NeuroImage 54 (2011) 681–688

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Weaving the (neuronal) web: Fear learning in spider phobia

Jan Schweckendiek [a,*], Tim Klucken [a], Christian J. Merz [a,b], Katharina Tabbert [a], Bertram Walter [a], Wolfgang Ambach [c], Dieter Vaitl [a], Rudolf Stark [a]

[a] Bender Institute of Neuroimaging, University of Giessen, Otto-Behaghel-Str. 10H, 35394 Giessen, Germany
[b] Department of Cognitive Psychology, Ruhr-University Bochum, Universitätsstr. 150, 44780 Bochum, Germany
[c] Institute for Frontier Areas of Psychology and Mental Health (IGPP), Clinical and Physiological Psychology, Wilhelmstr. 3a, 79098 Freiburg, Germany

ARTICLE INFO

Article history:
Received 3 May 2010
Revised 19 July 2010
Accepted 21 July 2010
Available online 27 July 2010

Keywords:
Specific phobia
Classical conditioning
fMRI
Fear learning
Amygdala
Medial prefrontal cortex

ABSTRACT

Theories of specific phobias consider classical conditioning as a central mechanism in the pathogenesis and maintenance of the disorder. Although the neuronal network underlying human fear conditioning is understood in considerable detail, no study to date has examined the neuronal correlates of fear conditioning directly in patients with specific phobias. Using functional magnet resonance imaging (fMRI) we investigated conditioned responses using phobia-relevant and non-phobia-relevant unconditioned stimuli in patients with specific phobias ($n=15$) and healthy controls ($n=14$) by means of a differential picture–picture conditioning paradigm: three neutral geometric figures (conditioned stimuli) were followed by either pictures of spiders, highly aversive scenes or household items (unconditioned stimuli), respectively. Enhanced activations within the fear network (medial prefrontal cortex, anterior cingulate cortex, amygdala, insula and thalamus) were observed in response to the phobia-related conditioned stimulus. Further, spider phobic subjects displayed higher amygdala activation in response to the phobia-related conditioned stimulus than to the non-phobia-related conditioned stimulus. Moreover, no differences between patients and healthy controls emerged regarding the non-phobia-related conditioned stimulus. The results imply that learned phobic fear is based on exaggerated responses in structures belonging to the fear network and emphasize the importance of the amygdala in the processing of phobic fear. Further, altered responding of the fear network in patients was only observed in response to the phobia-related conditioned stimulus but not to the non-phobia-related conditioned stimulus indicating no differences in general conditionability between patients with specific phobias and healthy controls.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Specific phobias are among the most common anxiety disorders with a lifetime prevalence of about 10% (Fyer, 1998). A specific phobia is characterized as a "marked and persistent fear that is excessive and unreasonable, cued by the presence or anticipation of a specific object or situation" (American Psychiatric Association, 1994). Classical fear conditioning is assumed to be an important mechanism in the pathogenesis of specific phobias (e.g. Antony and Barlow, 2002; Armfield, 2006; Mineka and Zinbarg, 2006; Mineka and Oehlberg, 2008). Classical fear conditioning describes the process by which an initially neutral stimulus (conditioned stimulus, CS) acquires emotional quality through the pairing with an emotionally salient aversive stimulus (unconditioned stimulus, UCS). After few pairings the CS elicits alterations in various behavioral and physiological measures (conditioned responses; CR), such as skin conductance responses (SCRs), startle amplitudes, brain activity and changes in valence in subjective evaluations (e.g. Büchel and Dolan, 2000; de Houwer et al., 2001; Hamm and Weike, 2005; Klucken et al., 2009a; Lang et al., 1998). Imaging studies typically utilize differential fear conditioning paradigms, in which a second unpaired CS (CS−) serves as control stimulus. The CR is then operationalized as the difference of responses to CS+ and CS−. Although a large body of research has led to a quite sophisticated understanding of the neuronal mechanisms underlying fear conditioning in animals and healthy control subjects, no study to date has examined the neuronal correlates of fear conditioning directly in patients with specific phobias.

Abbreviations: ACC, anterior cingulate cortex; BOLD, blood oxygen level dependant; CR, conditioned response; CS, conditioned stimulus; CS−, control conditioned stimulus; CS+A, conditioned stimulus predicting the aversive scenes; CS+S, conditioned stimulus predicting the spider scenes; FIR, first interval response; fMRI, functional magnet resonance imaging; FWHM, full width at half maximum; IAPS, International Affective Picture System; ITI, intertrial interval; M, mean; MNI, Montreal Neurological Institute; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; ROI, region of interest; SCR, skin conductance response; SD, standard deviation; SIR, second interval response; SPQ, spider phobia questionnaire; UCR, unconditioned response; UCS, unconditioned stimulus.

* Corresponding author. Fax: +49 641 9926309.
E-mail addresses: Jan.H.Schweckendiek@psychol.uni-giessen.de (J. Schweckendiek), Tim.Klucken@psychol.uni-giessen.de (T. Klucken), Christian.Merz-a3p@ruhr-uni-bochum.de (C.J. Merz), tabbert@bion.de (K. Tabbert), Bertram.Walter@psychol.uni-giessen.de (B. Walter), ambach@igpp.de (W. Ambach), vailt@bion.de (D. Vaitl), Rudolf.Stark@psychol.uni-giessen.de (R. Stark).

1053-8119/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2010.07.049

Regarding the neuronal correlates of conditioned fear, animal and human studies identified the amygdala as a key region within a neuronal network of fear learning and expression (e.g. Büchel and Dolan, 2000; LaBar and LeDoux, 1996; LeDoux, 2000; Phan et al., 2004).

Further, recent imaging studies point to the anterior cingulate cortex (ACC), the insula, the medial prefrontal cortex (mPFC), the orbitofrontal cortex (OFC) and the thalamus as neuronal correlates of conditioned fear responses (for a review see Sehlmeyer et al., 2009).

Imaging studies concerning fear elicitation in phobic patients have focussed mainly on symptom provocation, i.e. the confrontation with videoclips or pictures of their feared object (e.g. Dilger et al., 2003; Schienle et al., 2005; Veltman et al., 2004). A current meta-analysis of these studies documents exaggerated activations in the amygdala, the insula and the cingulate cortex in phobic patients (Etkin and Wager, 2007). In addition, two studies investigated phobic fear using anticipation paradigms (i.e. subjects were instructed that phobia-relevant material would follow a certain cue): An early positron emission tomography study found decreased regional cerebral blood flow in primary visual areas, which was interpreted as a neuronal correlate of avoidance behavior (Wik et al., 1996). A more recent study reported enhanced BOLD responses in the ACC, the insula, the thalamus and the fusiform gyrus (Straube et al., 2007). In addition, the strength of BOLD responses was correlated with self-reported anticipatory anxiety in the ACC and the mPFC. Taken together, these results implicate that phobic fear is based on exaggerated responses in structures that are also involved in fear conditioning in healthy controls.

Although it is of great relevance for the treatment of specific phobias to understand the neuronal correlates of fear learning processes elicited by phobia-relevant UCS, to our best knowledge no study to date has targeted this issue. To extend the current knowledge in this area we developed a novel conditioning paradigm using the picture–picture conditioning approach. Subjects were exposed to three different CS-types: One CS (CS+S) was paired with phobia-relevant pictures (spiders), a second CS (CS+A) with highly aversive scenes (e.g. mutilations) and a third CS (CS−) with neutral pictures (e.g. household items). The primary goal of the study was to examine the correlates of CRs to the CS+S in the spider phobic subjects relative to healthy control subjects. We hypothesized elevated responses in structures of the fear network (amygdala, ACC, insula, mPFC, thalamus). Further, we compared CRs elicited by the phobia-relevant CS+S to those elicited by the non-phobia-relevant CS+A. Additionally, we compared the contrast CS+A vs. CS− between spider phobic and healthy control subjects to test for group differences in general conditionability (cf. Lissek et al., 2005, 2008).

Methods

Subjects

Fifteen spider phobic subjects (two males; $M_{age} = 23.53$; $SD_{age} = 3.27$) and 14 healthy control subjects (two males, $M_{age} = 23.64$; $SD_{age} = 3.43$), were recruited from campus advertisements and received 8€/h for participation. All subjects were students at the University of Giessen, right-handed and had normal or corrected-to-normal vision. Subjects were classified as spider phobic if they met the criteria for specific phobia stated in the Diagnostic and Statistic Manual of Mental Disorders (animal type: spiders; 300.29; DSM-IV, American Psychiatric Association, 1994). Additionally, spider phobic subjects scored highly in the Spider Phobia Questionnaire (SPQ; $M_{SPQ} = 22.13$; $SD_{SPQ} = 2.53$; Klorman et al., 1974). Healthy control subjects did not show symptoms of spider phobia ($M_{SPQ} = 2.29$; $SD_{SPQ} = 1.54$). Additional psychopathological and neurological disorders were precluded by means of a short structured clinical interview (Margraf, 1994). No subject had ever received psychotropic medication. Participants were informed about the procedure in general and gave written informed consent. All experimental procedures were in accordance to the Declaration of Helsinki and were approved by the ethics committee of the German Psychological Society.

Stimuli

Three pictures of geometric figures (a rhomb, a square and a parallelogram) served as CS. All figures were gray in color, had identical luminance and were presented in 800×600 pixel resolution against a black background. Three sets, each consisting of 16 pictures, were employed as UCS. The first set contained close-up views of spiders (UCS+S), the second highly aversive scenes (UCS+A; e.g. mutilations), and the third household items (non-UCS). The highly aversive scenes were chosen because (1) they were successfully used as UCS in a previous study (Klucken et al., 2009a), (2) they had been judged nearly as aversive as spider pictures by spider phobic subjects (Schienle et al., 2005) and (3) received nearly identical ratings by spider phobic and healthy control subjects likewise (Schienle et al., 2005). Neutral pictures were included as "non-UCS" to control for visual processing. Pictures were taken from the International Affective Picture System (IAPS; Lang et al., 1999; picture numbers: 3000, 3071, 3150, 3400, 6150, 7000, 7002, 7004, 7006, 7009, 7010, 7034, 7035, 7041, 7042, 7043, 7052, 7056, 7059, 7080, 7090, 7100, 7175, 7233, 9405) or were collected by the authors. Pictures were comparable with regard to complexity as far as possible in order to prevent confounding effects. Stimuli were projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector (EPSON EMP-7250) and were viewed through a mirror mounted on the head coil.

Procedure

The experiment consisted of one acquisition and two extinction phases. One extinction phase immediately followed the acquisition with no delay (30 unreinforced trials, 10 per CS), the second extinction phase was conducted 24 h later. Only data of the acquisition phase, consisting of 48 trials, is reported here. Each CS was presented 16 times in pseudo-randomized order for 8 s with the restrictions that (1) the same CS could only appear twice in a row at most and (2) an equal quantity of each CS within the first and the second half of the experiment. The UCS was presented immediately after the CS offset for 3 s (100% reinforcement schedule). Each of the UCS pictures was shown only once during acquisition. The CS-UCS allocation was counterbalanced between subjects. In a random (equally distributed) interval of 1–2 s after the UCS offset, participants had to react to a simple distracter task. This procedure was chosen to (1) distract the attention away from the aversive pictures during the inter trial interval (ITI) and (2) to enhance overall vigilance (especially during the extinction phases). For this purpose, an adapted version of the flanker task (Eriksen and Eriksen, 1974) was used: five arrows appeared horizontally in the middle of the screen. The four outer arrows ("flanker stimuli") always pointed in the same direction (left or right), whereas the middle arrow ("target stimulus") pointed either in the same or in the opposite direction of the flanker arrows, resulting into four flanker stimulus variants. Each flanker stimulus was presented four times after each of the UCS-types in a pseudo-random order. Presentation time was 0.5 s at most or until the subject responded via button-press. The ITI was 14–17.5 s. A fixation cross was presented in the centre of the screen during the ITI and the interval between UCS offset and flanker task onset. Prior to scanning, subjects were familiarized with the flanker task and were instructed to attentively observe the different pictures and to look for regularities between the stimuli.

Contingency awareness

Immediately after the entire conditioning procedure (acquisition and extinction) subjects were asked to choose which of the three geometric figures preceded each of the three UCS-types using a forced-

choice questionnaire (cf. Dawson and Reardon, 1973). Subjects were classified as contingency aware if they stated the correct allocations for all three UCS-types. Subsequently, subjective estimations of the time point of the development of contingency awareness were assessed.

Subjective ratings of the UCS and evaluative conditioning

Afterwards, subjects rated their subjective fear, disgust, valence and arousal on a nine-point Likert scale, ranging from 1 ("not frightening at all"; "not disgusting at all"; "very unpleasant"; "calm and relaxed") to 9 ("very frightening"; "very disgusting"; 'very pleasant"; "very arousing") for each CS. Additionally, global ratings of the three UCS-types were assessed using the same rating scales. Difference scores of the three CS-types and the three UCS-types, respectively, were calculated for each individual for each rating dimension (e.g. $fear_{CS+A} - fear_{CS-}$). Statistical analyses of evaluative conditioning and UCS ratings were performed by testing these difference scores using one- and two sample *t*-tests as implemented in PASW for Windows (Release 18.0; SPSS Inc. Illinois).

Skin conductance responses

SCRs were recorded concurrently with the fMRI scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium from the hypothenar eminence of the left hand. Data of one healthy control subject was lost, due to technical difficulties. SCRs were analyzed in three different time windows (Prokasy and Ebel, 1967): The maximum response within 1–4.5 s after CS onset was defined as first interval response (FIR), the maximum response within 4.5–8 s after CS onset was defined as second interval response (SIR). The maximum response within 0.5–3.0 s after UCS onset was defined as the unconditioned reaction (UCR). Subjects that did not show at least two SCRs greater than .05 μS were classified as non-responders and excluded from further analysis leaving 8 subjects in the healthy control group and 13 subjects in the spider phobic group. Outliers were detected and discarded using a distribution-free approach for multivariate skewed data (Hubert and van der Veeken, 2008). To ensure comparability between subjects and to render the distribution more towards normal data of each response interval were individually *z*-transformed. Statistical analyses were performed using paired *t*-tests as implemented in PASW for Windows (Release 18.0; SPSS Inc. Illinois).

Magnetic resonance imaging

Functional and anatomical scans were obtained using a 1.5 T whole-body tomography (Siemens Symphony) with a standard head coil. Structural image acquisition consisted of 160 T1-weighted sagittal images (MPRage, 1 mm slice thickness). A gradient echo field map sequence was acquired before the functional image acquisition to obtain information for unwarping B_0 distortions. For functional imaging a total of 1012 volumes (acquisition phase: 612) were recorded using a T2*-weighted gradient echo-planar imaging sequence (EPI) with 25 slices covering the whole brain (slice thickness = 5 mm; gap = 1 mm; descending slice order; TA = 100 ms; TE = 55 ms; TR = 2.5 s; flip angle = 90°; field of view = 192 mm × 192 mm; matrix size = 64 × 64). The orientation of the axial slices was tilted to parallel the OFC tissue–bone transition to keep susceptibility artefacts to a minimum. Data were analyzed using Statistical Parametric Mapping (SPM5, Wellcome Department of Cognitive Neurology, London, UK; 2005) implemented in Matlab R2007b (Mathworks Inc., Sherborn, MA). Prior to all analyses, unwarping and realignment to the first volume (b-spline interpolation), slice time correction and normalization to the standard space of the Montreal Neurological Institute brain (MNI-brain) were performed. Smoothing was executed with an isotropic three dimensional Gaussian kernel with a full-width-at-half-maximum (FWHM) of 9 mm.

Following experimental conditions were modelled: CS+S, CS+A, CS− (first and second half each), UCS+S, UCS+A, non-UCS, the four flanker task stimuli and button-presses. Durations of CS and UCS were explicitly modelled. Three further regressors of no interest contained the first trial of each CS, as learning could not have happened yet (cf. Phelps et al., 2004). To coarsely account for possible temporal dynamics of the conditioned responses across trials we modelled CS+S, CS+A and CS− for the first and the second half of the experiment separately (referred to as first and second half of acquisition in the following; cf. LaBar et al., 1998; Tabbert et al., 2005). Further, the six movement parameters obtained by the realignment procedure were introduced as covariates in the model. Regressors were convolved with the canonical hemodynamic response function and the high pass filter was set at 128 s. These subject level models were analyzed using the general linear model with prewhitening. Contrasts of beta-estimates were calculated for each individual.

The individual contrasts were analyzed in random effects group analyses using one- and two-sample *t*-tests. The one-sample *t*-tests were computed for the contrasts UCS+S > non-UCS, CS+S > CS− in both groups separately and the contrasts UCS+S > UCS+A, CS+S > CS+A in the spider phobic group. Moreover, we tested for group differences in the contrasts UCS+S > non-UCS, UCS+A > non-UCS, CS+S > CS− and CS+A > CS− using two-sample *t*-tests. All tests involving CS were computed separately for the first and the second half of acquisition. In order to link BOLD responses to evaluative conditioning, we correlated (voxel-wise simple regression) the mean differential (negative) valence and arousal rating scores with neural activations in the contrast CS+A > CS− in the entire sample and the contrast CS+S > CS− in the spider phobic group.

Within all models, we used explorative as well as region of interest (ROI) analyses for the following structures: bilateral amygdala, ACC, mPFC, bilateral OFC, bilateral thalamus and bilateral insula. Masks were taken from the probabilistic Harvard-Oxford Cortical and Subcortical Structural Atlas (included in FSLView version 3.1; http://www.fmrib.ox.ac.uk/fsl/). Voxels were included in a mask if (1) the probability of belonging to the desired structure was higher than the probability for belonging to any other structure and (2) the probability for belonging to the desired structure was $p > .25$. The ACC mask was created by combining the masks for anterior cingulate cortex and paracingulate cortex. The significance threshold was set to $\alpha = .05$ on voxel level tests, corrected for multiple testing (family-wise-error correction; FWE). ROI analyses were carried out using the small volume correction option of SPM5. Additionally, trends until a value of $p_{corr} < .10$ are reported.

Results

Contingency awareness

Three subjects in the healthy control group and four subjects in the spider phobia group did not state the correct contingencies in the forced-choice questionnaire. Contingency aware participants achieved awareness during the initial trials of the experiment (Spider phobia group: $M_{trials} = 6.32$; $SD = 2.42$; healthy control group: $M_{trials} = 5.55$; $SD = 2.11$).

Subjective ratings

Subjective ratings of the UCS

One-sample *t*-tests revealed that the UCS ratings matched the expected rating patterns: spider phobic subjects rated the UCS+S and the UCS+A, respectively, as significantly more fear-inducing, more disgusting, more arousing and more unpleasant compared to the non-UCS (all $p < .01$). Further, spider phobic subjects rated the UCS+S as more fear-inducing as the UCS+A ($p < .01$), no differences were found in the other rating dimensions. Healthy control subjects rated the UCS+A

more fear-inducing, more disgust-inducing, more unpleasant and more arousing than the non-UCS and the UCS+S, respectively (all $p<.01$). Further, healthy control subjects rated UCS+S significantly more disgusting, more unpleasant and more arousing than the non-UCS (all $p<.05$). The two-sample t-tests revealed that the difference score UCS+S − non-UCS was significantly higher in spider phobic subjects compared to controls (all $p<.001$) and the difference score UCS+A − UCS+S was significantly higher in controls compared to spider phobic subjects in all rating dimensions (all $p<.001$). Figure S1 displays the mean post hoc disgust, fear, valence and arousal ratings for the UCS+A, the UCS+S and the non-UCS.

Evaluative conditioning

Fig. 1 displays the mean post hoc disgust, fear, valence and arousal ratings for the CS+A, the CS+S and the CS− in the spider phobic and the healthy control group. The one-sample t-tests revealed that spider phobic subjects rated the CS+S as significantly more fear-inducing, more disgusting, more arousing and more unpleasant compared to the CS− (all $p<.05$) and the CS+A as more disgust-inducing, more unpleasant and more arousing (all $p<.05$) and marginally more fear-inducing ($p<.10$) compared to the CS−. Further, spider phobic subjects rated the CS+S as marginally more fear-inducing compared to the CS+A ($p<.10$). Healthy control subjects rated the CS+A as more disgust-inducing, more arousing, more unpleasant (all $p<.05$) and marginally more fear inducing ($p<.10$) compared to the CS− and the CS+S, respectively. The two-sample t-tests revealed that the difference score CS+S − CS− was significantly higher in spider phobic subjects compared to controls (all $p<.005$) and the difference score CS+A − CS+S was significantly higher in controls compared to spider phobic subjects in all rating dimensions (all $p<.05$).

Skin conductance responses

Unconditioned responses

The spider phobic group showed significantly higher SCRs to the UCS+S compared to the non-UCS ($t_{12}=3.74$; $p=.003$) and significantly higher SCRs to the UCS+A compared to the non-UCS ($t_{12}=2.45$; $p=.031$). No significantly differences were found in the comparison UCS+A vs. UCS+S. The healthy control group showed significantly higher SCRs to the UCS+A compared to the non-UCS ($t_7=3.89$; $p=.006$) and significantly higher SCRs to the UCS+A compared to the UCS+S ($t_7=3.20$; $p=.015$). No significant differences were found in the healthy control group in the comparison UCS+S vs. non-UCS. Figure S2 displays mean z-transformed SCRs and standard errors of the mean for the UCS+A, the UCS+S and the non-UCS in the healthy control and the spider phobic group.

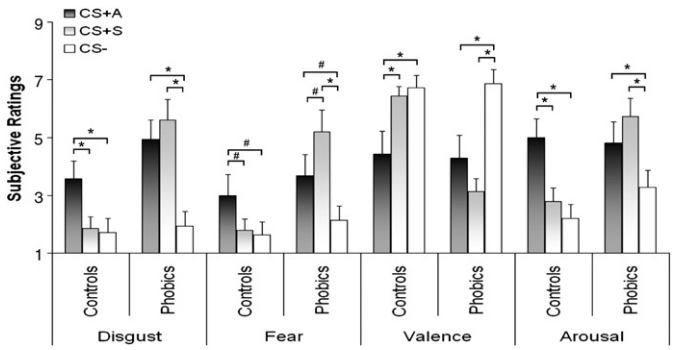

Fig. 1. Mean subjective ratings (and standard errors of the mean) for the CS+A, the CS+S and the CS− in the healthy control and the spider phobic group. * indicates $p<.05$; # indicates $p<.10$.

Conditioned responses

First interval responses. The spider phobic group showed significantly greater SCRs to the CS+S in the comparison CS+S vs. CS− ($t_{12}=2.38$; $p=.034$). No significant differences between the CS+A and the CS− nor the CS+S and the CS+A emerged in any group.

Second interval responses. No significant effects were found in any comparison in the SIR.

fMRI

Unconditioned responses

Structures, correction volume (whole brain and ROI), coordinates of peak voxels, t-values and p_{corr}-values for all UCS-contrasts are displayed in Table S1 of the supplementary material. In addition, two supplementary tables contain the analyses of the unconditioned responses separately for the first and the second half (Table S2 and S3).

Conditioned responses

Structures, correction volume (whole brain and ROI), coordinates of peak voxels, t-values and p_{corr}-values for all CS-contrasts in the first half of acquisition are displayed in Table 1.

CS+S>CS− (first half of acquisition). The between group comparison of this contrast revealed significantly greater activations of the spider phobic group in the mPFC and the right insula, as well as marginally significant effects in the ACC, bilaterally in the amygdala and bilaterally in the thalamus (see Fig. 2). In the separate analysis of the spider phobic group significant effects were found in the ACC, the mPFC, the right insula and the left amygdala, as well as marginally significant effects in the left OFC, and the left thalamus. Additionally, the exploratory whole

Table 1
Localization and statistics of the peak voxels of the contrasts CS+S>CS−, CS+A>CS−, CS+A>CS+S and CS+S>CS+A in the first half of acquisition. The threshold was $p_{corr}<.05$ (FWE-corrected according to SPM5; for ROI: Small volume correction). Additionally, trends until a value of $p_{corr}<.10$ are reported. All coordinates are given in MNI space. Degrees of freedom were $df_{phobics}=14$, $df_{controls}=13$ and $df_{all}=28$ for the one-sample t-tests and $df_{group}=27$ for the group comparisons.

Contrast	Group	Brain Structure	x	y	z	t_{max}	p_{corr}
CS+S>CS−	Phobia	Cerebellum*	−9	−63	−33	8.16	.037
		ACC	12	42	21	5.26	.042
		OFC	−12	36	−24	3.84	.096
		mPFC	3	39	−24	5.75	.004
		Insula	33	21	6	4.40	.035
		Amygdala	−24	−12	−12	3.64	.035
		Thalamus	15	−15	6	3.79	.077
	Control	No significant results					
	Phobia>Control	ACC	12	30	36	4.22	.056
		mPFC	0	51	−27	4.06	.016
		Insula	33	21	6	3.71	.043
		Amygdala	−24	−15	−12	2.81	.082
		Amygdala	30	0	−15	2.80	.083
		Thalamus	−15	−36	−3	3.46	.070
		Thalamus	15	−15	6	3.50	.061
	Control>Phobia	No significant results					
CS+A>CS−	Phobia>Control	No significant results					
	Control>Phobia	No significant results					
	All	Precuneus*	−15	−66	33	6.14	.033
		ACC	9	6	42	4.45	.036
		mPFC	9	42	−12	4.70	.004
		Insula	−39	9	0	3.51	.067
		OFC	−24	36	−21	4.95	.004
		OFC	12	9	−18	4.22	.017
		Thalamus	−9	−9	0	3.48	.070
		Thalamus	12	−27	0	3.44	.072
CS+A>CS+S	Phobia	No significant results					
CS+S>CS+A	Phobia	Amygdala	−24	−9	−12	2.88	.094
		Amygdala	24	−15	−12	2.95	.086

* indicates exploratory whole brain analyses.

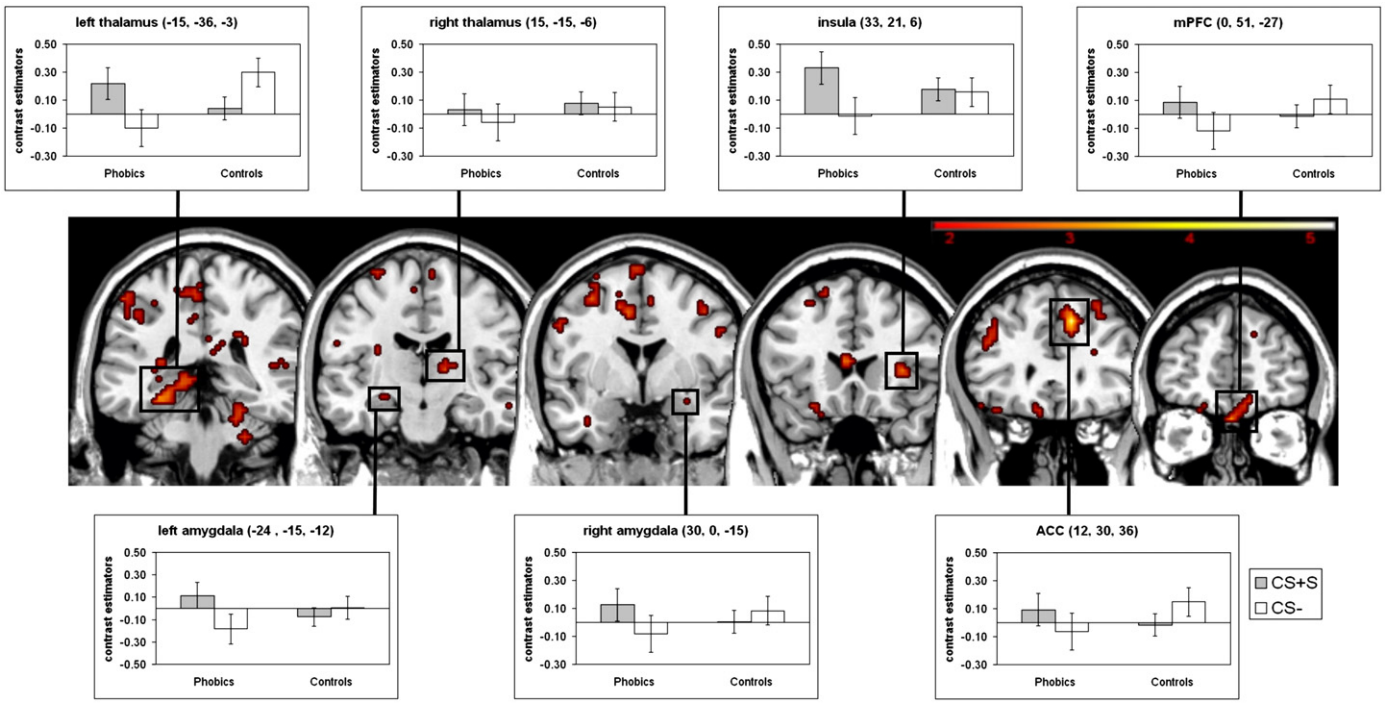

Fig. 2. Neural activations of group spider phobia>control for the contrast CS+S>CS− in the first half of acquisition (see color bars for exact *t*-values). Additionally, mean contrast estimates (and standard errors of the mean) to CS+S and CS− in the respective peak voxels are illustrated in the bar graphs. Threshold for displaying the images is set at $p = .005$ uncorrected.

brain analyses showed significant effects in the cerebellum. No significant effects were found in the separate analysis of the healthy control group.

CS+S>CS− (second half of acquisition). No significant effects were found in the between group comparison of this contrast and also in the separate analysis of the healthy control group. The spider phobic group showed marginally significant effects in the left thalamus ($x = -3$ $y = -12$ $z = 5$; $t_{max} = 4.26$; $p = .052$).

CS+A>CS− (first half of acquisition). No significant effects were found in the between group comparisons of this contrast. Thus, the two groups were merged into one. In the entire group, significant effects were found in the ACC, the mPFC, bilaterally in the OFC. Further, marginally significant effects were found bilaterally in the thalamus and in the left insula. In addition, the exploratory whole brain analysis revealed significant effects in the left precuneus.

CS+A>CS− (second half of acquisition). No significant effects were found in the between group comparison of this contrast. Thus, the two groups were merged into one. In the entire group marginally significant effects were found in the left OFC ($x = -30$ $y = 30$ $z = 0$; $t = 3.49$; $p = .086$) and in the thalamus ($x = 0$ $y = 12$ $z = 6$; $t_{max} = 3.58$; $p = .057$).

CS+S>CS+A (first half of acquisition). Marginally significant effects were detected in the spider phobic group bilaterally in the amygdala.

CS+S>CS+A (second half of acquisition). Significant effects were found in the spider phobic group bilaterally in the amygdala (left: $x = -24$ $y = -9$ $z = -18$; $t_{max} = 3.80$; $p = .031$; right: $x = 27$ $y = -6$ $z = -18$; $t_{max} = 4.26$; $p = .016$; see Fig. 3).

CS+A>CS+S. No significant effects were found in the spider phobic group in neither half of the acquisition.

Correlational analyses

The spider phobic group displayed significant positive correlations between the differential arousal rating scores (i.e. $arousal_{CS+S} - arousal_{CS-}$) and the contrast CS+S − CS− in the exploratory whole

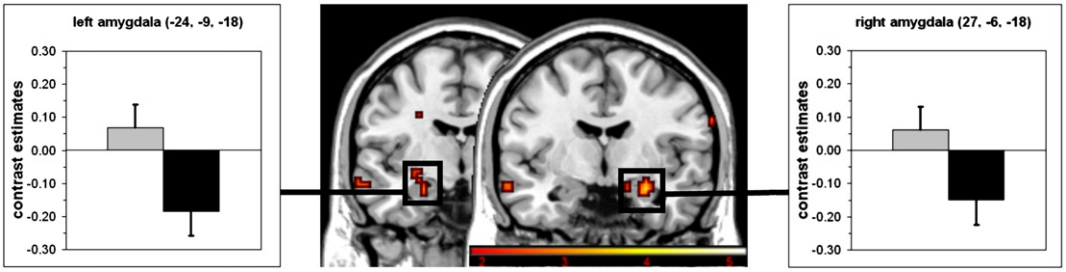

Fig. 3. Neural activations of the left and the right amygdala for the contrast CS+S>CS+A in the second half of acquisition in the spider phobia group. Additionally, mean contrast estimates (and standard errors of the mean) to CS+S (gray bars) and CS+A (black bars) in the respective peak voxels are illustrated in the bar graphs. Threshold for displaying the images is set at $p = .005$ uncorrected.

brain analyses in the parahippocampal gyrus ($r=.93$; $x=30$ $y=-27$ $z=-24$; $t_{max}=9.27$; $p=.015$) and the right amygdala ($r=.92$; $x=27$ $y=-12$ $z=-15$; $t_{max}=8.46$; $p=.041$). Further, the ROI analyses revealed significant correlations in the ACC ($r=.88$; $x=-12$ $y=54$ $z=6$; $t_{max}=6.83$; $p=.010$), the frontal medial cortex ($r=.82$; $x=3$ $y=51$ $z=-27$; $t_{max}=5.23$; $p=.015$); the right OFC ($r=.87$; $x=42$ $y=27$ $z=-21$; $t_{max}=6.34$; $p=.005$), the right insula ($r=.81$; $x=36$ $y=-9$ $z=15$; $t_{max}=4.90$; $p=.025$), the left amygdala ($r=.79$; $x=-30$ $y=-3$ $z=-27$; $t_{max}=4.58$; $p=.012$) and the bilateral thalamus (left: $r=.78$; $x=-3$ $y=-27$ $z=3$; $t_{max}=4.50$; $p=.044$; right: $r=.79$; $x=12$ $y=-12$ $z=18$; $t_{max}=4.69$; $p=.032$). Moreover, the spider phobic group showed significant correlations between the differential valence scores (i.e. $valence_{CS+S}-valence_{CS-}$) and the contrast CS+S−CS− in the bilateral insula (left: $r=.78$; $x=-42$ $y=9$ $z=-6$; $t_{max}=4.47$; $p=.041$; right: $r=.80$; $x=36$ $y=6$ $z=0$; $t_{max}=4.72$; $p=.030$) and the bilateral amygdala (left: $r=.71$; $x=-24$ $y=-9$ $z=-15$; $t_{max}=3.67$; $p=.041$; right: $r=.80$; $x=27$ $y=-12$ $z=-15$; $=4.77$; $p=.009$).

The correlational analyses of the differential arousal and valence ratings scores (i.e. $arousal_{CS+A}-arousal_{CS-}$ and $valence_{CS+A}-valence_{CS-}$), respectively, and the contrast CS+A−CS− in the entire sample revealed no significant results.

Discussion

The present study investigated CRs in spider phobic and healthy control subjects using phobia-relevant and highly aversive but non-phobia-relevant UCS. As the main finding, enhanced brain activations to the phobia-relevant CS+S were found in the spider phobic group in the fear network, including the amygdala, the insula, the ACC, the mPFC and the OFC. Further, the spider phobic group displayed stronger amygdala activations to the CS+S as compared to the CS+A reflecting exaggerated processing of disorder-relevant cues. In addition, spider phobic and healthy control subjects did not differ with regard to the responses to the CS+A, implicating no differences in general conditionability.

Subjective ratings clearly indicate successful evaluative conditioning in both groups. Regarding SCRs, spider phobic subjects displayed robust CRs to the CS+S, reflecting the high impact of phobia-relevant environmental cues in specific phobia. The picture is less clear for the CS+A. Neither group showed the expected differentiation. However, previous studies using pictorial UCS also failed to find differential SCRs (Wessa and Flor, 2007; Klucken et al., 2009b; Olatunji, 2006).

Concerning the conditioned neuronal responses, the contrast CS+S>CS− provoked significantly greater responses in spider phobic compared to healthy control subjects in the mPFC and the right insula as well as marginally enhanced activations in the bilateral amygdala, the ACC and the bilateral thalamus. Moreover, the comparison of the CS+S and the CS+A in the spider phobic group revealed greater amygdala activation to the phobia-relevant CS. These results are in line with models of phobic fear that emphasize the importance of the amygdala in the processing of phobic threat (Bishop, 2007; Öhman and Mineka, 2001; Bremner, 2004). The finding of enhanced amygdala responses only to the phobia-relevant CS+S supports the view that, in contrast to other anxiety disorders, e.g. posttraumatic stress disorder (Rauch et al., 2000), hyperreactive amygdala responses occur only in response to disorder-relevant cues (cf. Wright et al., 2003). This pattern of brain activity also fits well with the subjective experiences of patients with specific phobias, i.e. dysfunctionally elevated fear reactions to their phobic object, but “normal” fear reactions to non-phobic situations. This was also mirrored in the strong correlations between amygdala responses and subjective ratings of the CS.

In contrast to the long-term accepted role of the amygdala in the generation of pathological fear responses, the view of the insula as a key structure within the context of anxiety disorders has only recently emerged (Etkin and Wager, 2007). In addition to the finding of altered insula activity in symptom provocation, enhanced reactions of the insula have been observed in fear conditioning in social anxiety disorder (Veit et al., 2002), as well as in anticipatory anxiety in specific phobia (Straube et al., 2007) and social anxiety disorder (Lorberbaum et al., 2004; but see Tillfors et al., 2002). The insula has been implicated in processes related to self-awareness of bodily arousal states that may lead to anxiety-related behaviors (Phan et al., 2004). Recently, the insula has been proposed to be central to subjective anxiety proneness due to its ability to generate interoceptive prediction signals (Paulus and Stein, 2006). In line with this view of a close relation of insula activity to conscious behavior, we found significant correlations between the subjective valence and arousal ratings in the spider phobic group.

Enhanced ACC activations have been reported as a correlate of symptom provocation in specific phobia (Goossens et al., 2007; Straube et al., 2006) and have been shown to correlate with subjective fear ratings in the anticipation of phobic material (Straube et al., 2007). In accordance, ACC responses were related to subjective arousal ratings in this study. More generally, differential ACC responses are a typical finding in classical fear conditioning studies (for reviews see e.g. Büchel and Dolan, 2000; Sehlmeyer et al., 2009) and during anticipation of threat (Mechias et al., 2010). In a broader context, altered ACC responses have been observed during confrontation with aversive stimuli and have been shown to correlate with subjective emotion and emotional awareness (e.g. Lang et al., 1998; for reviews see Bush et al., 2000; Phan et al., 2004).

The finding of higher mPFC and amygdala responses in concert is in contrast to studies reporting decreased (pre)frontal activity during symptom provocation in specific phobia (Fredrikson et al., 1995; Johanson et al., 1998; Carlsson et al., 2004; Schienle et al., 2007). Further, the conscious anticipation of phobic stimuli did not result in enhanced mPFC and amygdala activations (Straube et al., 2007). A parsimonious explanation of this result would be that these activations could be a correlate of the association process between CS and UCS. Brain activity in the mPFC has been frequently observed in fear conditioning studies (Sehlmeyer et al., 2009), but not consistently when subjects were instructed about CS-UCS contingencies in forehand (Mechias et al., 2010). Activity in the mPFC has been associated to the extinction of conditioned fear responses and emotion regulation processes and, which are considered to be conveyed by the ability of the mPFC to exert inhibitory control over the amygdala (Rauch et al., 2006; Stein et al., 2008; Phan et al., 2004). It can be speculated, that the hyperactivation in the mPFC could be the result of an attempt to (down) regulate a hyper-functioning amygdala. However, this speculation is relativized by the recent finding of functional decoupling between prefrontal areas and the amygdala during the induction of phobic fear (Ahs et al., 2009).

Notably, the pattern of brain activity in response to the phobia-relevant CS in the spider phobic group resembles recent findings of symptom provocation studies (Etkin and Wager, 2007). This implies that pavlovian conditioning could be the mechanism that elicits the exaggerated fear network activity in response to disorder specific stimuli, which is in accordance to etiological models of specific phobia (Öhman and Mineka, 2001; Antony and Barlow, 2002; Mineka and Oehlberg, 2008).

Turning to the contrast CS+A>CS−, the observed pattern of results is largely in line with a previous study in our lab (Klucken et al., 2009a). Attempting to add to the ongoing debate, if subjects with anxiety disorders differ from healthy controls in terms of their general conditionability (see Lissek et al., 2005), we analyzed the contrast CS+A>CS− for group differences. The lack of significant effects favors the view that this is not the case; however, one should keep in mind, that absence of evidence is not evidence of absence. Instead, the presented data suggests that concerning specific phobia, the disorder-relevance of the UCS is the more important factor.

To coarsely account for possible temporal dynamics of the conditioned responses across trials we analyzed first and second half of the experiment separately. Strikingly, we found more broadly distributed activity in the first half as compared to the second half. However, amygdala responses in the contrast CS+S>CS+A in phobic subjects were larger in the second half. Possible interpretations of these results are differences in the speed of acquisition of CRs dependent on phobia-relevance. Alternatively, the observed pattern of results could be due to reduced habituation of the amygdala in spider phobic subjects to phobia-relevant CS. The lack of significant differentiations in the second half of the experiment in all structures except the amygdala slightly favors the latter interpretation, although it is not possible to definitively resolve this issue with the employed design.

In conclusion, the current study presented subjective, electrodermal and neuronal correlates of conditioned responses in specific phobia. Overall, the presented data confirm that the picture–picture conditioning paradigm is a suitable way of investigating the impact of affective learning on different response levels. This is the first study to investigate neuronal correlates of affective learning in specific phobia using phobia-relevant as well as generally aversive UCS. We found hyperactivation of brain areas belonging to the fear network in response to the phobia-relevant CS in spider phobic subjects, especially of the amygdala. The reported data is in line with models of the neuronal processes in specific phobia and further contributes to a more sophisticated understanding of the disorder.

Competing interests' statement

The authors declare that they have no competing financial interests.

Acknowledgments

This work was supported by the DFG Graduiertenkolleg GRK 885 “NeuroAct.”. We would like to thank Dr. Carlo Blecker for technical assistance with data collection and Dr. Andrea Hermann for helpful discussions on this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2010.07.049.

References

Ahs, F., Pissiota, A., Michelgård, A., Frans, O., Furmark, T., Appel, L., Fredrikson, M., 2009. Disentangling the web of fear: amygdala reactivity and functional connectivity in spider and snake phobia. Psychiatry Res. 172, 103–108.
American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Press, Washington, DC.
Antony, M.M., Barlow, D.H., 2002. Specific phobias. In: Barlow, D.H. (Ed.), Anxiety and Its Disorders: The Nature and Treatment of Anxiety and Panic. The Guilford Press, New York, pp. 380–417.
Armfield, J.M., 2006. Cognitive vulnerability: a model of the etiology of fear. Clin. Psychol. Rev. 26, 746–768.
Bishop, S.J., 2007. Neurocognitive mechanisms of anxiety: an integrative account. Trends Cogn. Sci. 11, 307–316.
Bremner, J.D., 2004. Brain imaging in anxiety disorders. Expert Rev. Neurother. 4, 275–284.
Büchel, C., Dolan, R.J., 2000. Classical fear conditioning in functional neuroimaging. Curr. Opin. Neurobiol. 10, 219–223.
Bush, G., Luu, P., Posner, M.I., 2000. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn. Sci. 4, 215–222.
Carlsson, K., Petersson, K.M., Lundqvist, D., Karlsson, A., Ingvar, M., Öhman, A., 2004. Fear and the amygdala: manipulation of awareness generates differential cerebral responses to phobic and fear-relevant (but nonfeared) stimuli. Emotion 4, 340–353.
Dawson, M.E., Reardon, P., 1973. Construct validity of recall and recognition postconditioning measures of awareness. J. Exp. Psychol. 98, 308–315.
Dilger, S., Straube, T., Mentzel, H.-J., Fitzek, C., Reichenbach, J.R., Hecht, H., et al., 2003. Brain activation to phobia-related pictures in spider phobic humans: an event-related functional magnetic resonance imaging study. Neurosci. Lett. 348, 29–32.
Eriksen, B.A., Eriksen, C.W., 1974. Effects of noise letters upon identification of a target letter in a nonsearch task. Percept. Psychophys. 16, 143–149.
Etkin, A., Wager, T.D., 2007. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am. J. Psychiatry 164, 1476–1488.
Fredrikson, M., Wik, G., Annas, P., Ericson, K., Stone-Elander, S., 1995. Functional neuroanatomy of visually elicited simple phobic fear: additional data and theoretical analysis. Psychophysiology 32, 43–48.
Fyer, A.J., 1998. Current approaches to etiology and pathophysiology of specific phobia. Biol. Psychiatry 44, 1295–1304.
Goossens, L., Sunaert, S., Peeters, R., Griez, E.J.L., Schruers, K.R.J., 2007. Amygdala hyperfunction in phobic fear normalizes after exposure. Biol. Psychiatry 62, 1119–1125.
Hamm, A.O., Weike, A.I., 2005. The neuropsychology of fear learning and fear regulation: neurobiology of fear and disgust. Int. J. Psychophysiol. 57, 5–14.
de Houwer, J., Thomas, S., Baeyens, F., 2001. Associative learning of likes and dislikes: a review of 25 years of research on human evaluative conditioning. Psychol. Bull. 127, 853–869.
Hubert, M., van der Veeken, S., 2008. Outlier detection for skewed data. J. Chemometr. 22, 235–246.
Johanson, A., Gustafson, L., Passant, U., Risberg, J., Smith, G., Warkentin, S., Tucker, D., 1998. Brain function in spider phobia. Psychiatry Res. 84, 101–111.
Klorman, R., Weerts, T.C., Hastings, J.E., Melamed, B.G., Lang, P.J., 1974. Psychometric description of some specific-fear questionnaires. Behav. Ther. 5, 401–409.
Klucken, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., Stark, R., 2009a. Neural, electrodermal and behavioral response patterns in contingency aware and unaware subjects during a picture–picture conditioning paradigm. Neuroscience 158, 721–731.
Klucken, T., Schweckendiek, J., Merz, C.J., Tabbert, K., Walter, B., Kagerer, S., Vaitl, D., Stark, R., 2009b. Neural activations of the acquisition of conditioned sexual arousal: effects of contingency awareness and sex. J. Sex. Med. 6, 3071–3085.
LaBar, K.S., LeDoux, J.E., 1996. Partial disruption of fear conditioning in rats with unilateral amygdala damage: correspondence with unilateral temporal lobectomy in humans. Behav. Neurosci. 110, 991–997.
LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., Phelps, E.A., 1998. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron 20, 937–945.
Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1998. Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. Biol. Psychiatry 44, 1248–1263.
Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1999. International Affective Picture System (IAPS): instruction manual and affective ratings: technical report A4.
LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184.
Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., Pine, D.S., 2005. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav. Res. Ther. 43, 1391–1424.
Lissek, S., Levenson, J., Biggs, A.L., Johnson, L.L., Ameli, R., Pine, D.S., Grillon, C., 2008. Elevated fear conditioning to socially relevant unconditioned stimuli in social anxiety disorder. Am. J. Psychiatry 165, 124–132.
Lorberbaum, J.P., Kose, S., Johnson, M.R., Arana, G.W., Sullivan, L.K., Hamner, M.B., et al., 2004. Neural correlates of speech anticipatory anxiety in generalized social phobia. NeuroReport 15, 2701–2705.
Margraf, J., 1994. Mini-DIPS: Diagnostisches Kurz-Interview bei psychischen Störungen. Springer-Verlag, Berlin.
Mechias, M.-L., Etkin, A., Kalisch, R., 2010. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. Neuroimage 49, 1760–1768.
Mineka, S., Oehlberg, K., 2008. The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. Acta Psychol. 127, 567–580.
Mineka, S., Zinbarg, R., 2006. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. Am. Psychol. 61, 10–26.
Öhman, A., Mineka, S., 2001. Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. Psychol. Rev. 108, 483–522.
Olatunji, B.O., 2006. Evaluative learning and emotional responding to fearful and disgusting stimuli in spider phobia. J. Anxiety Disord. 20, 858–876.
Paulus, M.P., Stein, M.B., 2006. An insular view of anxiety. Biol. Psychiatry 60, 383–387.
Phan, K.L., Wager, T.D., Taylor, S.F., Liberzon, I., 2004. Functional neuroimaging studies of human emotions. CNS Spectr. 9, 258–266.
Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E., 2004. Extinction learning in humans: role of the amygdala and vmPFC. Neuron 43, 897–905.
Prokasy, W.F., Ebel, H.C., 1967. Three components of the classically conditioned GSR in human subjects. J. Exp. Psychol. 73, 247–256.
Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., Orr, S.P., Pitman, R.K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.
Rauch, S.L., Shin, L.M., Phelps, E.A., 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research—past, present, and future. Biol. Psychiatry 60, 376–382.
Schienle, A., Schafer, A., Walter, B., Stark, R., Vaitl, D., 2005. Brain activation of spider phobics towards disorder-relevant, generally disgust- and fear-inducing pictures. Neurosci. Lett. 388, 1–6.
Schienle, A., Schäfer, A., Hermann, A., Rohrmann, S., Vaitl, D., 2007. Symptom provocation and reduction in patients suffering from spider phobia: an fMRI study on exposure therapy. Eur. Arch. Psychiatry Clin. Neurosci. 257, 486–493.
Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., Konrad, C., 2009. Human fear conditioning and extinction in neuroimaging: a systematic review. PLoS ONE 4, e5865.

Stein, J.L., Wiedholz, L.M., Bassett, D.S., Weinberger, Zink, C.F., Mattay, V.S., Meyer-Lindenberg, A., 2007. A validated network of effective amygdala connectivity. Neuroimage 36, 736–745.
Straube, T., Mentzel, H.-J., Miltner, W.H.R., 2006. Neural mechanisms of automatic and direct processing of phobogenic stimuli in specific phobia. Biol. Psychiatry 59, 162–170.
Straube, T., Mentzel, H.-J., Miltner, W.H.R., 2007. Waiting for spiders: brain activation during anticipatory anxiety in spider phobics. Neuroimage 37, 1427–1436.
Tabbert, K., Stark, R., Kirsch, P., Vaitl, D., 2005. Hemodynamic responses of the amygdala, the orbitofrontal cortex and the visual cortex during a fear conditioning paradigm: neurobiology of fear and disgust. Int. J. Psychophysiol. 57, 15–23.
Tillfors, M., Furmark, T., Marteinsdottir, I., Fredrikson, M., 2002. Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. Biol. Psychiatry 52, 1113–1119.
Veit, R., Flor, H., Erb, M., Hermann, C., Lotze, M., Grodd, W., Birbaumer, N., 2002. Brain circuits involved in emotional learning in antisocial behavior and social phobia in humans. Neurosci. Lett. 328, 233–236.
Veltman, D.J., Tuinebreijer, W.E., Winkelman, D., Lammertsma, A.A., Witter, M.P., Dolan, R.J., Emmelkamp, P.M.G., 2004. Neurophysiological correlates of habituation during exposure in spider phobia. Psychiatry Res. 132, 149–158.
Wessa, M., Flor, H., 2007. Failure of extinction of fear responses in posttraumatic stress disorder: evidence from second-order conditioning. Am. J. Psychiatry 164, 1684–1692.
Wik, G., Fredrikson, M., Fischer, H., 1996. Cerebral correlates of anticipated fear: a PET study of specific phobia. Int. J. Neurosci. 87, 267–276.
Wright, C.I., Martis, B., McMullin, K., Shin, L.M., Rauch, S.L., 2003. Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. Biol. Psychiatry 54, 1067–1076.