

## Original Article

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# Specific amygdala response to masked fearful faces in post-traumatic stress relative to other anxiety disorders

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## Abstract

**Background.** Altered amygdala activation to fear-related stimuli has been proposed to be a potential neural correlate of heightened threat sensitivity in anxiety- and stress-related disorders. However, the role of stimulus awareness and disorder specificity remains widely unclear. Here we investigated amygdala responses to conscious and unconscious fearful faces in patients suffering from panic disorder (PD), generalized anxiety disorder (GAD), or post-traumatic stress disorder (PTSD) and in a large sample of healthy controls (HC).

**Methods.** During event-related functional magnetic resonance imaging participants ( $n = 120$ ; 20 PD, 20 GAD, 20 PTSD, 60 HC) were confronted with briefly presented fearful faces, neutral faces, and non-faces in a backward masking paradigm. The design allowed for the analysis of trial-by-trial face detection performance and amygdala responses to fearful *v.* neutral faces.

**Results.** All participants exhibited increased amygdala activation to fearful *v.* neutral faces during conscious trials. Specifically during unconscious face processing, the PTSD, compared with all other groups, showed higher right basolateral (BLA) amygdala activity to fearful *v.* neutral faces.

**Conclusions.** The present study shows that BLA amygdala hyperactivity during unconscious, but not conscious, processing of fearful faces differentiates PTSD from the investigated disorders. This finding suggests an automatic and specific neural hyper-responsivity to general fear cues in PTSD and supports the idea of categorical differences between PTSD and other anxiety-related disorders.

## Introduction

Anxiety disorders are among the most prevalent psychopathologies (Kessler *et al.* 2005) and include panic disorder (PD), specific phobia (SP), social anxiety disorder (SAD), generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD) (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, DSM-IV; American Psychiatric Association, 2000). Current changes in research transferred PTSD into a new group of trauma- and stressor-related disorders, which distinguishes PTSD from the other anxiety disorders (DSM-5; American Psychiatric Association, 2013). There are still controversies about this new classification (Zoellner *et al.* 2011, 2013), mainly criticizing the insufficient evidence for PTSD being distinct from anxiety disorders.

One key region in the development, maintenance, and expression of pathological fear and anxiety is the amygdala (Janak & Tye, 2015; Marsh, 2016), which comprises the basolateral (BLA), centromedial (CeA), and superficial (SF) nucleus (Amunts *et al.* 2005; Ball *et al.* 2007; Janak & Tye, 2015). Especially the BLA and CeA hold special importance in healthy as well as pathological fear processing. The BLA is known as the main entrance center for information about the external environment, and therefore plays an important role in detecting relevant threat stimuli. Due to its strong connections to the CeA, which acts as an essential part in behavioral output processes, the BLA is able to potentiate fear responses via these projections (Janak & Tye, 2015). Thus, given the amygdala's critical role during threat processing (Öhman, 2005), its function in detecting relevant stimuli in the environment and initiation of subsequent behavioral reactions is pronounced concerning anxiety or anxiety-related disorders (Etkin & Wager, 2007; Patel *et al.* 2012; Duval *et al.* 2015). There are several studies suggesting abnormal amygdala hyperactivity in single patient groups, even during unconscious threat processing (Bryant *et al.* 2008; Kemp *et al.* 2009; Lipka *et al.* 2011; Ottaviani *et al.* 2012; Rabellino *et al.* 2016).

For investigation of conscious and unconscious amygdala responses to general fear-related stimuli, fearful facial expressions have proved to be a reliable probe in healthy controls (HC) and patients (Fusar-Poli *et al.* 2009; Mendez-Bertolo *et al.* 2016). Several single-disorder studies suggest abnormal amygdala responses to fearful faces in different disorders (Domschke

et al. 2008; Fonzo et al. 2010). However, to gain a better understanding of common and distinct processing mechanisms in different anxiety-related disorders, it is necessary to use the same experimental procedures and analytical methods for between-group comparisons within one study. To our knowledge, until now only two studies investigated fearful face processing across different anxiety-related disorders. One study demonstrated higher amygdala activity across all anxiety disorders (GAD, SAD, PD) compared with controls during conscious processing of fearful *v.* happy faces (Fonzo et al. 2015). Another study investigated unconscious affective face processing across different anxiety-related disorders (PD, SP, PTSD) using a backward masking design (Killgore et al. 2014). They showed differences during unconscious affective face processing between a combined patient group (PD, SP, PTSD) and HC and additionally between PTSD and HC but not between anxiety disorders, which was attributed to small samples sizes (PTSD and PD each  $n = 14$ , SP  $n = 15$ ).

These studies made important first steps in the understanding of shared and distinct amygdala involvement during fearful face processing in anxiety-related disorders. Nevertheless, besides the issue of sample sizes, some methodological and interpretative aspects may be critical in previous single-disorder and disorder-comparison (backward masking) studies (for discussion see Pessoa et al. 2006; Pessoa & Adolphs, 2010; Straube et al. 2010a, 2011). For example, both former disorder-comparison studies used block designs, which do not allow for the investigation of immediate trial-based responses, regardless of expectation and regulation aspects, or to control for visibility on a trial-by-trial basis. Furthermore, one study used a neutral face as mask for backward masking (Killgore et al. 2014), which clearly influences amygdala activity in backward masking studies due to target-mask effects (Kim et al. 2010; Straube et al. 2010a).

In the present study, we aimed to investigate amygdala activation during conscious and unconscious processing of fearful faces in different anxiety-related disorders (PD, GAD, PTSD, combined sample:  $n = 60$ ) and a large sample of HC ( $n = 60$ ) using event-related functional magnetic resonance imaging (fMRI). We used trial-by-trial responses of face awareness for a signal detection theory (SDT)-based analysis of face detection performance. To manipulate conscious visibility, we varied the inter-stimulus-interval (ISI) between target and mask, but kept all stimulus durations constant to avoid confounding conscious visibility and presentation duration. By means of probabilistic activation tracking, we also aimed to better characterize the anatomical amygdala subregion showing significant differential activation to fearful *v.* neutral faces.

## Methods and materials

### Subjects

One hundred and twenty right-handed (assessed by the Edinburgh Handedness Inventory; Oldfield, 1971) subjects participated in the study (group characteristics in Table 1). They were recruited via public announcements in newspapers and were paid for their participation. Since the study started in 2012, all subjects were screened with the German version of the Structured Clinical Interview for DSM-IV (SCID; Wittchen et al. 1997). As primary diagnosis, 20 subjects fulfilled the diagnostic criteria for PD with or without agoraphobia [16 female (f)], 20 patients for GAD (16

f) and 20 patients for PTSD after interpersonal violence (all f). The three single anxiety patient groups were matched according to age, gender, years of education, antidepressant medication (selective serotonin reuptake inhibitor, SSRI), and comorbid diagnosis. An exclusion criterion for patients was the comorbid diagnosis of at least one of the other two investigated disorders. Afterwards, 60 HC (51 f, 9 m) were matched for gender, age, and education (number of years in school) to the anxiety disorder group. HC were free of any psychiatric diagnosis. Common exclusion criteria for patients (PAT) and HC were presence or history of neurological, psychotic, or bipolar disorders, drug dependence or abuse within the last 10 years, suicidal ideations, psychotropic medication (except SSRI), and fMRI contraindications. All participants had normal or corrected-to-normal vision. Written informed consent was obtained from each subject. The study conformed to the Declaration of Helsinki and was approved by and conducted in accordance with guidelines of the Ethics Committee of Muenster (Germany).

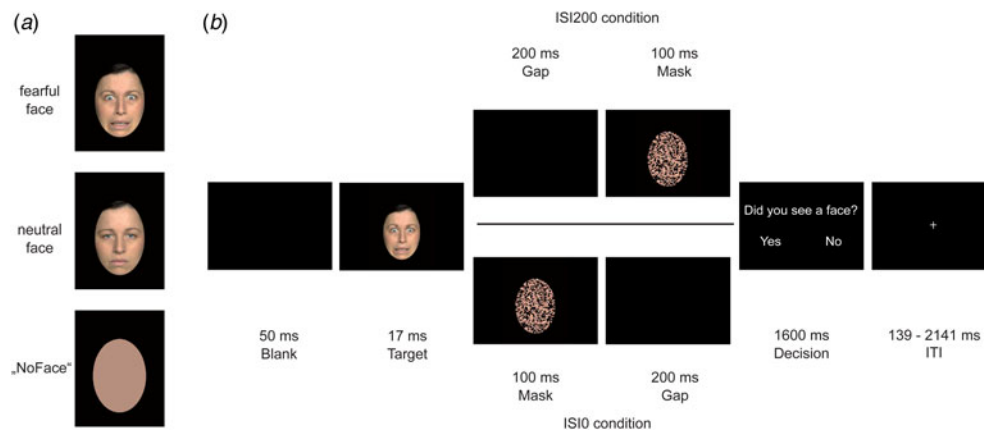
### Paradigm and stimuli

During the event-related functional run (13 min 8 s), participants underwent a backward masking design. Each trial began with a 50 ms blank screen after which a target face (fearful or neutral) was presented for 17 ms. As target faces, we used 40 female and 40 male different individuals, target faces' age ranged approximately between 18 and 40 years. The stimuli were taken from different face sets: FACES database, which was provided by the Max-Planck Institute for Biological Cybernetics in Tuebingen, Germany (Ebner et al. 2010), the NimStim set (Tottenham et al. 2002), and the Radbound Faces Database (Langner et al. 2010). Furthermore, artificially generated 'NoFaces' were presented as targets as well. These 'NoFaces' were originally neutral or fearful faces, which were converted into a skin-colored oval using a soft drawing filter of Adobe Photoshop CS6 (version 13.0.1, Adobe Systems Inc., San Jose, California, USA). After a variable ISI, a scrambled face mask (see below) was presented for 100 ms. In order to vary the degree of conscious visibility, we manipulated the ISI between the target face and the mask: in the ISI0 condition the masks onset coincided with target offset, whereas in the ISI200 condition target offset and mask onset were separated by a blank screen lasting for 200 ms. We expected the majority of target faces to be unconscious in the ISI0 condition and to be conscious in the ISI200 condition. As a mask, we used the same face of the target, either the same or other emotion distributed equally, in a scrambled shape (scramble filter) using Adobe Photoshop CS6. Afterwards, a decision screen ('Did you see a face?') was presented for 1600 ms. Participants were asked to press the 'yes' button (when seeing a face stimuli) or 'no' button (when not seeing a face stimuli or when seeing a 'NoFace'). We varied the inter-trial-interval by using a jitter that ranged between 139 ms and 2141 ms. Stimuli were presented using Presentation® software (Version 17.2, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com) and rear-projected via an LCoS (Liquid Crystal on Silicon) projector (DLA-RSxx, JVC Kenwood USA Corporation, Los Angeles, California, USA) onto a screen that the participants viewed through a mirror on the MRI head coil. Before fMRI scanning, participants were trained to perform the task in a 5-min training session outside the scanner. Figure 1 illustrates trial sequences for both ISI200 and ISI0 trials.

**Table 1.** Demographic and clinical characterization

	PD <i>M</i> ± s.d. (Range)	GAD <i>M</i> ± s.d. (Range)	PTSD <i>M</i> ± s.d. (Range)	HC <i>M</i> ± s.d. (Range)	Statistics for group difference
<i>N</i> (f/m)	20 (16/4)	20 (16/4)	20 (20)	60 (51/9)	$\chi^2_{(3)} = 4.455$ $p = 0.216$
Age (years)	24.45 ± 5.11 (18–35)	27.85 ± 8.76 (20–55)	26.5 ± 5.67 (19–38)	26.35 ± 5.04 (19–51)	$F_{(3)} = 4.105$ $p = 0.250$
Education (years)	12.65 ± 0.93 (11–15)	12.65 ± 1.53 (9–15)	12.78 ± 1.17 (10–14)	12.88 ± 1.02 (9–18)	$F_{(3)} = 1.629$ $p = 0.653$
Antidepressant medication	4/20	6/20	4/20	–	$\chi^2_{(2)} = 0.745$ $p = 0.689$
Questionnaire data					
BDI-II <sup>a</sup> score	11.1 ± 8.37 (0–36) HC < PD: $z = 4.899$ $p < 0.001$	16.2 ± 11.25 (0–47) HC < GAD: $z = 6.111$ $p < 0.001$	19.65 ± 10.99 (5–35) HC < PTSD: $z = 6.891$ $p < 0.001$	1.7 ± 2.49 (0–9) HC = HC: $z = 0.000$ $p < 0.001$	$F_{(3)} = 73.895$ $p < 0.001$
PAS <sup>b</sup> for PD	PAS <sub>PD</sub> : 20.65 ± 8.6 (1–32)			PAS <sub>HC</sub> : 0.25 ± 0.76 (0–4)	$U = -6.832$ $p < 0.001$
PSWQ <sup>c</sup> for GAD	PSWQ <sub>GAD</sub> : 51.8 ± 6.93 (32–61)			PSWQ <sub>HC</sub> : 31.92 ± 5.66 (21–48)	$U = -3.913$ $p < 0.001$
PDS <sup>d</sup> for PTSD				PDS <sub>PTSD</sub> : 23.55 ± 9.48 (7–43)	PDS <sub>HC</sub> : 1.26 ± 2.14 (0–8) $U = -6.248$ $p < 0.001$
No. of comorbidities (DSM-IV)					
Depressive disorders	5	1	1		$\chi^2_{(2)} = 5.175$ $p = 0.075$
Specific phobia	1	2			$\chi^2_{(2)} = 2.105$ $p = 0.349$
Social anxiety disorder	1		2		$\chi^2_{(2)} = 2.105$ $p = 0.349$
Eating disorders		1	1		$\chi^2_{(2)} = 1.034$ $p = 0.596$
Somatoform disorders			1		$\chi^2_{(2)} = 2.034$ $p = 0.362$

<sup>a</sup>German version (Hautzinger *et al.* 2009) of the Beck Depression Inventory (Beck *et al.* 1996).<sup>b</sup>German version of the Panic and Agoraphobia Scale (Bandelow, 1997).<sup>c</sup>German version of the Penn State Worry Questionnaire (Meyer *et al.* 1990).<sup>d</sup>German version by Anke Ehlers (unpublished) of the Posttraumatic Diagnostic Scale (Foa *et al.* 1997).



**Fig. 1.** Experimental trial procedure. In (a), exemplary target faces are displayed: fearful face, neutral face, and 'NoFace'. In (b), an exemplary trial procedure for the ISI200 and ISI0 condition is shown. Each trial began with a 50 ms blank, followed by a target face lasting for 17 ms. The ISI200 condition (upper path) differed from the ISI0 condition (lower path) due to a 200 ms gap that was either presented between target offset and mask onset (ISI200 condition) or after mask offset (ISI0 condition). In both trial variants, a decision screen followed lasting for 1600 ms.

### Behavioral data analysis

Behavioral data analysis was based on SDT methods (Green & Swets, 1966). Each participants' detection rate ( $d'$ ) was calculated based on the relative frequencies of hits and false alarms, separate for the ISI0 and ISI200 condition and for fearful and neutral faces, resulting in four  $d'$  values for each participant. To compare emotion processing between anxiety patients and HC in general, we performed, per ISI condition, a 2 (within; fearful/neutral)  $\times$  2 (between; PAT/HC) repeated measures analysis of variance (rANOVA), henceforth referred to as *overall rANOVA*. In order to compare anxiety disorders within the group of patients regarding emotion processing, we performed a 2 (within; fearful/neutral)  $\times$  3 (between; PD/GAD/PTSD) rANOVA in parallel, henceforth referred to as *differential rANOVA*. For reaction times, the same procedure was conducted after applying a log transformation to compensate for the skewed distribution of reaction times.

### Image acquisition and analysis

fMRI scanning was conducted on a 3-Tesla scanner (Magnetom Prisma; Siemens, Erlangen, Germany). First a T1-weighted anatomical scan was acquired. The functional run consisted of 375 volumes (36 axial slices per volume, thickness = 3 mm, gap = 0.3 mm, in-plane resolution = 2.26 mm  $\times$  2.26 mm, orientated at a tilted angle to the anterior–posterior commissural plane) using a T2\*-weighted echo planar sequence (repetition time = 2080 ms, echo time = 30 ms, flip angle = 90°, field of view = 208 mm, matrix = 92  $\times$  92 voxel). The first 10 volumes of the run were discarded to ensure steady-state tissue magnetization. Image preprocessing and analyses were performed using BrainVoyager QX (Version 2.8, Brain Innovation, Maastricht, The Netherlands). All volumes were realigned to the first volume, were corrected for slice time errors, and spatially (6-mm full-width half-maximum isotropic Gaussian kernel) and temporally (high-pass filter, 10 cycles per run; low-pass filter, 2.8 s) smoothed. Anatomical and functional images were coregistered and normalized to Talairach space (Talairach & Tournoux, 1988). Statistical analysis was performed using multiple linear regression of the signal time course at each voxel. The blood oxygen level-dependent signal change was modeled with a two  $\gamma$

hemodynamic response function for each event type. Based on each participants' behavioral data, predictors of interest for unconscious face processing were obtained from trials in the ISI0 condition where fearful or neutral faces were 'unseen' (button press 'no face'), and for conscious face processing by trials in the ISI200 condition where fearful or neutral faces were 'seen' (button press 'face'). The other events were treated as predictors of no interest. For second-level analysis, we conducted a small volume correction analysis for the *a priori* defined amygdala region of interest (ROI). The left and right amygdala ROIs were created according to the Automated Anatomical Labeling (AAL) atlas (and 1 mm dilating factor according to AAL; Tzourio-Mazoyer et al. 2002; Maldjian et al. 2003; Etkin et al. 2004) and were transformed into Talairach space (according to Lancaster et al. 2007) using ICBM2TAL in Matlab (MATLAB 2012, The MathWorks, Inc., Natick, Massachusetts, USA). The anatomical assignment of the observed activation patterns to an amygdala subregion was verified using anatomical probability maps of the Anatomy toolbox (Eickhoff et al. 2004; Amunts et al. 2005). Second-level analyses were performed for unconscious and conscious conditions separately. Based on a regression model for each patient, we calculated an overall 2 (within; fearful/neutral face)  $\times$  2 (between; PAT/HC) rANOVA for main effect of emotion and interaction effects of emotion by group (overall PAT v. HC). Furthermore, a differential 2 (within; fearful/neutral face)  $\times$  3 (between; PD/GAD/PTSD) rANOVA was calculated to further investigate interaction effects of emotion by anxiety-related group (PD, GAD v. PTSD). To further resolve interaction effects resulting from the differential rANOVA, average percent signal change across all voxels within each significant cluster was extracted for each regressor per subject. Afterwards, a 2 (within; fearful/neutral face)  $\times$  2 (within; conscious/unconscious)  $\times$  3 (between; PD, GAD v. PTSD) rANOVA was conducted in SPSS (Version 24; IBM, Armonk, New York, USA).

The issue of detectable amygdala effects to unconscious threat was further taken into account by statistical analyses based on estimated effect sizes. With regard to effect size estimates in a backward masking study ( $d = 0.58$ ) (Hoffmann et al. 2015), we performed a statistical power analysis of our design. This calculation yielded an unsatisfactorily voxel-level Power of  $1 - \beta = 0.74$  at an initial voxel threshold of  $p < 0.001$ , but not with voxel threshold of  $p < 0.005$  (Power of  $1 - \beta = 0.88$ ) (see also Lieberman &

Cunningham, 2009). Using the amygdala ROI, thresholded maps were submitted to an ROI-based correction criterion for multiple comparisons based on the estimate of the map's spatial smoothness and on an iterative procedure (Monte Carlo simulation as implemented in BrainVoyager), which do not use the Gaussian random-field approach for cluster-size thresholding. After 1000 iterations, the minimum cluster size threshold that yielded a cluster-level false-positive rate of 5% was applied to the statistical maps. *Post hoc* group comparisons of the differential rANOVA were calculated by (1) extraction of the  $\beta$  values of the significant effect and (2) statistical analysis using SPSS (Version 22.0, IBM Corp, 2013). *Post hoc* group comparisons for the differential rANOVA were calculated by Duncan (homogeneity of variance) or Games-Howell (inhomogeneity of variance) tests. To further check whether group differences differ from a healthy population, we calculated a Dunnett-*t* test to compare each anxiety group against the HC group. To further explore significant differential effects across patients, we calculated additional analyses of covariance (ANCOVAs), to test whether results were maintained after inclusion of Beck Depression Inventor (BDI)-II score and anti-depressant medication intake as covariates.

In this report, we only investigated activation of the amygdala. However, for the sake of completeness, whole brain analyses are included as online Supplementary material (method description Section S1 and S2; results online Supplementary Tables S2 and S3).

## Results

### Behavioral data – detection rate ( $d'$ )

#### ISI200

In the ISI200 condition, the overall rANOVA revealed a significant main effect of emotion [ $F_{(1,118)} = 25.558, p < 0.001$ ], resulting from a higher detection rate within trials with ISI200 for fearful compared with neutral faces ( $d'_{\text{fearful}} = 3.82, d'_{\text{neutral}} = 3.67$ ). Furthermore, we found a main effect of group [ $F_{(1,118)} = 12.908, p < 0.001$ ], showing that HC were more likely to detect faces than PAT, independent of emotional expression ( $d'_{\text{HC}} = 3.98, d'_{\text{PAT}} = 3.51$ ). We did not find any interaction effect ( $p > 0.290$ ).

In the ISI200 condition, the differential rANOVA revealed a main effect of emotion [ $F_{(1,57)} = 16.172, p < 0.001$ ], resulting from a higher detection rate within trials with ISI200 for fearful compared with neutral faces ( $d'_{\text{fearful}} = 3.60, d'_{\text{neutral}} = 3.42$ ). We did not find any main effect of group or an interaction effect between emotion and group (all  $p > 0.436$ ).

#### ISI0

Overall rANOVA for ISI0 during face processing revealed a significant main effect of emotion [ $F_{(1,118)} = 4.976, p = 0.028$ ]. Although  $d'_{\text{neutral}} = 0.082$  (0.51 of 40 trials = 1.28%) compared with  $d'_{\text{fearful}} = 0.034$  (0.33 of 40 trials = 0.83%) revealed to be significant, absolute numbers of trials for ISI0 but 'seen' neutral faces within participants ranged between 0 and 7 of 40 trials. For face detection during the ISI0 condition,  $d'$  of just three participants were slightly above chance level (0.85, 0.54, 0.54), while the  $d'$  confidence interval of chance performance is (-0.4774; 0.4795) as calculated by bootstrapping (Michey *et al.* 2008). We did not find any main effect of group (PAT/HC) or interaction effect emotion  $\times$  group (all  $p > 0.121$ ).

Concerning the ISI0 condition, in the differential rANOVA, we did not find any main or interaction effect (all  $p > 0.131$ ). For a presentation of the results, see also Table 2 and Fig. 2.

### Behavioral data – reaction time (RT)

#### ISI200

In the ISI200 condition, the overall rANOVA revealed a main effect of emotion [ $F_{(1,118)} = 15.790, p < 0.001$ ], indicating faster reactions within trials with ISI200 for fearful than neutral faces across all participants ( $RT_{\text{fearful}} = 453.75, RT_{\text{neutral}} = 463.57$ ). There was no main effect of group or an interaction effect (all  $p > 0.380$ ).

In the ISI200 condition, the differential rANOVA revealed a main effect of emotion [ $F_{(1,57)} = 9.708, p = 0.003$ ], indicating faster reactions to fearful than neutral faces ( $RT_{\text{fearful}} = 458.77, RT_{\text{neutral}} = 469.95$ ) in the ISI200 condition. There was no main effect of group or an interaction effect (all  $p > 0.450$ ).

#### ISI0

For the ISI0 condition, the overall rANOVA revealed neither main effects nor an interaction effect (all  $p > 0.279$ ). Concerning the ISI0 condition, in the differential rANOVA, no main effect or interaction effect was found (all  $p > 0.136$ ).

### FMRI data

**ISI200:** For the overall rANOVA, we found a main effect of emotion in bilateral amygdala [right amygdala: average  $F_{(1,118)} = 15.60, k = 73, p < 0.05$  corrected, peak voxel:  $x = 23, y = 2, z = -14$ ; left amygdala: average  $F_{(1,118)} = 17.07, p < 0.05$  corrected,  $k = 111$ , peak voxel:  $x = -30, y = -2, z = -12$ ], indicating more activation during fearful *v.* neutral face processing across all participants (see Fig. 3a). Both activation clusters comprise the BLA and SF subregion of the amygdala. The probability of the anatomical assignment of the respective maximal voxel suggests an activation of the SF amygdala subregion with  $p > 0.3$  (0.3–0.6) for the right activation cluster and of the SF amygdala subregion with  $p > 0.2$  (0–0.5) for the left activation cluster. There was no effect of or interaction with group during conscious face processing in the overall or differential rANOVA.

**ISI0:** There was neither a main effect of emotion nor a group or interaction effect in the overall rANOVA. For the differential rANOVA, a significant effect indicating differences between the disorders was found in the right amygdala [average  $F_{(2,57)} = 6.69, p < 0.05$  corrected,  $k = 5$ , peak voxel:  $x = 26, y = -3, z = -19$ ; see Fig. 3b and Table 3). *Post hoc* test Games-Howell revealed significant stronger activation in PTSD compared with PD (average difference  $M_{\text{PTSD}} - M_{\text{PD}} = 0.94, p = 0.026$ ) and GAD patients ( $M_{\text{PTSD}} - M_{\text{GAD}} = 0.73, p = 0.018$ ). Additional comparison with the HC group with *post hoc* test Dunnett-*t* showed that HC significantly differ from PTSD patients ( $M_{\text{HC}} - M_{\text{PTSD}} = 0.81, p = 0.035$ ), but not from PD ( $M_{\text{HC}} - M_{\text{PD}} = -0.13, p = 0.962$ ) and GAD patients ( $M_{\text{HC}} - M_{\text{GAD}} = 0.08, p = 0.992$ ). Finally, the probability of the anatomical assignment of the observed cluster suggests an activation of the BLA amygdala subregion with  $p > 0.7$  (0.6–0.8).

Furthermore, to investigate whether the effect in the amygdala represents differences between conscious and unconscious conditions, we conducted an additional 2 (within; fearful/neutral face)  $\times$  2 (within; conscious/unconscious)  $\times$  3 (between; PD, GAD *v.* PTSD) rANOVA for the average percent signal change

**Table 2.** Results for behavioral data (detection rate and reaction time)

	F-value	p value	Effect	F-value	p value	Effect
	Overall analysis: ISI200 condition face processing detection rate			Overall analysis: ISI0 condition face processing detection rate		
<i>d'</i> main effect emotion	25.558	<0.001	Fearful > neutral	4.976	0.028	Neutral > fearful
<i>d'</i> main effect group	12.908	<0.001	HC > PAT	2.439	0.121	–
<i>d'</i> interaction effect	1.130	0.290	–	0.559	0.456	–
RT main effect emotion	15.790	<0.001	Fearful < neutral <sup>a</sup>	0.017	0.895	–
RT main effect group	0.776	0.380	–	0.018	0.894	–
RT interaction effect	0.308	0.580	–	1.181	0.279	–
	Differential analysis: ISI200 condition face processing detection rate			Differential analysis: ISI0 condition face processing detection rate		
<i>d'</i> main effect emotion	16.172	<0.001	Fearful > neutral	0.982	0.326	–
<i>d'</i> main effect group	0.843	0.436	–	0.234	0.792	–
<i>d'</i> interaction effect	0.408	0.667	–	2.107	0.131	–
RT main effect emotion	9.708	0.003	Fearful < neutral <sup>a</sup>	0.409	0.525	–
RT main effect group	0.809	0.450	–	2.065	0.136	–
RT interaction effect	0.817	0.447	–	0.687	0.507	–

*d'*, *d* prime detection rate; RT, reaction time; HC, healthy controls; PAT, patients.

<sup>a</sup>Faster reaction time for fearful compared with neutral faces.

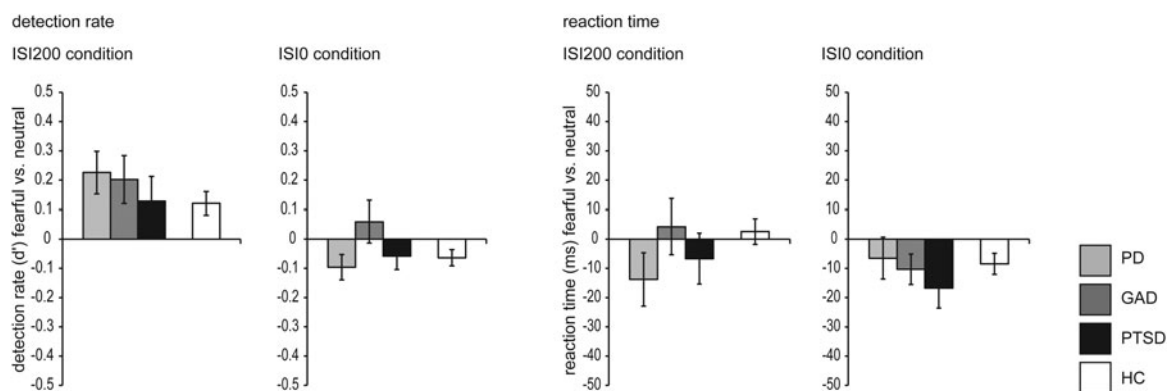
across all voxels within the significant amygdala cluster. Results revealed a significant emotion by group by condition interaction [ $F_{(1,57)} = 5.999, p = 0.004$ ] for this amygdala cluster.

Although only 'unseen' faces in the ISI0 condition were classified as unconscious, residual visibility of incompletely masked faces may still have contributed to the BLA effect (e.g. in trials where faces were actually seen but the subjects confused the response keys). Therefore, we checked whether the BLA interaction effect was modulated by the degree of visibility during unconscious processing by correlating individual BLA effects and *d'* values. There was no significant correlation ( $r = -0.147, p = 0.264$ ), and thus no evidence for a contribution of residual visibility to the BLA effect in the unconscious condition. The influences of depressive symptoms (using BDI-II scores) and

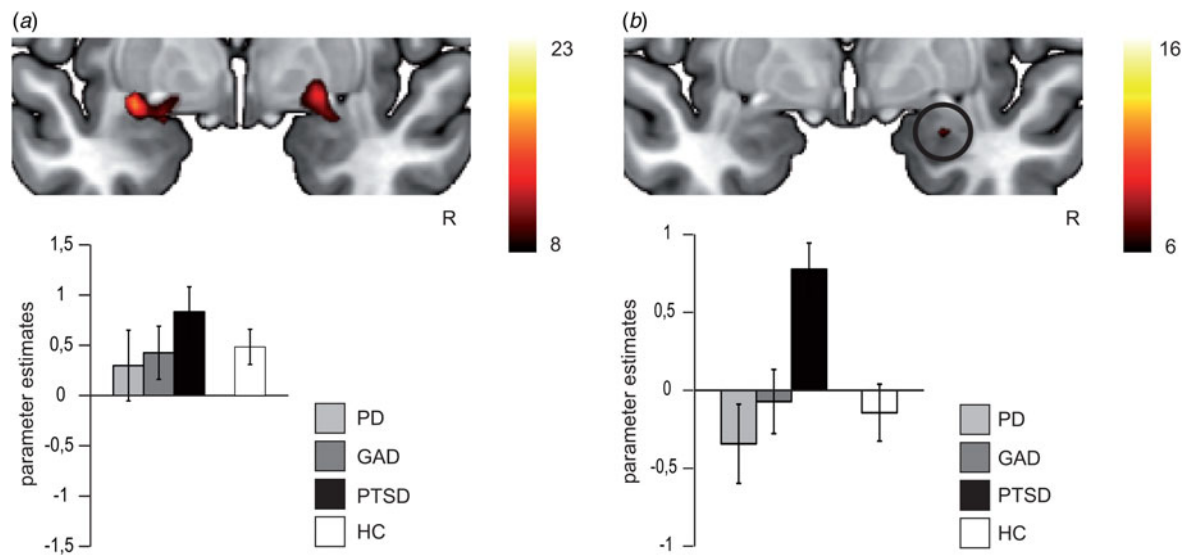
antidepressant medication intake were tested by means of ANCOVAs and revealed no significant effects of the covariates (all  $p > 0.473$ ) and maintained *group* effects (all  $F$ -values  $\geq 7.58$ , all  $p < 0.001$ ) for the significant amygdala cluster. An overview of antidepressant medication intake is found in the online Supplementary Table S1.

## Discussion

The present study is the first that allows for direct comparisons between three anxiety-related disorders during conscious and unconscious general threat processing. By meeting strong methodological requirements, we found increased BLA activation to



**Fig. 2.** Detection rate (left side) and reaction time (right side), respectively, for the ISI200 and ISI0 condition. Concerning detection rate in the ISI200 condition, the overall and differential ANOVA revealed main effects of emotion (fearful > neutral faces). Additionally, the overall ANOVA revealed a main effect of group (healthy controls > anxiety-related disorders). Concerning detection rate in the ISI0 condition, the overall ANOVA revealed a main effect of emotion (neutral > fearful faces). Analysis of reaction time in the ISI200 condition revealed main effects of emotion for the overall and differential ANOVA (faster reaction time for fearful than neutral faces). Concerning the ISI0 condition, there were no significant results.



**Fig. 3.** In (a), differential brain activation for fearful *v.* neutral faces in anxiety-related disorder patients and healthy controls in the bilateral amygdala ( $p < 0.05$  corrected). All participants show increased bilateral amygdala activation for fearful  $>$  neutral faces (main effect of emotion). In (b), differential brain activation for fearful *v.* neutral faces in anxiety-related disorder patients in the right amygdala ( $p < 0.05$  corrected). Post-traumatic stress disorder (PTSD) patients relative to panic disorder (PD) and generalized anxiety disorder (GAD) patients show increased right amygdala activation for fearful  $>$  neutral faces (interaction effect). Compared with healthy controls (HC), PTSD patients show increased right amygdala activation, whereas right amygdala activation of PD and GAD did not differ from HC. Note that in (a) and (b) scales for parameter estimates are different.

unconscious, but not conscious, fearful faces in PTSD compared with PD and GAD patients as well as with HC.

In the present study, all participants showed increased responses in several amygdala subregions, to fearful relative to neutral faces during conscious processing. This finding underlines

the amygdala’s key role in processing fear-related facial stimuli in both normal as well as pathological fear (Domschke *et al.* 2008; Fusar-Poli *et al.* 2009; Gamer & Büchel, 2009; Straube *et al.* 2010b; van den Bulk *et al.* 2014; Marsh, 2016; Mendez-Bertolo *et al.* 2016). The BLA is an important interface by sending

**Table 3.** Results for amygdala activation in the overall and differential analysis

	Region of interest	L	Talairach coord. of peak voxel			Cluster size (mm <sup>3</sup> )	F-value average	p value	Effect
			x	y	z				
Overall analysis: ISI200 condition face processing									
Main effect: emotion	Amygdala	L	-30	-2	-12	888	$F_{(1,118)} = 17.07$	$p < 0.05$	Fearful $>$ neutral
	Amygdala	R	23	2	-14	584	$F_{(1,118)} = 15.60$	$p < 0.05$	Fearful $>$ neutral
Interaction effect: emotion $\times$ group	Amygdala	L	-	-	-	-	-	-	-
	Amygdala	R	-	-	-	-	-	-	-
Differential analysis: ISI200 condition face processing									
Interaction effect: emotion $\times$ group	Amygdala	L	-	-	-	-	-	-	-
	Amygdala	R	-	-	-	-	-	-	-
Overall analysis: ISI0 condition face processing									
Main effect: emotion	Amygdala	L	-	-	-	-	-	-	-
	Amygdala	R	-	-	-	-	-	-	-
Interaction effect: emotion $\times$ group	Amygdala	L	-	-	-	-	-	-	-
	Amygdala	R	-	-	-	-	-	-	-
Differential analysis: ISI0 condition face processing									
Interaction effect: emotion $\times$ group	Amygdala	L	-	-	-	-	-	-	-
	Amygdala	R	26	-3	-19	40	$F_{(2,57)} = 6.69$	$p < 0.05$	PTSD $>$ PD = GAD = HC

information about potential threat to the CeA, which, e.g. modulates behavior reactions like startle response, freezing (Phelps & LeDoux, 2005; Janak & Tye, 2015; Perusini et al. 2016), or orienting responses (Gamer & Büchel, 2009; Straube et al. 2010b).

However, we found no evidence that patients are characterized by a hyper-responsivity to clearly seen stimuli relative to HC. This finding is in contrast to other studies (Domschke et al. 2008; Fonzo et al. 2010). It should be noted that in previous studies, target presentation time ranged between 500 and 5000 ms, while in the present work target faces were presented for 17 ms. Furthermore, previous studies used block designs, which on the one hand might have more power to detect potential effects. On the other hand, block designs are associated with expectation and regulation effects. Finally, our study did not require patients to attend to emotional information of faces at all. Thus, our study investigated very rapid and automatic responses to fearful faces. Under such conditions, we show comparable immediate amygdala responsivity to fearful faces in anxiety patients and controls.

Interestingly, when comparing different anxiety-related disorders during the unconscious condition, only patients suffering from PTSD exhibited exaggerated amygdala responsivity compared with other anxiety disorders and with HC. This finding is in line with previous PTSD studies, reporting heightened amygdala responsivity to general emotional stimuli during unconscious processing of threat stimuli (Bryant et al. 2008). In addition to these findings, the current study suggests a specificity of this response in PTSD compared with other anxiety disorders. In the current study, the specificity of this response in PTSD points to different neurobiological mechanisms in the processing of fear-related stimuli between anxiety-related disorders. Given the BLA's important role in detecting threat stimuli, this activation might be associated with increased hypervigilance to very subtle threat cues in PTSD relative to other anxiety disorders. Interestingly, behavioral data did not show any differential effects between the investigated anxiety-related disorders. Thus, PTSD patients might show an automatic reaction to emotionally relevant stimuli on the neural level, without altered behavioral reactions during the task. This dissociation could be interpreted by the irrelevance of facial expression (whether it is fearful or neutral) for solving the task (whether there was a face or not). It might also indicate that the automatic amygdala response represents a reduced neural threshold for the elicitation of a variety of threat-related responses, which might contribute to uncontrollable and automatic hyper-responsivity to threat cues in PTSD (Bryant et al. 2008; Kemp et al. 2009). Our findings suggest a difference between PTSD and other disorders at least for this kind of general fear-related stimulus. However, the implementation of an unconscious condition is necessary to differentiate between anxiety-related disorders (here in terms of amygdala responsivity).

Some limitations of this study need to be mentioned. First, with the present sample size of  $n = 20$  per anxiety-related group, we were able to discover effects of  $d > 0.53$  with a statistical power of  $1 - \beta = 0.8$ . More subtle differences (e.g.  $d = 0.3$ ) would require sample sizes of  $n = 60$  per anxiety-related group. Thus, future studies should implement larger samples within each patient group. Additionally, these studies should include more male patients than the current study. At present, we cannot draw any conclusions regarding gender influences on general threat processing due to the absence of male participants in the PTSD group and low number of male participants in the PD and GAD patient groups ( $0 \leq n_{\text{male}} < 5$ ).

To conclude, we found evidence for a PTSD-specific hyper-responsivity in the BLA during unconscious, but not conscious, fearful face processing. This finding provides evidence for PTSD being distinct from anxiety-related disorders based on a specific automatic BLA response to general fear-related cues, which might be associated with pronounced hypervigilance in these patients. Furthermore, the current study illustrates the importance of including automatic processing designs to investigate differences in neural responses between anxiety-related disorders.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0033291717002513>.

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**Declaration of Interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

## References

- American Psychiatric Association** (2000) *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Association.
- American Psychiatric Association** (2013) *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Association.
- Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ et al.** (2005) Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *Anatomy and Embryology* **210**, 343–352.
- Ball T, Rahm B, Eickhoff SB, Schulze-Bonhage A, Speck O and Mutschler I** (2007) Response properties of human amygdala subregions: evidence based on functional MRI combined with probabilistic anatomical maps. *PLoS ONE* **2**, e307.
- Bandelow B** (1997) *Panic and Agoraphobia-Scale (PAS)*. Seattle, Toronto, Bern, Göttingen: Hogrefe & Huber Publishers.
- Beck AT, Steer RA and Brown GK** (1996) *Beck Depression Inventory – Second Edition. Manual*. San Antonio, TX: The Psychological Corporation.
- Bryant RA, Kemp AH, Felmingham KL, Liddell B, Olivieri G, Peduto A et al.** (2008) Enhanced amygdala and medial prefrontal activation during nonconscious processing of fear in posttraumatic stress disorder: an fMRI study. *Human Brain Mapping* **29**, 517–523.
- Domschke K, Ohrmann P, Braun M, Suslow T, Bauer J, Hohoff C et al.** (2008) Influence of the catechol-O-methyltransferase val158met genotype on amygdala and prefrontal cortex emotional processing in panic disorder. *Psychiatry Research: Neuroimaging* **163**, 13–20.
- Duval ER, Javanbakht A and Liberzon I** (2015) Neural circuits in anxiety and stress disorders: a focused review. *Therapeutics and Clinical Risk Management* **11**, 115–126.
- Ebner NC, Riediger M and Lindenberger U** (2010) FACES – a database of facial expressions in young, middle-aged, and older women and men: development and validation. *Behavior Research Methods* **42**, 351–362.
- Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K et al.** (2004) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *NeuroImage* **25**, 1325–1335.
- Etkin A, Klemenhagen KC, Dudman JT, Rogan MT, Hen R, Kandel ER et al.** (2004) Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron* **44**, 1043–1055.
- Etkin A and Wager TD** (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Journal of Psychiatry* **164**, 1476–1488.



- Foa EB, Cashman L, Jaycox L and Perry K (1997) The validation of a self-report measure of posttraumatic stress disorder: the posttraumatic diagnostic scale. *Psychological Assessment* **9**, 445–451.
- Fonzo GA, Ramsawh HJ, Flagan TM, Sullivan SG, Letamendi A, Simmons AN *et al.* (2015) Common and disorder-specific neural responses to emotional faces in generalised anxiety, social anxiety and panic disorders. *The British Journal of Psychiatry* **206**, 206–215.
- Fonzo GA, Simmons AN, Thorp SR, Norman SB, Paulus MP and Stein MB (2010) Exaggerated and disconnected insular-amygdalar BOLD response to threat-related emotional faces in women with intimate-partner violence PTSD. *Biological Psychiatry* **68**, 433–441.
- Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S *et al.* (2009) Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *Journal of Psychiatry & Neuroscience: JPN* **34**, 418–432.
- Gamer M and Büchel C (2009) Amygdala activation predicts gaze toward fearful eyes. *The Journal of Neuroscience* **29**, 9123–9126.
- Green DM and Swets JA (1966) *Signal Detection Theory and Psychophysics*. New York: Wiley.
- Hautzinger M, Keller F and Kühner C (2009) *BDI II. Beck Depressions-Inventor. Manual*. Frankfurt a.M: Pearson Assessment & Information GmbH.
- Hoffmann M, Mothes-Lasch M, Miltner WHR and Straube T (2015) Brain activation to briefly presented emotional words: effects of stimulus awareness. *Human Brain Mapping* **36**, 655–665.
- IBM Corp (2013) *IBM SPSS Statistics for Windows*. Armonk, NY: IBM Corp..
- Janak PH and Tye KM (2015) From circuits to behaviour in the amygdala. *Nature* **517**, 284–292.
- Kemp AH, Felmingham KL, Falconer E, Liddell BJ, Bryant RA and Williams LM (2009) Heterogeneity of non-conscious fear perception in posttraumatic stress disorder as a function of physiological arousal: an fMRI study. *Psychiatry Research: Neuroimaging* **174**, 158–161.
- Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR and Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Archives of General Psychiatry* **62**, 593–602.
- Killgore WDS, Britton JC, Schwab ZJ, Price LM, Weiner MR, Gold AL *et al.* (2014) Cortico-limbic responses to masked affective faces across PTSD, panic disorder, and specific phobia. *Depression and Anxiety* **31**, 150–159.
- Kim MJ, Loucks RA, Neta M, Davis FC, Oler JA, Mazzulla EC *et al.* (2010) Behind the mask: the influence of mask-type on amygdala response to fearful faces. *Social Cognitive and Affective Neuroscience* **5**, 363–368.
- Lancaster JL, Tordesillas-Gutiérrez D, Martínez M, Salinas F, Evans A, Zilles K *et al.* (2007) Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. *Human Brain Mapping* **28**, 1194–1205.
- Langner O, Dotsch R, Bijlstra G, Wigboldus DHJ, Hawk ST and van Knippenberg A (2010) Presentation and validation of the Radboud Faces Database. *Cognition and Emotion* **24**, 1377–1388.
- Lieberman MD and Cunningham WA (2009) Type I and type II error concerns in fMRI research: re-balancing the scale. *Social Cognitive and Affective Neuroscience* **4**, 423–428.
- Lipka J, Miltner WHR and Straube T (2011) Vigilance for threat interacts with amygdala responses to subliminal threat cues in specific phobia. *Biological Psychiatry* **70**, 472–478.
- Maldjian JA, Laurienti PJ, Kraft RA and Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage* **19**, 1233–1239.
- Marsh AA (2016) Understanding amygdala responsiveness to fearful expressions through the lens of psychopathy and altruism. *Journal of Neuroscience Research* **94**, 513–525.
- MATLAB and Statistics Toolbox Release (2012) *The MathWorks, Inc.* USA, Natick, Massachusetts.
- Mendez-Bertolo C, Moratti S, Toledano R, Lopez-Sosa F, Martínez-Alvarez R, Mah YH *et al.* (2016) A fast pathway for fear in human amygdala. *Nature Neuroscience* **19**, 1041–1049.
- Meyer TJ, Miller ML, Metzger RL and Borkovec TD (1990) Development and validation of the Penn State Worry Questionnaire. *Behaviour Research and Therapy* **28**, 487–495.
- Micheyl C, Keaernbach C and Demany L (2008) An evaluation of psychophysical models of auditory change perception. *Psychological Review* **115**, 1069–1083.
- Öhman A (2005) The role of the amygdala in human fear: automatic detection of threat. *Psychoneuroendocrinology* **30**, 953–958.
- Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113.
- Ottaviani C, Cevolani D, Nucifora V, Borlimi R, Agati R, Leonardi M *et al.* (2012) Amygdala responses to masked and low spatial frequency fearful faces: a preliminary fMRI study in panic disorder. *Psychiatry Research: Neuroimaging* **203**, 159–165.
- Patel R, Spreng RN, Shin LM and Girard TA (2012) Neurocircuitry models of posttraumatic stress disorder and beyond: a meta-analysis of functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews* **36**, 2130–2142.
- Perusini JN, Meyer EM, Long VA, Rau V, Nocera N, Avershal J *et al.* (2016) Induction and expression of fear sensitization caused by acute traumatic stress. *Neuropsychopharmacology* **41**, 45–57.
- Pessoa L and Adolphs R (2010) Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. *Nature Reviews Neuroscience* **11**, 773–783.
- Pessoa L, Japee S, Sturman D and Ungerleider LG (2006) Target visibility and visual awareness modulate amygdala responses to fearful faces. *Cerebral Cortex* **16**, 366–375.
- Phelps EA and LeDoux JE (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* **48**, 175–187.
- Presentation® software, Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, [www.neurobs.com](http://www.neurobs.com).
- Rabellino D, Densmore M, Frewen PA, Théberge J and Lanius RA (2016) The innate alarm circuit in post-traumatic stress disorder: conscious and subconscious processing of fear- and trauma-related cues. *Psychiatry Research: Neuroimaging* **248**, 142–150.
- Straube T, Dietrich C, Mothes-Lasch M, Mentzel H-J and Miltner WHR (2010a) The volatility of the amygdala response to masked fearful eyes. *Human Brain Mapping* **31**, 1601–1608.
- Straube T, Langohr B, Schmidt S, Mentzel H-J and Miltner WHR (2010b) Increased amygdala activation to averted versus direct gaze in humans is independent of valence of facial expression. *NeuroImage* **49**, 2680–2686.
- Straube T, Mothes-Lasch M and Miltner WHR (2011) Neural mechanisms of the automatic processing of emotional information from faces and voices. *British Journal of Psychology* **102**, 830–848.
- Talairach J and Tournoux P (1988) *Co-Planar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. New York: Thieme.
- Tottenham N, Borscheid A, Ellertsen K, Marcus DJ and Nelson CA (2002) Categorization of facial expressions in children and adults: establishing a larger stimulus set. *Poster presented at the Cognitive Neuroscience Society Annual Meeting*, San Francisco.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N *et al.* (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* **15**, 273–289.
- van den Bulk BG, Meens PHF, van Lang NDJ, de Voogd EL, van der Wee NJA, Rombouts SARB *et al.* (2014) Amygdala activation during emotional face processing in adolescents with affective disorders: the role of underlying depression and anxiety symptoms. *Frontiers in Human Neuroscience* **8**, 393.
- Wittchen H-U, Zaudig M and Fydrich T (1997) *Strukturiertes Klinisches Interview für DSM-IV: SKID; eine deutschsprachige, erweiterte Bearbeitung der amerikanischen Originalversion des SCID*. Göttingen [u.a.]: Hogrefe.
- Zoellner LA, Bedard-Gilligan MA, Jun JJ, Marks LH and Garcia NM (2013) The evolving construct of posttraumatic stress disorder (PTSD): DSM-5 criteria changes and legal implications. *Psychological Injury and Law* **6**, 277–289.
- Zoellner LA, Rothbaum BO and Feeny NC (2011) PTSD not an anxiety disorder? DSM committee proposal turns back the hands of time. *Depression and Anxiety* **28**, 853–856.