.29	 2.19 (1.01–3.33) 2.36 (1.14–3.54) 2.32 (0.42–1.57) 3.18 (0.85) 2.08 (0.72)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Schienle et al. (2007)[33]	27 female phobics and 25 non-phobic females. Both groups were comparable (*M*/*S.D.* age = 24.6/6.3 years; years of education: *M* = 14.5; medication-naïve and right-handed).	Exposure to phobia-relevant, fear-inducing, disgust-inducing and affectively neutral pictures.	Relative to nonphobic participants, patients displayed increased activation in the amygdala and the fusiform gyrus as well as decreased activation in the medial orbitofrontal cortex (OFC) during exposure.	Phobics > controls Fusiform gyrus R 3.6 Fusiform gyrus L 3.7 Amygdala L 2.8 Controls > phobics IFG COF (med) L/R 5.7 IPG R 5.5 CCA L 4.4 CCA R 4.3 DLPFC R 4.1 OFC (med) R 5.3	 1 (0.42–1.57) 1.04 (0.45–1.62) 0.78 (0.21–1.34) 1.6 (0.97–2.22) 1.54 (0.91–2.15) 1.23 (0.63–1.82) 1.2 (0.60–1.79) 1.15 (0.56–1.73) 1 (0.42–1.57)
Straube et al. (2007)[34]	16 right-handed female phobics (*M*/*S.D.* age = 21.8/0.6 years) and 15 right-handed female controls (*M*/*S.D.* age = 22.7/0.9 years).	Anticipation of the blocks of spider and mushroom pictures after a cue presentation.	Results showed increased activation of the dorsal anterior cingulate cortex (ACC), insula, thalamus, and visual areas in phobics compared to controls during anticipation of phobia-relevant versus anticipation of neutral stimulation. Also, increased activation of the bed nucleus of the stria terminalis (BNST).	Phobics > controls ACC 3.59 Insula R $t(13) = 3.27$ Bed nucleus of the stria terminalis L t3.88 Fusiform gyrus R 4.12 Fusiform gyrus L 4.17 Thalamus R 4.14	 1.33 (0.54–2.10) 1.21 (0.43–1.97) 1.44 (0.63–2.22) 1.5 (0.69–2.29) 1.55 (0.73–2.35) 1.5 (0.69–2.29)
Wendt et al. (2008)[35]	16 phobics (*M*/*S.D.* age = 23.1/5 years) and 16 controls (*M*/*S.D.* age = 21.3/5 years)	Sustained exposure to phobia- relevant stimuli.	Insula activation was increased during sustained phobic exposure in phobics. It suggests that the activation of the amygdala in fMRI studies primarily indexes the detection of motivationally relevant stimuli whereas the insula might be more specifically linked to defensive response mobilization.	Phobics > controls Insula R 3.39 Insula L 3.24	 1.38 (0.59–2.15) 1.32 (0.54–2.08)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Britton et al. (2009)[36]	12 phobics (7 female; *M* age = 25.2 years) and 12 controls (8 female; *M* age = 26.7 years).	Emotional Stroop response to phobia-related videos, pictures, and words compared to neutral stimuli.	The SAP group showed greater activation than the HC group for the phobia-related versus neutral word contrast. HCs exhibited greater right amygdala and posterior insula activations as well as a greater thalamic deactivation than the SAP group.	ACC Rostral 2.7 Activation Phobics > controls ACC Rostral/dorsomedial prefrontal cortex 3.08 Controls > phobics Amygdala R 2.95 Posterior insula 3.09 Deactivation Controls > phobics Thalamus 3.12	1.15 (2.01–0.27) 1.61 (0.67–2.53) 1.51 (0.58–2.41) 1.62 (0.67–2.53) 1.65 (0.70–2.57)
Schienle et al. (2009)[37]	10 phobic females (*M*/*S.D.* age = 29.1/11.5) and 8 non-phobic females with a comparable age (*M*/*S.D.* age = 24/3.7 years).	Exposure to phobia-relevant and affectively neutral pictures.	Patients showed greater insula activation and less medial OFC activation than control subjects.	Phobics > controls Insula L 3 Control > phobics Medial OFC L 4.1 Medial OFC R 3.3	 1.5 (0.42–2.54) 2.05 (0.86–3.20) 1.65 (0.54–2.72)
Caseras et al. (2010)[10]	14 phobics (12 female) and 17 controls (15 female).	Viewing of phobic or neutral pictures.	Both phobia groups showed a quicker time-to-peak in the right amygdala than controls, but only spider phobics also differed from controls in this parameter within the left amygdala.	Spider phobia Amygdala L M/SD = 4.95/0.70 Amygdala R M/SD = 4.82/0.72 Insula R *M* = 5.35(0.71)	 0.90 (0.15–1.63) 0.91 (0.16–1.65) 0.94 (0.19–1.68)
Lipka et al. (2011)[38,a]	18 female phobics (*M*/*S.D.* age = 25.56/5.26 years) and 18 female controls (*M*/*S.D.* age = 24.72/5.00 years).	Exposure to phobia-relevant stimuli.	Compared with control subjects, phobic participants showed stronger responses of both amygdalae to consciously perceived spiders versus nonspider targets.	Phobics > controls Bilateral amygdala R 4.33 L 3.63	 1.48 (0.73–2.21) 1.24 (0.52–1.95)

Table 1 (*Continued*)

Author (year)	Participants	Procedure	Results	Comparison testing (t/F; $p < .05$)	ES (95% CI)
Lueken et al. (2011)[17,a]	41 subjects (phobics = 12; controls = 17).	Exposure to phobogenic video stimulation.	In an ROI approach, comparisons between SP and healthy controls exhibited substantial overlaps encompassing significant hemodynamic responses in the (dorsal) anterior cingulate gyrus, insula, middle frontal gyrus, and thalamus. Insula peak values differed significantly between SP and controls.	Phobics > controls ROI analysis Phobics > controls Thalamus R 4.65 Thalamus L 4.09 Insula R 4.65 Insula L 4.11 AC gyrus L 4.17 MFG R 5.17 MFG L 3.65	 1.79 (0.9–2.66) 1.57 (0.71–2.41) 1.79 (0.9–2.66) 1.58 (0.72–2.42) 1.61 (0.74–2.45) 1.99 (1.07–2.89) 1.4 (0.56–2.22)
Schweckendiek et al. (2011)[39]	15 phobics (2 males; *M*/*S.D.* age = 23.53/3.27) and 14 healthy control subjects (two males, *M*/*S.D.* age = 23.64/3.43 years).	Differential picture–picture conditioning paradigm.	No differences between patients and healthy controls emerged regarding the nonphobia-related conditioned stimulus. No differences in general conditionability between patients with specific phobias and healthy controls.	Phobics > controls PFC Med 4.06 Insula 3.7	 1.56 (0.71–2.39) 1.4 (0.57–2.21)
Killgore et al. (2014)[40,a]	65 right-handed adults, 22 controls (*M*/*S.D.* age = 30.7/9.2 years; 14 female) and 15 phobics (*M*/*S.D.* age = 35.6/8.7 years; 11 female)	Exposure to emotional faces.	Phobics showed no regions of greater activation compared to controls but did show significantly reduced activation within the vmPFC/OFC region.	Controls > phobics SOFG L 3.94	 1.33 (0.59–2.05)

M: mean; *S.D.*: standard deviation; *t*: Student's *t*; *F*: Snedecor's *F*; SAP: small animal phobia; HC: healthy control; R: right; L: left; ROI: regions of interest; PFC: prefrontal cortex; DM: dorsomedial; ACC: anterior cingulate cortex; VM: ventromedial; DLPFC: dorsolateral prefrontal cortex; DMPFC: dorsomedial prefrontal cortex; LOFC: lateral orbital prefrontal cortex; SMA: supplementary motor area; IPC: inferior parietal cortex; SPC: superior parietal cortex; VMPFC: ventromedial prefrontal cortex; OFC: orbitofrontal cortex; IFG: inferior frontal gyrus; SOFG: superior orbitofrontal gyrus; PCC: posterior cingulate cortex; MFG: middle frontal gyrus; IPG: inferior parietal gyrus.

[a] Studies that have used 3 T scanner.

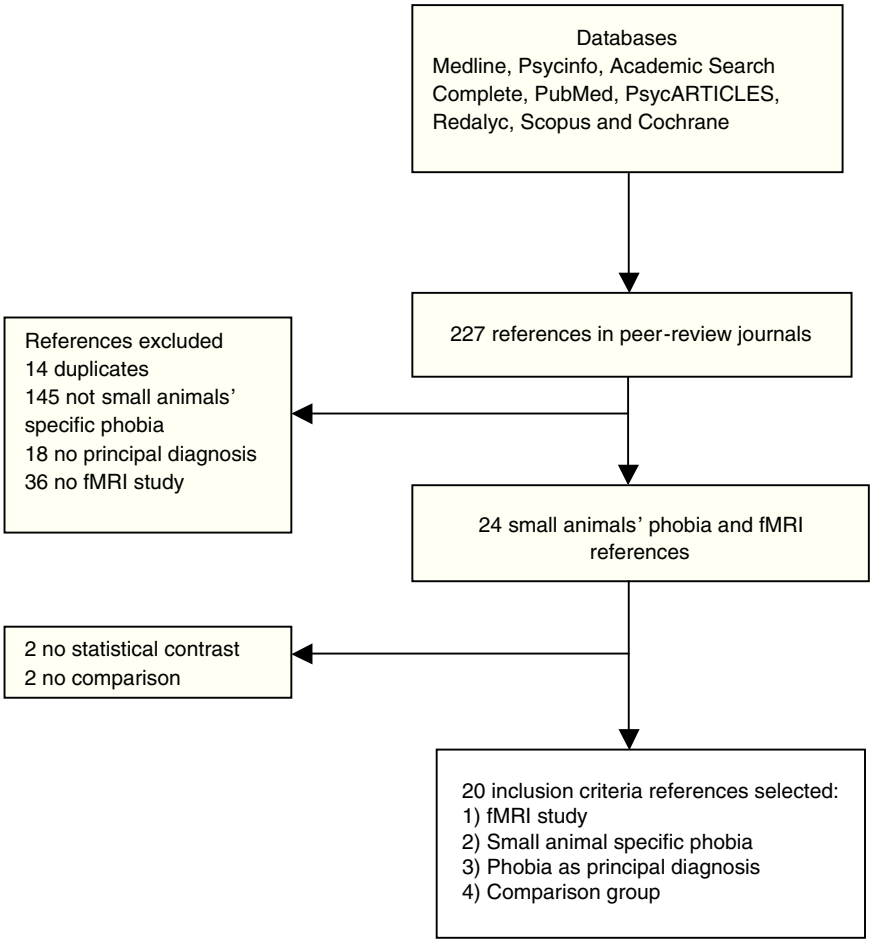

Figure 1 Flow chart of identification of studies' process and selection.

thalamus (0.11), the visual cortex (0.10), and the cerebellum (0.07). Only the amygdala obtained a coefficient close to moderate level.

Mean differences in brain activity in healthy controls as compared to individuals with SP were most frequently located in the left and right dorsolateral and medial prefrontal cortex (0.12), the left fusiform gyrus (0.13), and the bilateral orbitofrontal cortex (0.10). Yet, the most prominent result was the heterogeneity of healthy control participants, as only the right medial orbitofrontal cortex was found in more than one study.

Discussion

The main contributions of this meta-analytic study have been reducing the differences between studies adding greater methodological control and to provide data on the two pathway processing system phobic stimuli. The random-effect analysis confirmed the fear circuit in specific phobias and showed high overall effect size at the ROI or voxel as in previous studies.[41,42] The main results revealed an increase in brain activity in the left amygdala and insular cortex in phobic individuals exposed to phobia-related images. An increase in amygdala activation has been found both for static stimuli and moving stimuli. This is due to the presence of feared stimuli: phobic participants showed intense and short responses in the amygdala, while the control group showed weaker and sustained responses. Moreover, shorter starting time for the activation peak in this brain region in phobic individuals was observed.

Other structures involved in phobia responses are the fusiform gyrus, the left dorsolateral prefrontal cortex, and the left cingulate cortex. However, the role of frontal areas seems to be less stable than that of limbic areas. Previous research confirmed the hyperactivation of the fear circuit (amygdala, insula, anterior cingulate and prefrontal cortex).[41] Although other studies have found that anxiety patients would exhibit dysfunctions in the amygdala–prefrontal emotion regulation network using fMRI.[43] The results revealed abnormal connectivity between the OFC and the amygdala in patients in comparison with healthy controls. They assume a reduction of prefrontal control over amygdalar activation in patients. This inconsistency may indicate that these cognitive areas are not always present when phobic patients process the feared stimuli.

These results confirm the involvement of the amygdala and insular cortex, which play a central role in fear processing in phobic individuals. They may suggest that the activation of the amygdala primarily indicates the detection of motivationally relevant stimuli, whereas the insula might be more specifically connected to defensive response mobilization.[35]

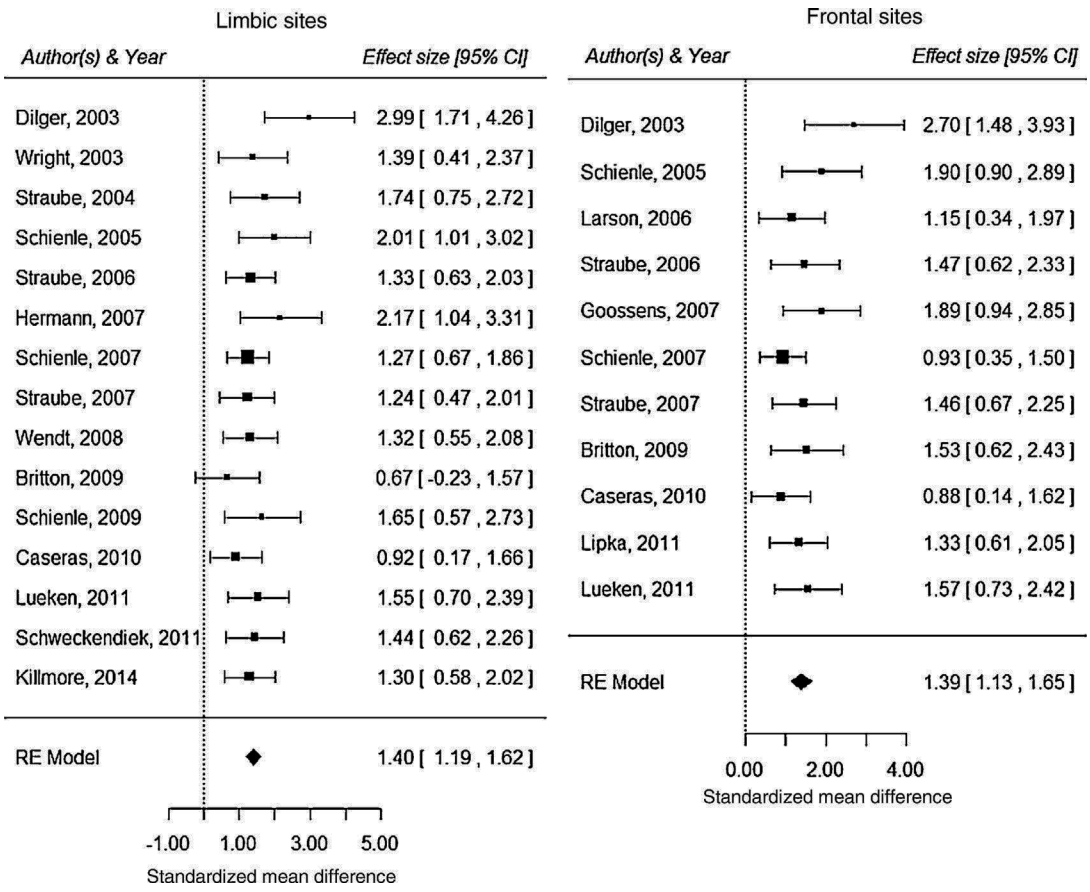

Figure 2 Forest plot.

These results are consistent with those reported in others systematic reviews[8,9] and meta-analyses.[10] Yet, if we consider the effect sizes found, we can highlight the relevance of the amygdala and insula in the processing of feared stimuli. Secondary structures such as prefrontal areas, the orbitofrontal cortex, and the cingulate cortex may play a differential role in the response to feared stimuli.

These results provided evidence for the hypothesis that there is increased activation in specific brain regions during anticipatory anxiety in individuals with SP. This confirms the results that reported an increase in activation of the dorsal ACC, insula, thalamus, and visual areas in phobic individuals during phobia relevant images compared to neutral pictures.[34]

It can be hypothesized that there is a functional network in the processing of phobic stimuli with two pathways whose central area is the amygdala, as expected. An initial network is represented by traces of information as a rapid response mechanism, with subcortical characteristics. A second network is represented by a detailed analysis of perceived stimuli. It implies a slower processing with the involvement of the sensory cortex and finally the prefrontal cortex. A phobic reaction occurs in both functional networks, but the second network implies a certain cognitive control over the emotional response. The two pathways processing phobic stimulus might suggest the existence of a double form of control response through the prefrontal cortex from the degree of accuracy in identifying the stimulus: an automatic response from the ventral prefrontal cortex and an intentional response mediated by dorsal anterior cingulate and prefrontal cortex. The deficit in this system appears to be associated with altered activity in phobic subjects medial prefrontal cortex.[44] This also could explain that the phobic stimuli produce different peaks of activation between subjects due to that the effort to achieve emotional regulation may be lesser whether the stimuli are less threatening.

If these two pathways are present in the processing of feared contexts, a better knowledge of cognitive involvement in that processing will be especially helpful: knowing the conditions in which cognitive processing is activated can have clear clinical implications, since cognitive mechanisms can mediate (and eventually suppress) the anxiety reaction.

Although this paper attempted to reduce the variability of methodological characteristics of studies about the neural basis of phobias, some methodological differences still remain and can be seen as limitations of our results. Specifically, not all the studies used a similar fMRI machine. Most studies used 1.5 T scanner, and only 4 (20%) used 3 T. A 1.5 T is completely adequate for most studies but higher spatial resolution (3 T) can provide a better delimitation of the brain structures detected.[45] The 3 T have detected mainly additional areas of activation with the motor paradigm[46] and should offer better results for frontal pathway. Moreover, specific effects may occur depending on whether pictures

or videos are presented. Future reviews could clarify the different ways of presenting stimuli blocks and perhaps the specific content of those blocks.

In conclusion, this meta-analysis confirms the neural structure of fears, with limbic areas playing the main role but with a differentiated participation of frontal areas as well. The existence of a slow cognitive processing of feared stimuli by phobic individuals provides an opportunity to learn how this pathway is activated, which would have implications for the treatment of specific phobias.

Conflict of interest

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the paper.

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