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Brain activation to phobia-related pictures in spider phobic humans: an event-related functional magnetic resonance imaging study

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Abstract

Using event-related functional magnetic resonance imaging we investigated blood oxygen level dependent brain activation in spider phobic and non-phobic subjects while exposed to phobia-related pictures (spiders) and non-phobia-related pictures (snakes and mushrooms). In contrast to previous studies, we show significantly increased amygdala activation in spider phobics, but not in controls, during presentation of phobia-relevant visual stimuli. Furthermore, phobia-specific increased activation was also found in the insula, the orbitofrontal cortex and the uncus. Our study confirms the role of the amygdala in fear processing and provides insights into brain activation patterns when animal phobics are confronted with phobia-related stimuli.

Keywords: Spider phobia; Anxiety; Amygdala; Event-related functional magnetic resonance imaging; Insula

Although the role of the amygdala in the processing of fear-related stimuli is widely recognized (see for review refs. [13,15]), previous neuroimaging studies did not find increased amygdala activation when animal phobics were exposed to phobia-related visual stimuli. Using positron emission tomography (PET), Wik et al. [23] showed increased regional cerebral blood flow (rCBF) in the secondary visual cortex but reduced rCBF in different temporal and frontal regions of the brain during processing of phobia-related as compared to neutral visual stimuli. Similar PET results were reported by Fredrikson et al. [8,9]. A previous blocked functional magnetic resonance imaging (fMRI)-study found activation of the prefrontal, parahippocampal, and visual cortex but reported no evidence of amygdala activation when spider phobics were exposed to film excerpts depicting spiders [16]. In contrast to these results, several fMRI-studies have shown specific activation of the amygdala to conditioned fear-eliciting stimuli (see for review ref. [3]) and in social phobics in response to face pictures [1,20,22].

Using an event-related fMRI design, the present study reexamined the question of whether the amygdala is significantly activated when animal phobics are exposed to phobia-related stimuli. Besides the amygdala, we also investigated whether additional brain regions show significant phobia-specific blood oxygen level dependent (BOLD) signal increases. Ten female spider phobic subjects (age: 25 ± 2.3 years) and ten female healthy control subjects (age: 21.3 ± 0.6 years) participated in the study. One subject of each group had to be excluded from analysis due to fMRI signal artefacts. Subjects were diagnosed as spider-phobics prior to the experiment if they obtained high scores on a spider phobia questionnaire (SPQ, [11]: $M = 70.1$, $SD = 12.0$) and met the criteria of the diagnostic and statistic manual of mental disorders for spider phobia [24]. Control subjects did not reveal any sign of phobia (SPQ: $M = 4.3$, $SD = 4.6$). Furthermore, according to the outcome of the diagnostic interview and scores of a symptom-checklist (SCL-90-R, [7]) all subjects were free from additional psychopathological disorders. The study was approved by the ethics committee of the University of Jena

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and written informed consent was obtained from each participant prior to the experiment.

In the 1.5 T magnetic resonance scanner ('Magnetom Vision plus', Siemens, Medical Systems) subjects were exposed to a series of phobia-relevant, potentially fear-relevant, and neutral stimuli (pictures of spiders, snakes and mushrooms) and – as a 'null event' (see ref. [10]) – to a fixation cross (56 pictures of each category). Pictures were comparable in spatial extent and background colour and were presented in pseudo-random order via a back-projection screen on an overhead mirror for 1 s each with a variable inter-stimulus interval of 2.27–10.54 s between succeeding stimuli. While showing the pictures, 300 volumes were acquired using a T2* weighted echo-planar sequence (TE = 60 ms, flip angle = 90°, matrix = 64 × 64, FOV 192 mm, TR = 2.27 s). Each volume comprised 20 axial slices (thickness = 3 mm, gap = 0.9 mm, in plane resolution = 3 × 3 mm), covering the temporal lobe, the occipital lobe and the inferior frontal lobe. Analysis of the functional data showed sufficiently imaging of the amygdala region when using a voxel size of 3 × 3 × 3 mm. Additionally, a high-resolution T1-weighted anatomical volume (192 slices, TE = 5 ms, TR = 15 ms, matrix = 256 × 256, voxel size = 1 × 1 × 1 mm) was recorded. Preprocessing, statistical analysis and presentation of the results was done using the software Brain Voyager 2000 (Version 4.6; Brain Innovation, Maastricht, Holland). The first five functional volumes were discarded to allow for signal equilibration. All volumes were realigned to the first volume and corrected for slice time errors, temporally filtered with a high-pass filter (allowing a minimum of 10 cycles per session) and a low-pass filter (3.8 s full width at half maximum [FWHM] Gaussian kernel), and spatially smoothed (6 mm FWHM isotropic Gaussian kernel). Anatomical and functional volumes were coregistered and normalized to the Talairach space [21]. Statistical analysis was performed using the general linear model. The expected BOLD signal change for each event type was modelled by a canonical hemodynamic response function (modified gamma function; delta = 2.5, tau = 1.25). After z-transformation of the signal values, within- and between-group statistical comparisons were conducted using a fixed effect multi-subject-multi-study model as implemented in Brain Voyager. Because of our main question, the amygdala was defined a priori as a region of interest (ROI) using a sphere of 9 mm radius. For the ROI analysis a significance level of $P < 0.0005$ was chosen (cluster threshold: 15 voxel). In exploratory statistical analysis Bonferroni correction for multiple comparisons was applied ($P < 0.05$, corrected; cluster threshold: 15 voxel).

ROI analysis showed specific activation of the amygdala when phobic subjects were exposed to phobia-related stimuli. In spider phobics, the within-group linear contrasts *Spider* > *Mushroom* as well as *Spider* > *Snake* revealed activation of the left amygdala (see Table 1; for the contrast *Spider* > *Snake* see Fig. 1a). In controls, the same contrasts failed to show any significant amygdala activation (for the contrast *Spider* > *Snake* see Fig. 1b). These results were confirmed by contrasting the same stimulus categories between phobics and controls. There was a significant

Table 1
Summary of results[a]

	Side	Coordinates	*t*-value
Within-group contrast			
Controls			
Spider > *Mushroom*			
Primary visual cortex	R	22, −97, 8	6.07[b]
Mushroom > *Spider*	No differential activation		
Spider > *Snake*	No differential activation		
Snake > *Spider*	No differential activation		
Within-group contrast			
Spider phobics			
Spider > *Mushroom*			
Amygdala	L	−19, 0, −14	3.96[c]
Insula	R	37, 15, 11	6.84[b]
Insula	L	−50, 9, 2	6.36[b]
Orbitofrontal cortex	R	34, 20, −7	5.32[b]
Fusiform gyrus	R	46, −48, −22	7.28[b]
Fusiform gyrus	L	−38, −36, −25	6.08[b]
Uncus	L	−26, −3, −37	5.61[b]
Mushroom > *spider*			
Posterior cingulate gyrus		4, −48, 24	6.81[b]
Spider > *Snake*			
Amygdala	L	−17, −3, −19	4.42[c]
Insula	R	40, 15, 8	5.88[b]
Insula	L	−50, 12, 5	6.52[b]
Snake > *Spider*			
Posterior cingulate gyrus	L	−11, −66, 14	5.64[b]
Temporal gyrus		−57, −9, −10	5.20[b]
Between-group contrast			
Phobics > Controls			
Spider			
Amygdala	L	−17, −3, −22	4.41[c]
Posterior cingulate		−8, −17, 32	5.98[b]
Gyrus insula	R	34, 9, −4	6.87[b]
Insula	L	−50, 9, 2	6.60[b]
Orbitofrontal cortex	L	−38, 27, −13	7.03[b]
Fusiform gyrus	R	34, −39, −25	8.82[b]
Fusiform gyrus	L	−29, −36, −28	6.45[b]
Uncus	L	−26, −9, −34	5.84[b]
Snake			
Fusiform gyrus	R	43, −58, −22	6.01[b]
Mushroom			
Fusiform gyrus	L	−38, −51, −25	5.58[b]
Uncus	L	−29, −14, −35	5.58[b]
Between-group contrast			
Controls > Phobics			
Spider	No differential activation		
Snake	No differential activation		
Mushroom			
Fusiform gyrus	L	−31, −44, −17	5.21[b]

[a] L = left, R = right; only clusters >15 significant voxels are shown.
[b] *P*(corr.) < 0.05.
[c] *P*(uncorr.) < 0.0005.

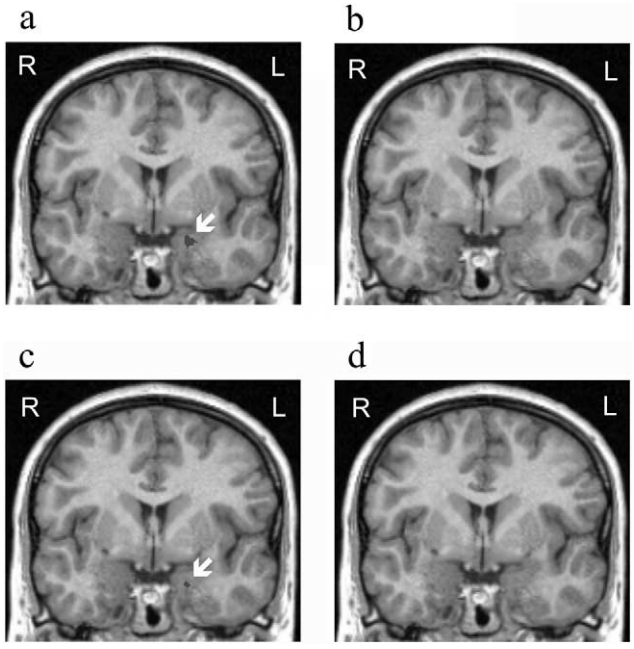

Fig. 1. Phobia-specific activation of the amygdala. For the within-group contrast *Spider* > *Snake* significant amygdala activation was found in spider phobic subjects (a) but not in control subjects (b). Between-group comparisons revealed significant amygdala activation for the contrast *Spider/phobics* > *Spider/controls* (c) but not for the contrast *Snake/phobics* > *Snake/controls* (d). Amygdala activation is indicated by an arrow. Talairach coordinate for all pictures y = −2.

activation of the left amygdala for spider pictures in phobics, whereas no between-group differences in amygdala activation were found for the other stimulus categories (see Table 1; for the contrasts *Spider/phobics* > *Spider/controls* and *Snake/phobics* > *Snake/controls* see Figs. 1c,d).

Besides the result from the ROI analysis, the exploratory within-group analysis revealed significant activations of the right and left insula in phobic subjects when BOLD-responses to spider pictures were compared to BOLD-responses to snake or mushroom pictures (see Table 1). In addition, for phobic subjects the contrast *Spider* > *Mushroom* indicated a significantly stronger activation of the bilateral fusiform gyrus, the left uncus and the right orbitofrontal cortex (see Table 1). In control subjects, the contrast *Spider* > *Mushroom* revealed activation of the primary visual cortex only (see Table 1).

The between-group contrast for spider pictures showed stronger activation in phobic subjects in the right and left insula, the right orbitofrontal cortex, the left uncus, the right and left fusiform gyrus and the posterior cingulate cortex (see Table 1). Between-group contrasts 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 (see Table 1).

To our knowledge, the present study provides the first evidence for a phobia-specific amygdala activation in animal phobics when subjects are exposed to phobia-related stimuli. Similar results seem difficult to obtain in PET-studies, possibly due to the low temporal resolution of PET (see also ref. [8]). However, as described above, the recent fMRI-study by Paquette et al. [16] found no evidence of amygdala activation in spider phobics during phobia-related visual stimulation. The authors suggested that the amygdala might not be as important for fear expression and/or experience as for the acquisition and pathogenesis of specific phobias [16]. However, our results show that brief exposure of animal phobics to phobia-related stimuli is associated with increased amygdala activity. A failure to demonstrate animal phobia-specific activation of the amygdala in previous neuroimaging studies might also be related to stronger habituation effects in block designs [2] on which all of the earlier studies were based. Furthermore, the corrected *P*-value used by Paquette et al. may have been too conservative to detect amygdala activation. Currently, we investigate the relation between study design and BOLD intensity changes during phobia-related stimulation in more detail.

The significant differences in amygdala activity were limited to the left amygdala. This is consistent with previous evidence for a left-lateralized activation of the amygdala during negative emotion processing (e.g. refs. [2,17]), although some studies have reported opposite results [4, 25]. Interestingly, Morris et al. [14] showed that left amygdala activation was specifically associated with the presentation of unmasked and thus consciously perceived pictures of angry faces, while the right amygdala was activated when subjects did not consciously perceive masked pictures. At least for conscious processing of briefly presented animal phobia-related stimuli our data support a special role of the left amygdala.

Besides amygdala activation, we found evidence for an involvement of further brain regions in the processing of phobia-related stimuli, such as the insula and the orbitofrontal cortex. Insula activation can be induced by a variety of aversive stimuli like disgust- or fear-related pictures [18, 19] and seems to be implicated in the representation of internal bodily states of arousal [5]. The finding of an activation of orbitofrontal cortex is in line with studies suggesting that this area is part of an emotion regulation circuitry [6]. Interestingly, visual areas showed stronger activation in spider phobics as well as in controls when spider pictures were compared with mushroom pictures. This observation confirms previous studies indicating higher activation of visual areas in response to emotional pictures (including spiders) as compared to neutral pictures (e.g. ref. [12]). Additionally, the between group comparison revealed that mushroom pictures activated spatially separated clusters within the fusiform gyrus, an area important for visual object processing.

In summary, our findings are consistent with models predicting a specific function of the amygdala in the processing of fear-related stimuli [13,15]. Furthermore, it

was shown that various brain regions known to be involved in the processing of emotional stimuli, such as the insula or the orbitofrontal cortex, are also activated by phobia-related stimuli in animal phobics.

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