Initial and sustained brain responses to threat anticipation in blood-injection-injury phobia

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Blood-injection-injury (BII) phobia differs from other subtypes of specific phobia in that it is associated with elevated disgust-sensitivity as well as specific autonomic and brain responses during processing of phobia-relevant stimuli. To what extent these features play a role already during threat anticipation is unclear. In the current fMRI experiment, 16 female BII phobics and 16 female healthy controls anticipated the presentation of phobia-specific and neutral pictures. On the behavioral level, anxiety dominated the anticipatory period in BII phobics relative to controls, while both anxiety and disgust were elevated during picture presentation. By applying two different models for the analysis of brain responses to anticipation of phobia-specific versus neutral stimuli, we found initial and sustained increases of activation in anterior cingulate cortex (ACC), insula, lateral and medial prefrontal cortex (PFC), thalamus and visual areas, as well as initial activation in the amygdala for BII phobics as compared to healthy controls. These results suggest that BII phobia is characterized by activation of a typical neural defense network during threat anticipation, with anxiety as the predominant emotion.

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1. Introduction

Specific phobia is characterized by rapid, intense and uncontrollable fear in response to phobia-relevant objects and situations (American Psychiatric Association, 2000). Besides that, most phobics show anticipatory anxiety during expectation of phobia-related situations (Aue and Hoeppli, 2011) that triggers avoidance behavior which in turn prevents fear extinction (Gray and McNaughton, 2000).

A common subtype of specific phobia is blood-injection-injury (BII) phobia with an estimated prevalence of 3–4% in the general population (Wani and Ara, 2014) and a higher prevalence in women (Oosterink et al., 2009). In BII phobia, phobic fears and anxiety emerge in relation to blood withdrawal, medical interventions and the confrontation with a person’s own blood or blood of others, especially in the context of injuries. A feature that distinguishes BII phobia from other specific phobias is vasovagal syncope during exposure to phobia-specific objects or situations (Marks, 1988; Page, 1994), which affects approximately 75% of BII phobics (Marks, 1988). This reaction has been attributed to a biphasic autonomic response with a short increase followed by a marked decrease of heart rate (Page, 1994). Furthermore, besides fear and anxiety, BII phobics generally also experience strong disgust during symptom provocation (de Jong and Merckelbach, 1998; Sawchuk et al., 2002; Tolin et al., 1997). BII phobics often avoid medical treatment and even decline necessary treatment (Wani and Ara, 2014). Consequently, BII phobia can have detrimental effects and the investigation of its neural correlates could provide important insights for the development of an effective treatment.

In general, functional magnetic resonance imaging (fMRI) studies in specific phobia point towards involvement of amygdala, insula, anterior cingulate cortex (ACC) as well as prefrontal and orbitofrontal cortex during fear and anxiety, although the neural correlates of specific phobia are still not definitive and most studies were concerned with the animal subtype of specific phobia (for review see Del Casale et al., 2012; Ipser et al., 2013; Linares et al., 2012). To date, BII phobia has received little attention in neuroscientific research (Del Casale et al., 2012). Unfortunately, results are rather inconclusive and seem to critically depend on experimental designs and procedures. Confrontation with phobia-relevant or generally disgusting images has been associated with diminished medial prefrontal cortex (PFC) activity (Hermann et al., 2007) and relatively unspecific activations in thalamus and occipital cortex in BII phobics (Caseras et al., 2010a; Schienle et al., 2003). Direct comparison between BII and animal phobics revealed that only spider phobics showed activations in key areas for emotional processing, i.e. insula and ACC, when confronted with phobia-specific pictures (Caseras et al., 2010a; Lueken et al., 2011). In contrast, another study reported...
so-called extended amygdala in anticipatory anxiety in animal phobia activation in the bed nucleus of the stria terminalis (BNST) during threat when anticipating public speaking (Boehme et al., 2014; Lorberbaum et al., 2004).

Eminent threat and the BNST modulating sustained anxiety states in un- and BNST, with the amygdala being involved in rapid processing of im- mune responses. Based on previous research, as well as changes in heart rate were examined to control for character- stic emotional and autonomic responses. Based on previous research, we were interested in brain activations in amygdala, BNST, ACC, insula, MFC, thalamus and visual areas during anticipation of phobia-specific in contrast to neutral pictures. Especially with regard to amygdala and BNST, we used an initial as well as a sustained model for BOLD responses in order to separate phasic and sustained brain activation, respectively. We hypothesized that initial amygdala activation and sustained BNST activation would be evident in phobic participants as compared to healthy controls during anticipation of phobia-specific versus neutral stimuli.

2. Material and methods

2.1. Subjects

Sixteen right-handed female subjects with BII phobia (age: 24.1 ± 3.82 years) and 16 right-handed female healthy control subjects (age: 23.7 ± 4.44 years) participated in the study. Only female participants were included since BII phobia is most common in young women (Miloyan and Eaton, 2016; Wani and Ara, 2014) and the majority of studies in specific phobia investigated female samples (for review see Del Casale et al., 2012; Ipser et al., 2013; Van Houtem et al., 2013), which makes the current study more comparable to other studies, especially to the anticipation study by Straube et al. (2007). Participants were recruited by public advertisement and received monetary reimbursement (10 €) or course credit for participation. BII-phobic subjects were selected by means of a short clinical interview (mini-DIPS, Margraf, 1994) based on DSM-IV (American Psychiatric Association, 2000) and ICD-10 (World Health Organization, 1992). Patients and controls were matched with regard to age and level of education. Phobics scored significantly higher than non-phobic controls on the Mutilation Questionnaire (MQ, Klorman et al., 1974) (phobics: mean = 21.5, S.D. = 3.27; controls: mean = 5.94, S.D. = 2.29; t[30] = 15.6, p < 0.001, d = 5.52) and the Disgust Propensity and Sensitivity Scale (DPSS-R, van Overveld et al., 2006) (phobics: mean = 48.06, S.D. = 7.13; controls: mean = 31.25, S.D. = 7.37; t[30] = 6.56, p < 0.001, d = 2.32). In one of the control subject, the clinical interview indicated diagnostic criteria for spider phobia, but without sufficient psychological strain. For none of the reported effects, this subject was an outlier. Participants with psychopathological disorders other than BII phobia were excluded. Additionally, participants had no history of or current neurological disorder and traumatic brain injury. None of the partici- pants had taken psychotropic drugs or beta-blockers for a period of at least three months prior to the study (also see Del Casale et al., 2012). The study conforms to the Declaration of Helsinki and was approved by the ethics committee of the University of Jena, Germany. All partici- pants gave informed consent prior to the experiment.

2.2. Experimental design

In the scanner, participants anticipated the presentation of phobia- specific or neutral pictures. Pictures were selected from the Internation- al Affective Picture System (IAPS, Lang et al., 2008). Phobia-specific pictures showed bloody injuries of people and limbs as well as blood-withdrawal (# 3010, 3030, 3051, 3060, 3071, 3100, 3130, 3150, 9405, 9592). Neutral pictures showed people or objects (# 2200, 2214, 2215, 2270, 2383, 5395, 5520, 7010, 7090, 7503) and were matched to the phobia-specific pictures with regard to features, complexity, and color scheme (Adobe Photoshop, Version 13.0.1; Adobe Systems Software Ireland Limited, Ireland; see Supplementary Table 1). Phobia-specific and neutral pictures were used in previous studies on BII phobia (Buodo et al., 2006; Hamm et al., 1997). During the anticipatory period, one of two white cues (circle or square) was presented on black back- ground, indicating whether the following picture would be phobia-spe- cific or neutral. Assignment of the symbols to the conditions was counterbalanced across subjects, and participants were informed about cue-condition association before the session. The experiment comprised 10 phobia-specific and 10 neutral trials presented in pseudo-random order. The anticipatory period lasted 10, 12, or 14 s to ensure unpredictability of stimulus onset. The subsequent picture presentation lasted 12 s to establish a sufficiently threatening context and to be able to detect alterations in heart rate. Between trials, a fixation cross was shown for 16 s. In total, the experiment lasted 13 min.

After the scanning session, participants rated phobia-specific and neutral pictures as well as the respective anticipatory periods on the dim-ensions anxiety (1 = “not anxious at all” to 9 = “very anxious”) and disgust (1 = “not disgusting at all” to 9 = “very disgusting”) using a nine-point Likert-scale. Furthermore, as a measure of avoidance behav- ior, participants were requested to answer the question “How long did you look at the unpleasant pictures on average?” (“very briefly”, “a few seconds”, “almost the entire time”, or “I never looked away”). Behav- ioral data were analyzed by means of mixed-model analyses of var- iance (ANOVA)s using IBM SPSS software (Version 22; IBM, Armonk, New York, USA), with group (BII phobics vs. healthy controls) as be- tween-subject factor and condition (phobia-specific vs. neutral) as within-subject factor. Post-hoc t-tests were performed to resolve interac- tions when appropriate. Generally, a p-value of < 0.05 was considered statistically significant. Data are presented as mean ± standard error.
2.3. Pulse oximetry

A pulse oximeter (Pulse Oximeter 8600FO; Nonin Medical Inc., Amsterdam, the Netherlands) was used to determine the participants’ heart rate as an indirect measure of blood oxygen saturation and blood volume (Bowes et al., 1989). The signal was recorded together with the experimental events. Data were analyzed with Brain Vision Analyzer (Version 1.03; BrainProducts, Munich, Germany). Due to signal disturbances, one phobic and one control participant had to be excluded from further analysis. For all other subjects, the peak of each pulse wave was detected, and the time interval between two peaks was considered an index for the heart rate. This analysis was conducted separately for anticipation and presentation periods, covering a time window from 500 ms before trial onset until the end of the respective trial. The 500 ms before trial onset were used as baseline. Finally, the mean changes in heart rate for phobia-specific and neutral anticipatory and presentation periods for each participant were calculated. Resulting means were analyzed with mixed-model ANOVAs using SPSS (Version 22; IBM, Armonk, New York, USA) with group serving as between-subject and condition as within-subject factor. Post-hoc t-tests were used to resolve interactions. A p-value of < 0.05 was considered statistically significant. Additionally, heart rate variability was calculated (root mean square of successive differences [RMSSD]; see Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) to control for influence of autonomic data on fMRI data.

2.4. FMRI

Data were collected with a 1.5 T magnetic resonance scanner ("Magneton Vision Plus"; Siemens Medical Systems, Erlangen, Germany). The scanning session started with a high-resolution T1-weighted anatomical scan. Afterwards functional data were acquired with a T2*-weighted echo-planar sequence (TE = 50 ms, flip angle = 90°, matrix = 64 x 64, FOV = 192 mm, TR = 4000 ms) with 40 axial slices (thickness = 3 mm, gap = 1 mm, in plane resolution = 3 x 3 mm) in each volume. One run consisted of 205 volumes. FMRI data were preprocessed and analyzed with BrainVoyager QX (Version 2.8; Brain Innovation, Maastricht, the Netherlands). During preprocessing, the first four volumes of each run were discarded to ensure adequate saturation. Data were corrected for slice time errors as well as movement artifacts. Anatomical and functional data were co-registered and normalized to fit Talairach space (Talairach and Tournoux, 1988). Subsequently, data were smoothed spatially (6 mm full-width-half maximum [FWHM] Gaussian kernel) and temporally (high pass filter: 3 cycles per run; low pass filter: 2.8 s; linear trend removal). Statistical analysis comprised multiple linear regression of the signal time course at each voxel. The expected BOLD signal change for each condition was modelled with a canonical hemodynamic response function (HRF). For the anticipatory period, we calculated two different general linear models (GLM) (also see Herrmann et al., 2016). In the first GLM, the HRF was modelled over the whole phobia-specific and neutral anticipatory periods (sustained response). In the second GLM, the response was modelled as the HRF initiated by the first second of the anticipatory periods (initial response), while the rest of the anticipatory period was modelled separately as a predictor of no interest. Due to the majority of the phobic subjects stating to have avoided at least some part of the picture presentation phase, and due to predictability of the experimental conditions based on the cues presented at the beginning of each trial (phobia-specific or neutral), FMRI analysis of the picture presentation phase was not informative. Picture presentations were thus defined as predictors of no interest in both GLMs. As a first analysis step, percent-standardized predictor estimates were calculated for each participant by dividing the signal in a given voxel at each time point by the mean of the signal time course. Next, random effects analysis with adjustment for autocorrelation following a global AR(2) model across the individual predictor estimates for planned contrasts was performed.

The analyses were conducted for defined regions of interest (ROIs) as defined a priori. The amygdala ROI was extracted from the anatomy toolbox (Eickhoff et al., 2005) and consisted of amygdala maximum probability maps as recommended by Eickhoff et al. (2006) (also see Herrmann et al., 2016). ROIs for ACC, insula, PFC (dorsolateral superior frontal gyrus, medial superior frontal gyrus, middle frontal gyrus), OFC (orbital superior frontal gyrus, orbital middle frontal gyrus, orbital inferior frontal gyrus), thalamus and visual cortex (cuneus, fusiform gyrus) were defined on the basis of the Automated Anatomical Labeling (AAL) atlas included in the Wake Forest University (WFU) PickAtlas software (Maldjian et al., 2004; Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002). Obtained MNI-coordinates were converted into Talairach space with ICBM2tal (Lancaster et al., 2007). Coordinates for bilateral BNST were defined according to an anatomical atlas (Maï et al., 1997).

To correct for multiple comparisons, the cluster-level statistical threshold estimator plugin for BrainVoyager (Goebel et al., 2006) was used. After setting the voxel-level threshold to p < 0.005 (uncorrected; Lieberman and Cunningham, 2009), a mask consisting of all ROIs was applied to the thresholded maps with an estimated full width at half maximum for spatial smoothness following the approach by Forman et al. (1995) and an iterative procedure (Monte Carlo simulation) with 1000 iterations. The minimum cluster size threshold (189 mm³) with a cluster-level false positive rate of 5% was applied to the statistical maps. The potential influence of autonomic data was tested by correlating mean heart rate and heart rate variability across trials with parameter estimates (anticipation of phobia-specific vs. neutral pictures) within a mask, created on the basis of significant activation clusters found in the ROI analyses.

To further analyze temporal dynamics of differential activations, we first z-standardized differences in parameter estimates (anticipation of phobia-specific vs. neutral stimuli) resulting from the initial and sustained model within clusters of activation in our main regions of interest (amygdala, BNST, ACC and insula). Standardized data were then analyzed for within- (phasic vs. sustained) and between-group contrasts (model by group interaction) by means of t-tests using SPSS (Version 22; IBM, Armonk, New York, USA).

3. Results

3.1. Behavioral data

The ANOVA for anxiety ratings during anticipation (Fig. 1) yielded significant main effects for group (F[1, 30] = 25.45, p < 0.001, d = 1.84) and condition (F[1, 30] = 36.89, p < 0.001, d = 2.22) as well as a significant group by condition interaction (F[1, 30] = 14.94, p = 0.001, d = 1.41). Post-hoc analysis revealed significantly higher anxiety ratings for phobia-specific anticipation for phobic as compared to control subjects (t[30] = 5.81, p < 0.001, d = 2.05), while there was no significant group difference for anticipation of neutral pictures. Additionally, phobics, but not control subjects, rated anticipation of phobia-specific stimuli as significantly more anxiety-inducing than anticipation of neutral stimuli (t[15] = 5.96, p < 0.001, d = 2.11). Analysis of disgust ratings for the anticipatory period (Fig. 1) yielded a significant main effect for condition (F[1, 30] = 13.24, p = 0.001, d = 1.33), indicating higher disgust ratings for anticipation of phobia-specific in contrast to neutral pictures. The main effect for group and the interaction effect did not reach significance.

The ANOVA for anxiety ratings in response to picture presentation (Fig. 1) showed significant main effects for group (F[1, 30] = 58.13, p < 0.001, d = 2.78) and condition (F[1, 30] = 369.97, p < 0.001, d = 7.02), as well as a significant group by condition interaction (F[1, 30] = 43.48, p < 0.001, d = 2.41). Post-hoc analysis revealed that anxiety ratings for phobia-specific pictures were significantly higher in the phobic group than in the control group (t[30] = 7.63, p < 0.001, d = 2.70), while
this was not the case for the neutral pictures. Additionally, phobic as well as control participants rated phobia-specific pictures as significantly more anxiety-inducing than neutral pictures (phobics: $t_{[15]} = 27.98$, $p < 0.001$, $d = 9.89$; controls: $t_{[15]} = 7.13$, $p < 0.001$, $d = 2.52$). Disgust ratings for pictures (Fig. 1) also resulted in significant main effects for group ($F_{[1, 30]} = 37.14$, $p < 0.001$, $d = 2.23$) and condition ($F_{[1, 30]} = 744.09$, $p < 0.001$, $d = 9.96$) as well as a significant group by condition interaction ($F_{[1, 30]} = 31.28$, $p < 0.001$, $d = 2.04$). Post-hoc analysis showed significantly higher disgust ratings for phobia-specific pictures in phobic as compared to control subjects ($t_{[30]} = 5.98$, $p < 0.001$, $d = 2.11$), while ratings did not differ between groups for neutral pictures. In both groups, phobia-specific pictures were rated as significantly more disgust-inducing than neutral pictures (phobics: $t_{[15]} = 60.49$, $p < 0.001$, $d = 21.37$; controls: $t_{[15]} = 11.27$, $p < 0.001$, $d = 3.99$).

Answers on avoidance behavior during the experiment (“How long did you look at the unpleasant pictures on average?”) differed between groups. The majority of phobic subjects answered with “almost the entire time” (56.25%), while only few phobics chose “a few seconds” (25%), “I never looked away” (12.5%), or “very briefly” (6.25%). In the control group, most of the subjects answered “I never looked away” (87.5%), while only two subjects answered with “almost the entire time” (12.5%). Statistical analysis showed a significant association between group and chosen answer (Fisher’s exact test: $p < 0.001$).

3.2. Heart rate

With regard to the anticipatory period, there were no significant main effects for group or condition, and no significant interaction. For the presentation phase, there was a significant main effect for group ($F_{[1, 28]} = 5.99$, $p = 0.021$, $d = 0.89$) and a significant interaction between group and condition ($F_{[1, 28]} = 7.24$, $p = 0.012$, $d = 0.98$), but no significant main effect for condition. Post-hoc analysis revealed that phobic subjects had a significantly lower change in heart rate during presentation of phobia-specific pictures than healthy controls ($0.37 \pm 0.59$ vs. $-1.78 \pm 0.40$; $t_{[28]} = 3.00$, $p = 0.006$, $d = 1.06$), while there was no significant difference between groups during presentation of neutral pictures ($-0.28 \pm 0.25$ vs. $-0.01 \pm 0.35$). Furthermore, controls had a significantly decreased heart rate in phobia-specific as compared to neutral presentation phases ($t_{[14]} = 3.38$, $p = 0.005$, $d = 1.20$), while there was no significant difference within the phobic group (Fig. 2).

3.3. FMRI data

3.3.1. Sustained response

In the first analysis step, the GLM covering the whole anticipatory period was analyzed. ROI analyses for group effects resulted in several activation differences for the contrast phobia-specific $>$ neutral anticipa-
tion (Table 1, Fig. 3). Compared to healthy control subjects, phobic subjects showed increased activation in dorsal ACC, posterior insula, dorsolateral prefrontal cortex (dlPFC), dorsomedial prefrontal cortex (dmPFC), thalamus, cuneus and fusiform gyrus as well as decreased activation in medial OFC. There were no significant correlations between these differential activations and heart rate or heart rate variability. The $t$-tests for standardized parameter estimates for activation clusters in dorsal ACC and posterior insula showed a significant model by
group interaction in the dorsal ACC ($t_{30} = 1.83, p = 0.039, d = 0.65$) (Fig. 5).

### 3.3.2. Initial response

In the ROI analyses for the initial response model of the anticipatory period, phobic as compared to control subjects showed increased activation for the contrast phobia-specific > neutral anticipation in the amygdala, rostral ACC, anterior insula, dmPFC, ventrolateral prefrontal cortex (vlPFC), ventromedial prefrontal cortex (vmPFC), thalamus and fusiform gyrus as well as decreased activation in dmPFC (Table 2, Fig. 4). Again, these differential activations did not correlate with heart rate or heart rate variability. The $t$-tests for standardized parameter estimates for activation clusters in amygdala, rostral ACC and anterior insula showed significant model by group interactions (amygdala: $t_{30} = 2.84, p = 0.004, d = 1.00$; rostral ACC: $t_{30} = 3.47, p = 0.001, d = 1.23$; anterior insula: $t_{30} = 1.78, p = 0.043, d = 0.63$) (Fig. 5). Furthermore, phobics showed significantly higher parameter estimates in the initial relative to the sustained model in the rostral ACC ($t_{15} = 2.16, p = 0.024, d = 0.76$) and healthy controls showed significantly higher parameter estimates in the sustained relative to the initial model in the amygdala ($t_{15} = 2.76, p = 0.007, d = 0.98$), rostral ACC ($t_{15} = 2.90, p = 0.006, d = 1.03$) and anterior insula ($t_{15} = 1.81, p = 0.045, d = 0.64$).

### 4. Discussion

The present study demonstrates increased activation of BII phobics in several brain regions during anticipatory anxiety. Specifically, sustained phobia-specific relative to neutral picture anticipation was associated with hyperactivations in ACC, insula, PFC, thalamus and visual areas in phobics as compared to controls. For initial phobia-specific relative to neutral anticipation, phobic subjects as compared to controls showed increased activation in amygdala, ACC, insula, PFC, thalamus and fusiform gyrus. On the behavioral level, the anticipatory period
was characterized by increased anxiety but not disgust and measures of heart rate did not indicate biphasic response patterns in phobic subjects.

BII phobia is associated with high disgust-sensitivity (de Jong and Merckelbach, 1998; Sawchuk et al., 2002; Tolin et al., 1997). This is confirmed by the present rating data for picture presentation. However, no such effect was found for the anticipatory period. This stands in contrast to increased anxiety ratings in BII phobia for both anticipation and presentation of phobia-specific pictures. Consequently, anxiety seems to be the dominant emotion during threat anticipation, which makes the emotional experience similar to other specific phobias (Straube et al., 2007) and other anxiety disorders (Andrews et al., 1994; Grillon et al., 2008; Grillon et al., 2009). The current fMRI results support this assumption, as findings replicate activation patterns previously shown for anticipatory anxiety in spider phobia (Münsterkötter et al., 2012; Ipser et al., 2013; Linares et al., 2013). Several studies have also shown insula activation during anticipation in patients with specific phobia (Del Casale et al., 2012; Ipser et al., 2013; Linares et al., 2012). This is reflected by the current results, with increased activation of dorsal ACC and posterior insula during sustained anticipation of phobia-specific pictures in BII phobics. ACC has been shown to be active during threat processing in animal phobia (Britton et al., 2008; Carlsson et al., 2004; Goossens et al., 2007; Straube et al., 2006a; Straube et al., 2006b). More specifically, dorsal ACC has been associated with increased drive for action, scanning of the environment and adaptive control during anticipation of aversive stimuli (Grupe et al., 2013; Straube et al., 2007). Insula hyperactivation, on the other hand, has been implicated in interoception and representation of bodily states (Craig, 2002, 2009; Critchley et al., 2004; Grupe and Nitschke, 2013). Several studies have also shown insula activation during anticipation in patients with specific phobia (Straube et al., 2007), social phobia (Boehme et al., 2014; Lorberbaum et al., 2004), or panic disorder with agoraphobia (Wittmann et al., 2014), and also in healthy controls (e.g. Carlson et al., 2011; Chua et al., 1999; Plöghaus et al., 1999).

**Table 2**

Significant initial activations during anticipation of phobia-specific vs. neutral pictures as revealed by ROI analysis.

| Region          | Phobics > controls | t-Value | mm^3 | Controls > phobics | t-Value | mm^3 |
|-----------------|---------------------|---------|------|--------------------|---------|------|
| Amygdala        | L                   | −30     | −7   | −10                | 4.68    | 297  |
| ACC             | L/R                 | −1      | 46   | 7                  | 3.79    | 351* |
| Insula          | L                   | −33     | −7   | −9                 | 4.08    | 378  |
|                 | R                   | 31      | 5    | −7                 | 4.15    | 324  |
| PFC             | dmPFC               | L       | 9    | −6                 | 3.24    | 270  |
|                 |                     | R       | −29  | 38                 | 3.11    | 189  |
|                 | vPFC                | L       | 31   | 57                 | 3.48    | 594  |
|                 |                     | R       | 18   | 38                 | 3.41    | 513  |
|                 | vmPFC               | L/R     | −2   | 48                 | 4.29    | 1836*|
| Thalamus        | L                   | −9      | −13  | 11                 | 3.29    | 540  |
| Visual cortex   | Fusiform gyrus      | L       | −41  | −48                | 4.39    | 1107 |
|                 |                     | L       | −38  | −69                | 4.79    | 945  |
|                 |                     | R       | 26   | −44                | 4.54    | 3510 |

**ACC**, anterior cingulate cortex; PFC, prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; vPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; L, left; R, right; (x,y,z), Talairach coordinates of maximally activated voxel (activation threshold: p < 0.05 corrected)

* These activation clusters are interconnected.

**Fig. 4.** Phobic subjects showed increased initial activation during anticipation of phobia-specific in contrast to neutral pictures in left amygdala, right and left anterior insula and a cluster of increased activation extending from the ventromedial prefrontal cortex (vmPFC) to the rostral anterior cingulate cortex (rACC). Statistical parametric maps are overlaid on an averaged T1 scan (radiological convention: left = right). Graphs display contrasts of parameter estimates (phobia-specific vs. neutral picture anticipation; mean ± standard error for activation cluster).
The combination of ACC and insula activation in the current study suggests that BII phobics allocate more attention, externally as well as internally, during anticipation of phobia-specific pictures. In line with this notion, increased activation in visual areas (fusiform gyrus and cuneus) was observed in BII phobics. This is consistent with previous symptom provocation studies in specific phobia (Caseres et al., 2010a; Schienle et al., 2003; Straube et al., 2005; Straube et al., 2006b), and has been attributed to increased visual attention in the expectation of behaviorally relevant visual input during anticipatory anxiety (Straube et al., 2007). Furthermore, in accordance with results for other anxiety disorders (Boshuisen et al., 2002; Straube et al., 2007), increased thalamus activation was found during phobia-specific anticipation in BII phobics, which is suggested to reflect general arousal states and stress (Vertes et al., 2015).

Current findings include several clusters of increased activation during sustained anticipation in medial and lateral PFC for BII phobics as compared to healthy controls. This region has generally been suggested to integrate emotional and cognitive processing (Pessoa, 2008), and seems to be important for appraisal and regulation of emotionally relevant situations (Amadio and Frith, 2006; Hermann et al., 2007; Kalisch et al., 2006; Kerr et al., 2012; Ochsner and Gross, 2005), also with regard to specific phobia (Del Casale et al., 2012; Ipser et al., 2013; Linares et al., 2012). Additionally, mPFC is part of the so-called default mode network under baseline and resting state conditions, and is involved in introspection as well as self-referential mental activity (Raichle, 2015; Raichle et al., 2001). In previous studies, medial and lateral PFC have been shown to be involved in anticipatory anxiety in healthy subjects (Drabant et al., 2011; Nitschke et al., 2006; Ploghaus et al., 1999). On the contrary, the current findings show decreased activation of the medial part of the OFC in BII phobics in contrast to healthy controls. Similar deactivation has been reported in BII phobics during symptom provocation, likely reflecting reduced control over emotional responses (Hermann et al., 2007). The simultaneous increase and decrease of activation in PFC subregions in the present study indicates differential functions of the PFC in sustained anticipatory anxiety in BII phobics. Enhanced prefrontal activity could reflect increased appraisal of the anticipatory context as well as increased self-perception in BII phobics. Deactivation of medial OFC, on the other hand, indicates that BII phobics might have difficulties down-regulating negative emotions. Furthermore, it has been suggested that the default mode network is directly competing with other systems and as a consequence focused attention on external stimuli tends to reduce activation in the default mode network (Buckner et al., 2008). An alternative explanation could thus be that the activation increases and decreases in PFC subregions reflect competing systems. Consequently, the medial OFC as part of the default mode network might show decreased activation because the anticipation of phobia-specific stimuli demands more attentional resources in BII phobics as compared to controls.

Beyond that, the current analysis revealed activation of the amygdala in the initial period. Such an effect has not been found in studies which only considered sustained anticipatory anxiety (e.g. Boshuisen et al., 2002; Chua et al., 1999; Jensen et al., 2003; Ploghaus et al., 1999; Straube et al., 2007). In line with previous studies in healthy subjects (Alvarez et al., 2011; Grupe et al., 2013; Somerville et al., 2013), analysis of initial brain responses facilitated the detection of amygdala activation, even though a prolonged anticipation design was used. This supports involvement of the amygdala in rapid threat processing (LeDoux, 1998; Öhman and Mineka, 2001) and again demonstrates that it is possible to meet temporal characteristics with specific analysis strategies (Grupe et al., 2013; Somerville et al., 2013).

There was no differential activation of BNST. This stands in contrast to findings reported for spider phobia (Münsterkötter et al., 2015;
5. Conclusions

The current study demonstrates brain activations related to anticipatory anxiety in BII phobia. While previous studies on symptom provocation in specific phobia reported inconsistent results, neural correlates during anticipatory anxiety could constitute a common denominator for specific phobia subtypes. In the future, it would be interesting to test the current results by directly comparing different subtypes of specific phobia during anticipation of phobia-specific stimuli. Furthermore, modeling of initial and sustained brain responses revealed distinct temporal dynamics of the brain regions involved in anticipatory anxiety, also within one and the same brain region. These temporal dynamics should be considered during conceptualization of study designs and data analysis in future studies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2016.12.015.

Conflicts of interest

The authors report no financial interests with commercial interests.

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