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Individual differences in anxiety and automatic amygdala response to fearful faces: A replication and extension of Etkin et al. (2004)

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ABSTRACT

Trait anxiety refers to the stable tendency to attend to threats and experience fears and worries across many situations. According to the widely noticed, pioneering investigation by Etkin et al. (2004) trait anxiety is strongly associated with reactivity in the right basolateral amygdala to non-conscious threat. Although this observation was based on a sample of only 17 individuals, no replication effort has been reported yet. We reexamined automatic amygdala responsiveness as a function of anxiety in a large sample of 107 participants. Besides self-report instruments, we administered an indirect test to assess implicit anxiety. To assess early, automatic stages of emotion processing, we used a color-decision paradigm presenting brief (33 ms) and backward-masked fearful facial expressions. $N = 56$ participants were unaware of the presence of masked faces. In this subset of unaware participants, the relationship between trait anxiety and basolateral amygdala activation by fearful faces was successfully replicated in region of interest analyses. Additionally, a relation of implicit anxiety with masked fear processing in the amygdala and temporal gyrus was observed. We provide evidence that implicit measures of affect can be valuable predictors of automatic brain responsiveness and may represent useful additions to explicit measures. Our findings support a central role of amygdala reactivity to non-consciously perceived threat in understanding and predicting dispositional anxiety, i.e. the frequency of spontaneously occurring anxiety in everyday life.

1. Introduction

Trait anxiety refers to a general disposition to experience anxiety and respond fearfully to a variety of unspecific threats and novel situations (Spielberger et al., 1983). High trait anxious individuals appear to demonstrate a processing advantage for threatening environmental cues. It has been shown that anxiety is associated with attentional preferences for threat-related stimuli (Bar-Haim et al., 2007) and facilitates threat detection (Doty et al., 2013). It has been assumed that this higher vigilance and sensitivity to threatening information may increase the susceptibility for developing anxiety disorders (Beck and Clark, 1997). The amygdala plays a key role in enhancing vigilance, recruiting attentional resources, and guiding attention toward emotional stimuli (Davis and Whalen, 2001; Phelps and LeDoux, 2005). Thus, the amygdala has been proposed as a neural substrate, which influences processing of threat in anxiety (Cisler and Koster, 2010; Grupe and Nitschke, 2013). The amygdala has demonstrated functional alterations in anxiety disorders (Etkin and Wager, 2007; Fonzo et al., 2015) and is a key component in the anxiety network (Tovote et al., 2015). In particular, the basolateral amygdala has been implicated in the generation of anxiety behaviors (Tovote et al., 2015). As a main input site for sensory information (Duvarci and Pare, 2014), the basolateral amygdala contributes to behavioral outputs of fear and anxiety, via its direct projections to the central nucleus of the

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amygdala (Janak and Tye, 2015). In general, the amygdala is assumed to operate at an early and automatic stage in the visual encoding of emotional stimuli and therefore enables a fast detection of potential threats (Tamietto and de Gelder, 2010). The amygdala receives sensory information by two pathways: one leading directly through the thalamus and the other one originating in sensory cortices (LeDoux, 1996). The subcortical pathway provides a rapid and coarse analysis and evaluation of emotional contents, which need not to be accessible to conscious awareness. The cortical pathway is slower, providing more detailed and elaborated information. This corresponds to the view that emotional responses are modulated by fast bottom-up processes driven by subcortical networks, as well as top-down processes involving cognitive appraisal (e.g., Izard, 1993; LeDoux, 1995; Ochsner et al., 2009). The amygdala is considered a key structure in bottom-up processes for generating emotional responses to stimuli, but was also activated during top-down emotion generation along with the medial prefrontal cortex (Ochsner et al., 2009).

There is robust evidence that fearful faces elicit activation in the amygdala (Fusar-Poli et al., 2009). However, when fearful faces are presented below the threshold of conscious awareness, findings are more controversial. While some studies have reported significant activation in the amygdala (Whalen et al., 1998; Liddell et al., 2005; Williams et al., 2006; Yang et al., 2012; Chen et al., 2017), others have failed to find this effect (Etkin et al., 2004; Phillips et al., 2004; Pessoa et al., 2006; Pichon et al., 2016). Etkin et al. have argued that individuals differ in the extent to which they attend and respond to non-conscious threat signals. The authors provided evidence that trait anxiety is closely linked to the automatic responsivity to threat in the amygdala. By manipulating backward masking and exposure duration to fearful faces in a color identification task, Etkin et al. have demonstrated in a sample of 17 subjects that high trait anxiety was associated with increased activation in the right basolateral amygdala during non-conscious processing, but not during conscious processing. Since its publication in 2004, this influential work on neural threat sensitivity and trait anxiety has been cited 413 times (according to Web of Science Core Collection; 13. January 2020). This study has improved our understanding of the neurobiological correlates of a non-conscious vigilance for threat in anxiety and has inspired later research in many ways. To our knowledge, no effort has been made to replicate Etkin et al.'s pioneering finding in a large sample. There has been growing concern that many published scientific observations are false positives (e.g., Ioannidis, 2005), leading to a biased view of reality in the research literature. The claim for rigorous replication studies has arisen as a hallmark of good scientific practice (Open Science Collaboration, 2015; Munafò et al., 2017). It has even been suggested that empirical findings may be considered as preliminary as long as they lack replication (Forstmeier et al., 2017). Recently, the extensive attempt to replicate genetic association studies of amygdala reactivity to threatening facial expressions resulted in low reproducibility rates (Avinun et al., 2018), leading to the assumption that many results are not reliable. In order to prevent false assumptions in sciences, replication efforts appear all the more reasonable and necessary.

Only few imaging studies investigated automatic (i.e., unconscious, fast, efficient or unintentional, see Moors, 2016) processing of fearful faces in non-clinical trait anxiety. Etkin et al.'s (2004) finding of altered amygdala activation was partly corroborated by these studies. Highly anxious individuals appear to demonstrate hyper-responsiveness of the amygdala to clearly visible, but unattended (Ewbank et al., 2009) and briefly displayed (Ohrmann et al., 2007) fearful faces. In contrast, Bishop et al. (2007) applied a character-identification task, with visible fearful faces being super-imposed by letters, and did not find associations between trait anxiety and automatic amygdala activation. Also Ewbank et al. (2010) failed to reveal an impact of trait anxiety on amygdala activation during the processing of clearly visible fearful faces in a gender-decision task. Thus, evidence for an overall automatic processing advantage for threat-related stimuli in the amygdala is rather inconsistent.

The currently predominant measure of trait anxiety is the State-Trait-Anxiety-Inventory (STAI; Spielberger et al., 1983). As a self-report measure, the STAI assesses conscious cognitive representations of anxious experiences, namely *explicit* anxiety. Explicit measures of anxiety presuppose introspective access to one's own feelings and rely on reflective reasoning processes (Quirin et al., 2009a). However, conscious awareness of one's own mental processes, thoughts, and emotional reactions can be restricted (Wilson and Dunn, 2004). Emotional responses that occur automatically, no matter whether consciously experienced or not, have been termed *implicit* affect (e.g., Quirin et al., 2009a; Quirin and Lane, 2012). Implicit affect can be measured by using indirect assessment methods, which do not explicitly ask individuals about their emotional experiences. The administration of direct and indirect measures of anxiety can be promising for increasing the accuracy of reports of anxious experiences. The Implicit Positive and Negative Affect Test (IPANAT), introduced by Quirin et al. (2009a), is a reliable and well-validated indirect measure of emotions. The IPANAT assesses implicit affect, which has been defined as automatic activation of cognitive representations of affective experiences. It has been argued that indirect measures predict spontaneous behaviors that are driven by automatic and impulsive tendencies (Friese et al., 2008). In line with this assumption, implicit negative affect as measured by the IPANAT predicted stress-induced cortisol release (Quirin et al., 2009b) and cardiovascular responses (van der Ploeg et al., 2016), as well as unintentionally occurring gaze behavior (Bodenschatz et al., 2018). In the mentioned studies, implicit negative affect explained variance in psychophysiological and behavioral reactions above and beyond explicit negative affect.

The amygdala is involved in the induction of sympathetic nervous system responses and secretion of stress hormones (Rodrigues et al., 2009; Orem et al., 2019), as well as in the guidance of attention toward emotionally salient stimuli (Phelps and LeDoux, 2005; Gamer and Büchel, 2009). Therefore, this brain region might be of particular interest when investigating neurobiological underpinnings of implicit anxiety. Moreover, negative implicit affect predicted neural responses to threat-related body postures in brain regions involved in fear and flight behavior (Suslow et al., 2015). These studies highlight the usefulness of the IPANAT in emotion research as a valuable predictor of spontaneous behavioral and psychophysiological reactions to negative emotional stimuli and stress above and beyond explicit measures of affect.

In the present study, functional magnetic resonance imaging scans were obtained from young, healthy adults with varying degrees of explicit and implicit anxiety. Our study was designed to replicate findings from Etkin et al. (2004) of a relationship between explicit trait anxiety (assessed with the STAI) and increased basolateral amygdala responsiveness to masked threat. Therefore, we administered a color-identification task with briefly presented, backward-masked fearful faces. As in the original study, a correlation between explicit anxiety and faster reactions to masked fearful faces was expected. Furthermore, we are the first to administer an additional indirect measure of anxiety, the IPANAT, to investigate associations with brain activation and reaction time to masked fearful faces. It was hypothesized that implicit anxiety could explain an incremental proportion of variance in brain responsiveness, particularly in the basolateral amygdala, relative to explicit anxiety.

2. Methods and materials

2.1. Participants and psychometric measures

One hundred and eighteen healthy volunteers took part in our study. All participants were right-handed, native German speakers and had normal or corrected-to-normal visual acuity. They were recruited via public notices that were posted in canteens, libraries and student

halls of residence. A history of neurological or psychiatric diseases, head trauma involving loss of consciousness, and contraindications for magnetic resonance imaging (MRI) were exclusion criteria for study participation. The Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I; Wittchen et al., 1997) was administered to determine diagnoses of current or past Axis I disorders. Moreover, individuals were required to have a score < 19 in the revised Beck Depression Inventory (BDI-II; Hautzinger et al., 2009) since higher scores were considered as extreme scores (> 3 SDs from mean). In general, our participants were medication free, besides the intake of oral contraceptives (49% of women) and antiallergic agents (5 participants). Three participants had to be excluded due to high BDI scores, four participants did not complete the study and for four participants imaging data were missing due to technical problems during the scanning procedure. Our final sample included 107 participants (59 women).

In accordance with Etkin et al. (2004) levels of general anxiety were assessed with the German trait version of the State-Trait Anxiety Inventory (STAI; Laux et al., 1981). Participants' preference of the right hand was determined by the Handedness Questionnaire (HQ; Raczkowski et al., 1974). The BDI-II (Hautzinger et al., 2009) was administered to assess level of depressive symptoms via self-report.

Implicit anxiety was assessed by a discrete emotion version of the Implicit Positive and Negative Affect Test (IPANAT, see Quirin and Bode, 2014). In contrast to explicit questionnaires, participants are not directly asked to report their anxious experiences. Thus, the IPANAT is an indirect measure of affect, assessing *implicit* affect. Participants have to rate the degree to which artificial words express certain moods. In our study, each of three negatively and three positively charged adjectives (scared, afraid, and frightened for the anxiety subscale; happy, joyful, cheerful for the happiness subscale) are presented along with each of six artificial words from a putative language (e.g., VIKES, BELNI). For example, based on their gut feelings, participants had to rate how well the word "VIKES" could express the word "afraid" in an artificial language. Judgments were provided for the 36 word pairs on a 6-point scale (1 = doesn't fit at all, 6 = fits well). Depending on their chronic accessibility, the emotional adjectives are thought to activate implicit affect, which in turn influences the individuals' judgements of artificial words (Quirin et al. 2009a). Therefore, the IPANAT measures implicit affect via affective priming of judgements for nonsense words.

Using anxiety-related and happiness-related adjectives in the present study, the IPANAT measures implicit anxiety, as well as implicit happiness. However, we were only interested in the anxiety subscale. Thus, individual mean item scores of fittingness ratings were calculated only for anxiety related adjectives, as an indicator for implicit anxiety.

In our sample, the internal consistency (Cronbach's alpha) was $\alpha = 0.76$. In general, the IPANAT is a reliable instrument capturing trait as well as state aspects of implicit affectivity (Quirin and Bode, 2014).

In our sample, explicit (STAI) and implicit anxiety (IPANAT) were not significantly correlated ($r = .06$, $p = .56$).

Following the methodological approach of Etkin et al. (2004), participants were divided into unaware ($N = 56$) and aware ($N = 51$) subjects. Groups were built based on individuals' objective awareness for briefly presented, masked fearful faces (see 2.2. stimuli and procedure). According to Simonsohn (2015), a replication sample should be 2.5-fold the size of the original sample in order to achieve a reasonable statistical power. Thus, in our replication sample, at least 43 participants in the unaware group were required. Demographic and questionnaire characteristics of the final sample, and separated for the aware and unaware group, are presented in Table 1.

The study was approved by the local ethics committee of the University of Leipzig and written informed consent was obtained from all participants. After completion of all tasks, they received financial compensation.

Table 1
Demographic, questionnaire and experimental performance characteristics of the total sample, unaware, and aware individuals (means and SD (in brackets)).

Variable	Total sample ($N = 107$)	Unaware group ($N = 56$)	Aware group ($N = 51$)	p
Age	25.55 (3.34)	26.11 (3.44)	24.94 (3.16)	n.s.
School education (years)	12.38 (0.97)	12.32 (1.22)	12.44 (0.57)	n.s.
STAI	35.29 (7.19)	35.61 (7.48)	34.94 (6.91)	n.s.
IPANAT anxiety	2.53 (0.60)	2.42 (0.58)	2.64 (0.59)	n.s.
BDI-II	4.61 (3.67)	4.09 (3.49)	5.18 (3.82)	n.s.
d' masked	0.42 (0.38)	0.14 (0.13)	0.73 (0.33)	< .001
RT-effect masked (ms)	24.68 (29.07)	24.63 (32.29)	24.74 (25.41)	n.s.

BDI-II = Beck Depression Inventory II; d' = sensitivity index d prime for the masked condition; IPANAT anxiety = mean item score in the anxiety subscale of the Implicit Positive and Negative Affect Test; RT = reaction time, the RT effect (for the masked condition) is calculated by subtracting mean RTs for neutral from fearful trials; STAI = State-Trait Anxiety Inventory, total score.

2.2. Stimuli and procedure

2.2.1. Face processing task

The face processing task, with a masked and non-masked condition, in the MRI scanner was derived from Etkin et al. (2004). Stimuli consisted of photographs of 64 actors (32 women) depicting either fearful or neutral facial expressions, chosen from the FACES database (Ebner et al., 2010). Faces were cropped into an elliptical shape to eliminate background and hair, and were then artificially colorized (red, yellow, green or blue). Subjects were told they would perform a color identification task and were instructed to respond to the color of presented faces as quickly and accurately as possible. Unlike in the study of Etkin et al., we choose four colors (instead of three), so the response rates for the index and middle finger of the left and right hand were balanced.

Each trial had a duration of 2 s and started with a fixation cross shown for 200 ms, followed by a 400 ms blank screen and 200 ms of face presentation. A gray screen depicting the color response scale at the bottom followed for 1.2 s. In this time frame, participants had to respond by a button press. In each hand, participants held a fiber optic response pad with two buttons each.

In the non-masked condition, a neutral or fearful face was presented for 200 ms during the face presentation period. In the masked condition, a fearful or neutral face was briefly presented (33 ms) and immediately masked for 167 ms by a neutral face belonging to a different individual, but of the same color and gender (see Fig. 1). The task comprised 16 blocks (4 per condition) of 8 trials each. Trials within a block were randomized with respect to color and gender. However, presentation frequencies of actors and colors were balanced across the experiment. Trials were presented in two fixed counterbalanced sequences to avoid stimulus order effects. Order of blocks is presented in Fig. S1 of the supplemental material. Each block lasted for 16 s and was followed by a 16 s blank screen.

In order to avoid learning effects during the task, participants were trained prior to the functional run, using unrelated neutral face stimuli. Presentation® software (Version 16.3, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com) was used to control stimulus presentation and to record task performance.

2.2.2. Objective awareness task

To ensure the success of backward masking, participants' objective awareness of masked fearful faces was checked after fMRI scanning. Participants were administered a forced-choice detection task under identical presentation conditions as during scanning. Subjects were informed about the presence of briefly presented fearful faces and were asked to indicate for each trial whether they saw a fearful face or not.

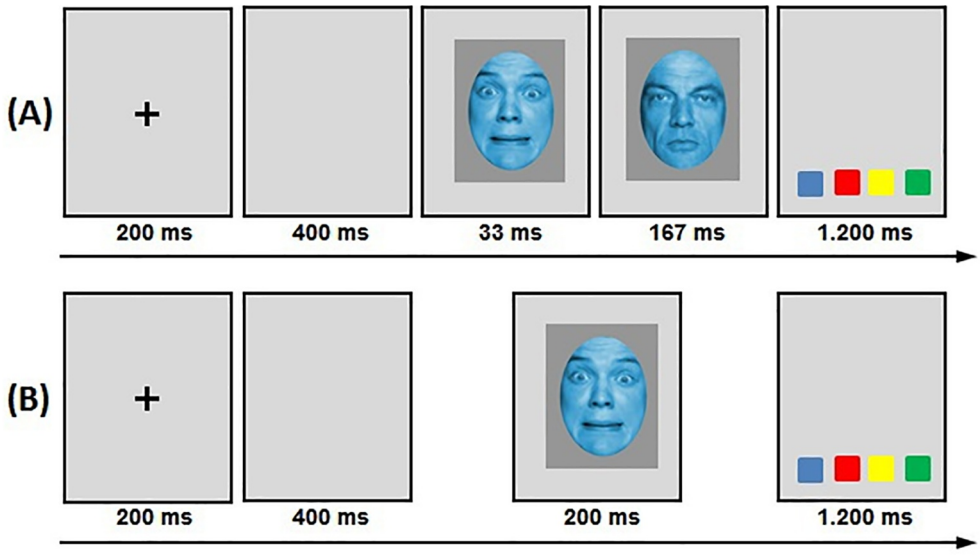

Fig. 1. Experimental paradigm. Depicted is the sequence of events within a trial of the masked (A) and non-masked (B) condition of the fMRI experiment. Stimuli were either fearful or neutral faces artificially colored in blue, red, yellow or green. In the example of a masked trial (A), a fearful face is masked by a neutral face of the same color and gender, but different identity. The neutral face mask could also be preceded by a briefly presented neutral face. In the non-masked condition (B), either fearful or neutral faces were presented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Accuracy and d' values as a sensitivity index were calculated for the masked and non-masked condition by using the formula proposed by Macmillan and Creelman (2008, p. 7 and appendix Table A5.7). Performance above chance in the masked condition was determined according to the one-tailed ($p < .05$) binomial model. Thus, subjects with an accuracy of 61% or higher were considered as objectively aware, since their recognition performance of masked faces was above chance. This corresponds to a d' of 0.40 or higher. Fifty-one participants (48%) exceeded the objective threshold and were assigned to the aware group. Thus, the unaware group consisted of 56 participants. In these subjects, fearful face processing occurred outside of conscious awareness according to an objective threshold.

Aware and unaware subjects significantly differed in their d' values for masked faces, $t(64.20) = -12.05, p < .001$, with the aware group demonstrating a better recognition performance. No group differences occurred in other psychometric and task performance measures (all $ps > .05$), see Table 1. Thus, the grouping did not lead to selective assignment of subjects with skewed anxiety scores. In general, more anxious individuals were not more able to detect masked fearful faces.

2.3. MRI acquisition and preprocessing

Structural and functional MR images were acquired using a 3 T scanner (Magnetom Trio, Siemens, Erlangen, Germany). Structural images were obtained with a T1-weighted 3D MP-RAGE (Mugler and Brookeman, 1990). The imaging parameters were TI 900 ms, TR 1900 ms, TE 2.65 ms, flip angle 9°, spatial resolution of $0.8 \times 0.8 \times 1$ mm^3, two averages. Blood oxygen level dependent (BOLD) contrast sensitive images were collected using T2*-weighted echo-planar imaging (EPI) sequence [matrix 64^2; resolution $3.5 \times 3.5 \times 3.5$ mm^3; TR 2.54 s; TE 30 ms; flip angle 90°; interleaved acquisition of 40 slices along the AC-PC plane; 206 images]. MRI data were preprocessed and analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). The initial four functional volumes were discarded. Further preprocessing included slice time-correction, realignment, motion-correction, and co-registration. Anatomical images were segmented, including normalization to the Montreal Neurological Institute (MNI) template. The normalization parameters were then applied to the functional EPI series (resulting in a re-sampled voxel size of $2 \times 2 \times 2$ mm^3). A temporal high-pass filter (128 s) was applied to remove slow signal drifts. Functional data were smoothed (Gaussian kernel size = 8 mm).

2.4. Data analyses

Reaction time (RT) data of the color identification task were only analyzed for trials with correct responses. Mean accuracy was 98% ($SD = 0.02\%$) and error rates were not correlated with the STAI or IPANAT anxiety ($r = .12, p = .22$ and $r = .01, p = .95$, respectively). In accordance with Etkin et al. (2004) the RT difference score (RT-effect) for the masked condition was calculated by subtracting mean RTs for neutral from fearful trials. The RT-effect can be considered as a measure of attention allocation, and lower scores indicate enhanced performance (faster reactions) for fearful faces as compared to neutral faces. Given our a priori hypotheses, a significance level of $p < .05$ (one-tailed) was used for correlation analyses with masked performance and STAI.

Functional MRI data were analyzed by modeling the onset and duration of 15 s for each block. Regressors were convolved with a hemodynamic response function for the four conditions (masked fear, masked neutral, non-masked fear, non-masked neutral). First level t-contrasts were calculated for masked and non-masked activity by contrasting the fearful condition to the neutral one, respectively. These contrasts were chosen to allow clear conclusions whether activation can be uniquely attributed to the emotional content of a facial expression.

Second level analyses were performed separately for each group. Within each group, one-sample t-tests across all participants were calculated to determine main effects of masked fearful expressions (vs. masked neutral ones). Moreover, contrast images were entered into regression models with the individual STAI and IPANAT anxiety score as regressors of interest. One regression model was calculated per anxiety measure for the masked condition.

Exploratory whole-brain analyses were conducted with a voxel-wise threshold at $p < .001$ (uncorrected) and a cluster-level threshold of $p < .05$, family-wise-error (FWE) corrected. Region of Interest (ROI) analyses were carried out for the right basolateral amygdala. The Anatomy Toolbox (Institute of Neuroscience and Medicine, Jülich, Germany) was used to create the anatomically defined mask. For ROI analyses, the statistical threshold was set to $p = .05$, FWE-corrected. Following the procedure of Etkin et al. (2004) for the non-smoothed functional data in the masked condition, we extracted the averaged signal (contrast estimates) across all voxels in our ROI for each participant (by means of the MarsBaR toolbox, see Brett et al., 2002) and calculated additional correlation analyses in SPSS.

To investigate whether implicit anxiety (IPANAT) shares an incremental proportion of variance with reaction time effects and brain activation, relative to explicit anxiety (STAI), additional two-stage

hierarchical regression analyses were calculated with masked RT-effect and mean brain activation as dependent variable, respectively. Within the aware group implicit anxiety (IPANAT) was significantly correlated with d' sensitivity scores for masked fearful faces ($r = .38$, $p = .01$), indicating better detection in individuals with high implicit anxiety. Given this association in the aware group, d' was also included in the regression analyses. This method was chosen to control for potential modulatory effects of explicit trait anxiety and d' on the relationship between implicit anxiety and RT-effect/brain activation. Therefore, explicit trait anxiety (STAI) and d' scores were entered as predictors in the first step of the regression model to regress out their possible influence. In a second step, implicit anxiety was entered as predictor of interest.

For exploratory purposes, additional hierarchical regression analyses with masked RT-effect and extracted right basolateral amygdala activity as dependent variable, respectively, were carried out separately for implicit and explicit anxiety in the whole sample ($N = 107$). In the first step, awareness and explicit (or implicit) anxiety were entered as predictors. In a second step, the interaction of awareness with explicit (or implicit) anxiety was included to formally test for interaction effects. Findings from these analyses are reported first in the respective results section.

Since our hypotheses were restricted to masked fear processing, we report data only for the masked condition. However, for the purpose of meta-analyses and for interested readers, our results for the non-masked condition are provided in the Supplemental material.

3. Results

3.1. Reaction times

3.1.1. Whole sample

Behavioral performance data in the masked condition for the whole sample are presented in Table 1. Hierarchical regression analyses on RT-effects with anxiety, awareness, and their interaction term as regressors were performed for exploratory purposes. Regression analyses revealed no significant effect of awareness ($p = .96$) or STAI ($p = .11$) in the first step of the model, $R^2 = .02$; $F(2,104) = 1.30$, $p = .28$. The inclusion of their interaction in a second step did not significantly increase the predictive value of the model ($\Delta R^2 = .01$, $p = .24$; $F(3,103) = 1.33$, $p = .27$), indicating that the relation between RT-effect and explicit anxiety was not modulated by awareness.

Across the whole sample, variance in RT-effect was not significantly explained by awareness ($p = .90$) or IPANAT ($p = .58$) in the first step of hierarchical regression analyses ($R^2 = .00$; $F(2,104) = 0.16$, $p = .86$). However, entering the interaction in the second step significantly increased the predictive power of the model ($\Delta R^2 = .04$, $p = .04$; $F(3,103) = 1.53$, $p = .21$). This means that awareness significantly modulated the relationship between IPANAT and masked RT-effect. Thus, unaware and aware groups were analyzed separately.

3.1.2. Unaware group

Behavioral performance data in the masked condition for unaware and aware groups are presented in Table 1. As expected, in the unaware group during masked fear presentation our analyses yielded a significant negative correlation between STAI and the RT-effect ($r = -.24$, $p = .04$, one-tailed). Highly anxious individuals gave faster color identification responses during the presentation of masked fearful faces as compared to masked neutral faces. In the unaware group, implicit anxiety (IPANAT) was not significantly associated with behavioral performance during the masked condition ($r = .13$, $p = .18$).

3.1.3. Aware group

In the aware group, implicit anxiety was significantly negatively correlated with the masked RT-effect ($r = -.30$, $p = .03$). No relationship was revealed between the STAI and masked RT-effects ($r = -.03$, $p = .84$).

In the first step of the hierarchical regression analysis, variance in the RT-effect was not significantly explained by STAI or sensitivity index d' (both $ps > .51$), $R^2 = .01$; $F(2,48) = 0.25$, $p = .78$. Entering implicit anxiety (IPANAT) in the second step did significantly increase the predictive value of the model ($\Delta R^2 = .08$, $p = .04$; $F(3,47) = 1.63$, $p = .20$). According to this result, the prediction of the RT-effect by implicit anxiety was not accounted for by explicit trait anxiety or a higher discriminability of masked faces.

3.2. Neural main effects of masked fearful compared to masked neutral faces

3.2.1. Whole sample

Results from one-sample *t*-tests in the whole sample indicate that presentation of masked fearful faces (vs. masked neutral faces) produced no significant brain activations in ROI and whole-brain analyses.

3.2.2. Unaware group

In the unaware group, presentation of masked fearful faces (vs. masked neutral faces) produced no significant brain activations in ROI and whole-brain analyses.

3.2.3. Aware group

In the aware group, ROI and whole-brain analyses revealed no significant activations in response to masked fearful vs. masked neutral faces.

3.3. Relationships between brain activation to masked fearful faces and anxiety

3.3.1. Whole sample

In order to analyze associations between amygdala activation and anxiety, awareness, and their interaction for the whole sample, explorative hierarchical regression analyses with extracted mean right basolateral amygdala activity were calculated in SPSS. For explicit anxiety, hierarchical regression analyses demonstrated that variance in amygdala activity was significantly explained by STAI ($p = .02$) but not awareness ($p = .77$) in the first step of the model ($R^2 = .06$; $F(2,104) = 3.04$, $p = .05$). Higher scores in the STAI predicted increased amygdala activation to masked fearful faces in the whole sample. However, entering the interaction between awareness and STAI in the second step did significantly increase the predictive value of the model ($\Delta R^2 = .04$, $p = .03$; $F(3,103) = 3.74$, $p = .01$). This finding indicates that the relationship between explicit anxiety and right basolateral amygdala activation differed as a function of awareness. This finding supports Etkin et al.'s (2004) and our methodological approach to analyze unaware and aware participants separately.

For implicit anxiety, hierarchical regression analyses within the whole sample yielded the following results for extracted mean basolateral amygdala activation: In the first step, variance in amygdala responsiveness was significantly explained by the IPANAT ($p = .01$), but not awareness ($p = .35$), ($R^2 = .07$; $F(2,104) = 4.08$, $p = .02$). Thus, higher implicit anxiety predicted higher amygdala responsiveness to masked fearful faces in the whole sample. Entering the interaction between awareness and IPANAT in the second step did only marginally increase the predictive value of the model ($\Delta R^2 = .03$, $p = .07$; $F(3,103) = 3.09$, $p = .01$). The association between implicit anxiety and right basolateral amygdala activity differed only marginally as a function of awareness. Thus, the relationship in aware individuals was not significantly stronger than in unaware individuals.

Next, we investigated whether implicit anxiety (IPANAT) shares an incremental proportion of variance with right basolateral amygdala activation, relative to explicit anxiety (STAI) and awareness in the whole sample. As reported above, STAI ($p = .02$), but not awareness ($p = .77$), was a significant predictor in the first step of the regression

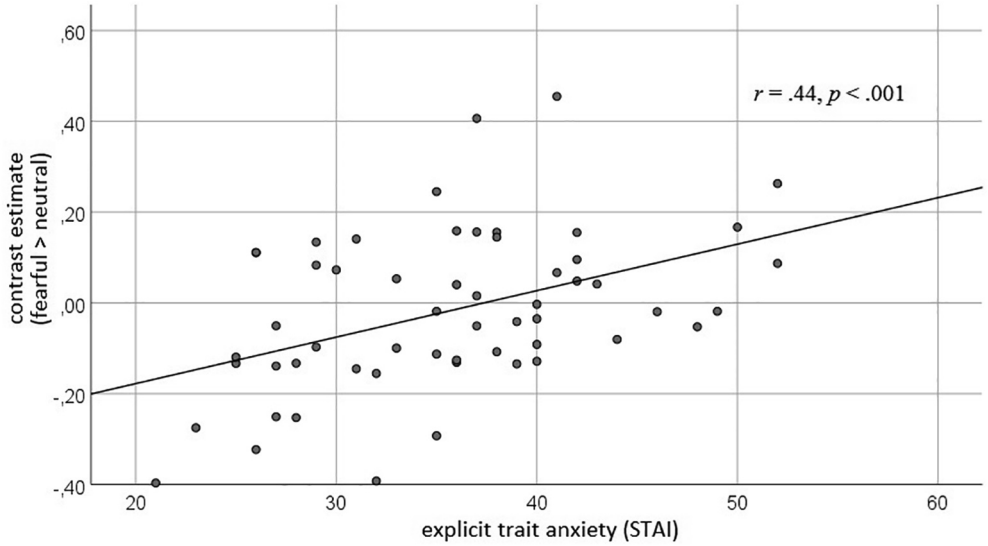

Fig. 2. Relationship between amygdala activation to masked fearful faces and explicit trait anxiety. In the unaware group (N = 56) mean activation of the right basolateral amygdala in response to masked fearful (vs. neutral) faces is significantly correlated with explicit trait anxiety, as measured with the STAI (mean total score).

model. Entering IPANAT scores in the second step did significantly improve the predictive power (ΔR^2 = .06, p = .01; F(3,103) = 4.63, p = .001). Thus, implicit anxiety remained a significant predictor of amygdala responsiveness to masked fearful faces, even after accounting for the effect of explicit anxiety and awareness.

3.3.2. Unaware group

In the unaware group, ROI-based regression analyses with the STAI yielded a significant cluster in the right basolateral amygdala (peak voxel *xyz*: 24–10 −12, cluster size: 7, T-score = 3.78, p_{FWE} = .01), indicating a positive correlation between brain activation and explicit anxiety. No significant correlation was revealed between implicit anxiety (IPANAT) and basolateral amygdala activation.

Furthermore, correlation analysis between STAI and the extracted mean activation (fearful vs. neutral contrast) for the overall right basolateral amygdala was calculated in SPSS. The STAI was significantly associated with activity in the basolateral amygdala (r = .44, p < .001, 95% CI [.20, .63]), see Fig. 2, but not with the IPANAT (r = .10, p = .47). Etkin et al. (2004, p. 1046) have reported a large effect size (r = .74, p < .001, 95% CI [.41, .90]). We calculated the 95% confidence interval of the original correlation in order to compare both coefficients. The overlap between the confidence intervals suggests that our correlation does not significantly differ from the original one.

For the unaware group, exploratory whole-brain regression analyses with explicit and implicit anxiety did not reveal any positive correlations with brain activity to masked fearful faces (vs. neutral faces). At a more lenient threshold at p < .001 (uncorrected) and a cluster of k > 10 contiguous voxels (corresponding to Etkin et al., 2004), the STAI was correlated with brain activity in the right fusiform gyrus (peak voxel *xyz*: 40–46 −12, cluster size: 19, T-score = 3.61, p < .001), left middle temporal gyrus (peak voxel *xyz*: −48–50 2, cluster size: 60, T-score = 4.19, p < .001) and right amygdala (peak voxel *xyz*: 24–10 −12, cluster size: 52, T-score = 3.78, p < .001).

3.3.3. Aware group

In the aware group, the STAI was not associated with right basolateral amygdala activity to masked fearful (vs. neutral) faces in ROI-based regression analyses. However, implicit anxiety (IPANAT) was found to be positively related to right basolateral amygdala activity (peak voxel *xyz*: 32–6 −14, cluster size: 45, T-score = 4.07, p_{FWE} = .01). Extracted mean activation of the overall right basolateral amygdala demonstrated a medium-size correlation with the IPANAT (r = .44, p = .001), see Fig. 3, but not with the STAI (r = .00, p = .98). In the first step of hierarchical regression analyses, variance in the basolateral amygdala activity was not significantly explained by the STAI or sensitivity index d' (both ps > .19), R^2 = .04; F(2,48) = 0.87, p = .43. Entering implicit anxiety (IPANAT) in the second step did significantly increase the predictive value of the model (ΔR^2 = .16, p = .003; F(3,47) = 3.84, p = .02).

In exploratory whole-brain analyses at a conservative cluster level corrected threshold, only implicit anxiety (IPANAT) was significantly and positively associated with brain activity to masked fear in the right and left middle, extending to superior temporal gyrus (BA21 and BA22) (peak voxel *xyz*: −64–50 4, cluster size: 424, T-score = 4.91, p < .001, and peak voxel *xyz*: 66–46 2, cluster size: 240, T-score = 4.91, p < .001).

Mean activations in the clusters located in the left and right temporal gyrus were extracted for additional hierarchical regression analyses. In the first step, variance in the right temporal gyrus was significantly explained by d' (p = .03) but not STAI (p = .96), (R^2 = .09; F(2,48) = 2.49, p = .09). Thus, a higher sensitivity for masked fearful faces predicted brain activation in the right temporal gyrus. For the left temporal gyrus, STAI and d' did not significantly contribute to the regression model in the first step (both ps > .15), (R^2 = 0.04; F(2,48) = 1.10, p = .34). Entering implicit anxiety (IPANAT) in the second step for right and left temporal gyrus did significantly increase the predictive values of both models (ΔR^2 = .21, p < .001; F(3,47) = 6.76, p < .001 and ΔR^2 = .24, p < .001; F(3,47) = 6.29, p = .001, respectively). Hence, implicit anxiety remained a significant predictor of brain activation even after accounting for the effect of explicit anxiety and higher sensitivity for masked fearful faces.

4. Discussion

We investigated automatic amygdala responsiveness as a function of anxiety in a large sample of 107 healthy participants. The present study primarily aimed to replicate findings from Etkin et al. (2004), who demonstrated in a sample of 17 subjects a relationship between explicit trait anxiety and basolateral amygdala activity in response to non-consciously processed fearful faces. Following the methodological approach of Etkin et al., participants were selected based on their awareness about the presence of briefly presented fearful faces. Consistent with the results by Etkin et al., greater responsiveness to masked fearful faces in the right basolateral amygdala was modulated by explicit trait anxiety in 56 unaware individuals. The use of a German sample stemming from a different cultural and linguistic background compared to the original North American sample also substantially

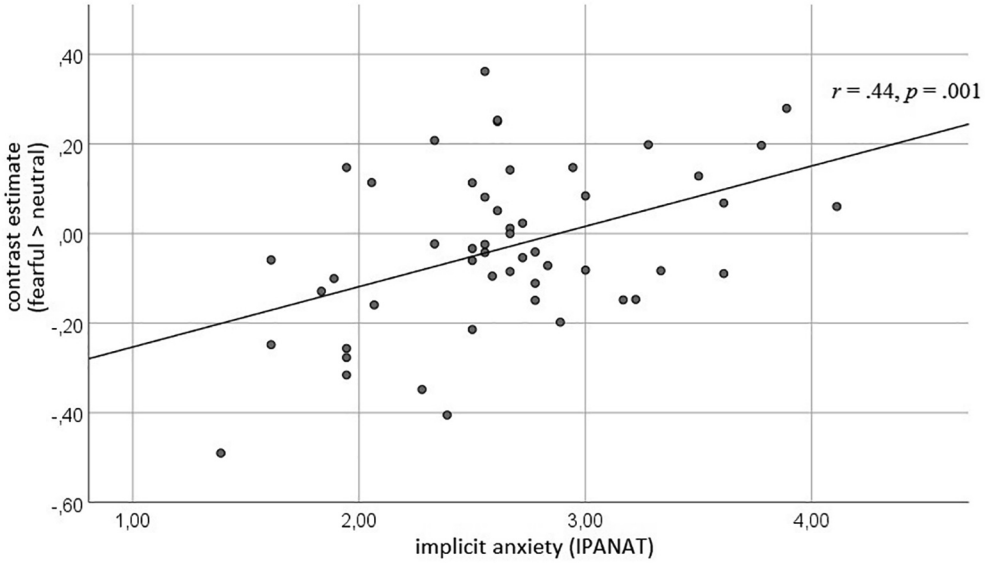

Fig. 3. Relationship between amygdala activation to masked fearful faces and implicit anxiety. In the aware group ($N = 51$) mean activation of the right basolateral amygdala in response to masked fearful (vs. neutral) faces is significantly correlated with implicit anxiety, as measured with the IPANAT (mean item score).

strengthens the generalizability of the observations. While Etkin et al. have reported a large effect size, our data suggest a more moderate relationship between trait anxiety and amygdala activity. It is not unusual that large effect sizes of original studies appear to be only half the magnitude in replication samples (Open Science Collaboration, 2015). Nevertheless, our correlation coefficient did not significantly differ from the original one. Our data indicate that subjects respond in the amygdala to a non-conscious danger signal with the magnitude determined by their individual level of trait anxiety. They give further support for the idea formulated by Etkin et al. that unconscious neural processes may underlie information processing biases in trait-anxious individuals. In our study, explicit trait anxiety only significantly moderated amygdala activity when subjects were entirely unaware of the presence of fearful faces, i.e., during non-conscious threat processing. Hierarchical regression analyses confirmed that awareness modulated the relationship between trait anxiety and amygdala responsiveness significantly, as they are more strongly related in unaware subjects. We assume that automatic or implicit processing of masked fearful faces occurred in both, the unaware and aware group. In our color decision task threat-related information was presented very briefly and was goal irrelevant and unattended. Therefore, processing was fast, efficient, and uncontrolled, and met several criteria of automaticity according to Moors (2016). Groups only differed with respect to the feature of non-consciousness in automaticity. Etkin et al. have proposed that in anxious individuals the basolateral amygdala may be initially recruited by non-conscious threat signals, and due to the uncertain nature of the masked stimulus the activation could be sustained. However, during the conscious perception of threat, where the emotional content of the stimuli becomes clear, active top down inhibition of the amygdala might occur. Thus, bottom-up activations in the amygdala might have triggered additional top-down attentional or regulatory processes in individuals with conscious perceptual awareness of fearful faces. Hence, one could speculate that non-conscious processing of threatening faces in unaware individuals is qualitatively different from automatic, but potentially conscious processing in aware individuals. For explicit anxiety, there was no significant relationship with brain response in the aware group.

Etkin et al. (2004) have suggested that non-conscious processing reflects trait anxiety, as measured with the STAI, more strongly than conscious processing. Possibly, the link between trait anxiety and automatic amygdala response to fear signals can be detected most reliably under strict control of subjects' unawareness of threat signals. This is in line with the cognitive model of Beck and Clark (1997), who proposed that the initial stage of biased information processing in anxiety operates at an automatic level, which involves the rapid, involuntary, and non-conscious recognition of stimuli. This mode of information processing serves as an early detection system for potential danger, is perceptually driven by the stimulus, and is assumed to be over-reactive in anxiety. This is consistent with the notion that the evaluation of stimulus valence and generation of emotional responses can take place at early and non-conscious processing stages, particularly in the amygdala (LeDoux, 1995; 1996;; Janak and Tye, 2015).

High trait anxiety is characterized by a disposition to experience anxiety in the face of uncertain situations, to overestimate potential dangers, as well as being hypervigilant and apprehensive (Sylvers et al., 2011). Our results suggest that these individuals exhibit exaggerated automatic reactivity in the amygdala during non-conscious processing of fearful faces. Our finding is in line with the assumption of a dysregulated responsivity of the amygdala to threatening cues as a neurocognitive function associated with trait vulnerability to anxiety (Indovina et al., 2011). The involvement of the amygdala in threat detection and evaluation has been intensively studied in previous research on anxiety (Adhikari, 2014; Calhoon and Tye, 2015). The amygdala has been discussed as part of a hypersensitive appraisal circuit in high trait anxiety (Sylvers et al., 2011). Moreover, prior research has demonstrated that patients with posttraumatic stress disorder (PTSD), a severe anxiety disorder, also exhibited a hyper-responsiveness in the right basolateral amygdala to non-conscious threat (Neumeister et al., 2018). This finding has corroborated earlier reports of heightened reactivity in the amygdala to masked fearful faces in PTSD (Rauch et al., 2000; Killgore et al., 2014). In youth with generalized anxiety disorder, there is additional evidence for greater amygdala responsiveness to non-conscious threat faces, which also correlated with anxiety symptoms (Monk et al., 2008). In contrast, when threatening faces were processed rather consciously, patients did not differ from healthy controls in amygdala reactivity (Monk et al., 2006).

Interestingly, increased activity in the amygdala, along with impaired downregulation by prefrontal areas, has also been linked to rumination (Ray et al., 2005; Disner et al., 2011; Mandell et al., 2014). According to several items of the STAI, anxious individuals report pronounced worrying, brooding, and reverberant thinking. These ruminative thoughts may be considered as cognitive aspects of anxiety. Hence, our findings might provide further evidence that the amygdala

is an important component of the neural network involved in habitual brooding, which is accompanied by feelings of nervousness and arousal.

It has been proposed that high trait anxiety is a vulnerability factor for the development of depression, anxiety disorders, and stress-induced psychopathologies (e.g., Calvo and Cano-Vindel, 1997; Chambers et al., 2004; Sandi and Richter-Levin, 2009; Weger and Sandi, 2018). It might be concluded that trait anxious individuals have an enhanced automatic threat detection system in the basolateral amygdala, which may underlie individual differences in vulnerability to mood and anxiety disorders. Such automatic processing biases might contribute to the involuntary and uncontrollable nature of anxiety-related thoughts and symptoms, which has been often described by anxious individuals (Capitão et al., 2014). Interestingly, in a longitudinal study, evidence emerged that amygdala responsiveness to clearly visible threatening faces serves as a biomarker of future risk for stress-related psychopathology (Swartz et al., 2015). Whether heightened reactivity in the amygdala to non-consciously processed fearful faces, as an objective indicator of threat sensitivity, can predict future anxiety disorders and can be modified by psychotherapeutic interventions remains to be investigated. In PTSD, no treatment-related changes have been revealed in automatic amygdala responsiveness to non-conscious threat after psychotherapy, but symptom improvement was accompanied by increased frontopolar activation during deliberate emotion regulation (Fonzo et al., 2017).

A second objective of our study was to shed light on the association between automatic brain responsiveness and implicit anxiety, as identified by an indirect measure. Negative implicit affect, as measured by the IPANAT, has been previously proven as a valuable predictor of spontaneous psychophysiological responses, above and beyond the effect of explicit measures of affect (e.g., Quirin et al., 2009b). Since brain responsiveness to non-consciously processed fearful faces can be assumed as highly automatic, we expected that implicit anxiety can explain an incremental proportion of variance in basolateral amygdala reactivity in unaware subjects, but this hypothesis was not confirmed. Thus, indirectly assessed anxiety does not enhance our understanding of inter-individual differences in brain activity during non-conscious threat processing.

However, our analyses revealed a significant association between hyper-responsiveness to masked fearful faces in the right basolateral amygdala and implicit anxiety in aware subjects. Hierarchical regression analyses indicated that this effect was not driven by explicit anxiety or enhanced sensitivity in masked fear detection. This means that relative to self-reported anxiety and the better ability to consciously perceive masked threat-related information, implicit anxiety shared an incremental proportion of variance with automatic right basolateral amygdala reactivity.

Moreover, implicit anxiety was associated with heightened activity in the bilateral middle, extending to superior temporal gyrus. This temporal activity appears to be a function of implicit anxiety, rather than only a correlate of the level of conscious perceptual awareness or explicit anxiety. Interestingly, there is evidence from diffusion-weighted imaging studies in humans, that the basolateral amygdala and superior temporal gyrus are substantially and directly interconnected (Abivardi and Bach, 2017). The temporal gyrus is supposed to be involved in the processing of emotions and faces in general (Fusar-Poli et al., 2009; Sabatinelli et al., 2011; Kirby and Robinson, 2017). Automatic activation in this brain area has previously been observed in response to briefly presented, masked faces with ambiguous or fearful expressions (e.g., Phillips et al., 2004; Liddell et al., 2005; Günther et al., 2017). Our data suggest that individuals with high implicit anxiety, as well as pronounced perceptual awareness, exhibit increased responsiveness in the temporal gyrus to threat-related information. This could reflect an upregulation of the face processing system with a more detailed representation and processing of facial threat and danger signals in high implicit anxiety.

Our findings are in line with previous studies which have demonstrated that implicit affect, as measured by the IPANAT, can predict spontaneous psychophysiological and brain responses to emotional stimuli and stress (Quirin et al., 2009b; Suslow et al., 2015; van der Ploeg et al., 2016). In contrast, a previous study on emotional face recognition suggests that implicit anxiety, as determined by a performance based Implicit Association Test (IAT), is not associated with brain activity during controlled processing of briefly presented, masked fearful expressions (Suslow et al., 2019). With regard to these null results and based on our findings, it can be argued that implicit anxiety rather facilitates automatic processing of fearful faces in the amygdala and temporal gyrus. When analyzing aware and unaware groups separately, our observed association between implicit anxiety and amygdala responsiveness was restricted to aware individuals, thus, subjects with a pronounced ability to detect subliminal threat. By analyzing unaware subjects separately, we followed the procedure of Etkin et al. (2004). Notably, explorative hierarchical regression analyses for the whole sample indicated that implicit anxiety can significantly predict automatic amygdala responsiveness to masked fearful faces, above and beyond the influence of explicit anxiety, and irrespective of subjects' awareness. Awareness modulated only marginally the strength of the association between implicit anxiety and amygdala activation. This means that the relationship in aware individuals was higher, but not significantly different from the relationship in unaware individuals. Overall, implicit anxiety appears to be associated with automatic amygdala responsiveness to masked fearful faces in individuals with higher and lower detection sensitivity for threat.

Subjects' awareness in our study was determined in a forced-choice task after the scanning procedure. One cannot clearly conclude whether all subjects in the aware group were conscious of the presence of masked fearful faces during the scanning. Some individuals might have been preoccupied with the instruction to identify the color of faces and awareness was triggered afterwards by questioning. However, their detection performance was above chance when attention to masked fearful faces was explicitly raised. Thus, individuals in the aware group can be characterized by decreased thresholds of consciousness for low intensity signals of threat.

As an indicator of implicit affect, the IPANAT measures automatic components of emotional responses that occur spontaneously and are accompanied by physiological and behavioral changes (Quirin and Lane, 2012). These emotional responses do not necessarily involve reflective cognitive processes. The principle of the test is based on the assumption that anxious affect automatically exerts an influence on the evaluation of artificial word stimuli. Its goal is to capture automatic affective processes expressed in the participants' biased judgments (Weil et al., 2019). It is speculative, but one might argue that this measure is a better indicator of implicit anxiety in individuals with a high sensitivity for subtle external and internal stimuli, such as brief threat signals or bodily processes accompanying anxious responses. This might explain the marginally higher predictive value of the IPANAT for brain responsivity in aware subjects, than in unaware participants with a lower sensitivity.

In line with our prediction, higher explicit and implicit anxiety was also associated with enhanced behavioral performance. In unaware subjects, increased explicit anxiety levels were related to faster color identification when the neutral target face was preceded by a masked fearful face, as compared to a masked neutral face. Similar observations have been reported by Etkin et al. (2004). However, our results were only significant for one-tailed testing and should be interpreted with caution. In aware subjects, implicit anxiety significantly predicted faster reactions during the presentation of masked fearful faces, as compared to masked neutral faces. This relationship remained significant when accounting for a potential influence of explicit trait anxiety and detection of masked fear. Our results correspond to findings from Dodd et al. (2017) for a visual search task with threatening faces. Interestingly, the authors did not find an influence of high explicit trait anxiety on reaction time when the emotional content of the face was

task relevant. Here, anxious and non-anxious individuals demonstrated similar response patterns. Only during automatic processing of facial expression, when the emotional content was irrelevant for the search goal, trait anxiety exhibited an influence on performance. This finding was interpreted as an indicator that particularly anxious individuals automatically attend to threat, possibly because the goal to search for threatening information is habitually activated. Overall, our behavioral data provide further evidence that anxiety appears to be linked to an attentional bias and increased vigilance for threatening stimuli (Bar-Haim et al., 2007; Armstrong and Olatunji, 2012). This is in line with cognitive models of anxiety, where the presence of automatic biases at a preattentive level of information processing has been highlighted (Beck and Clark, 1997).

In line with previous research (Phillips et al., 2004; Pessoa et al., 2006) results from our main effect analyses failed to show brain activations in response to briefly presented and backward-masked fearful faces in unaware subjects. This supports the view that objectively successful masking of briefly presented threatening faces eliminates activation in the amygdala (e.g., Etkin et al., 2004; Pessoa, 2005). Even when masking has failed in the aware group, we did not find significant activations by fearful faces in the amygdala or other brain areas. This contradicts earlier reports (e.g., Whalen et al., 1998; Liddell et al., 2005), where non-consciously processed fearful faces elicited significant amygdala activation.

This study has few limitations. In the original work, Etkin et al. (2004) isolated functional data from the amygdala separately based on each individual's anatomical image. Here, we followed a more standardized procedure and extracted contrast estimates of the basolateral amygdala based on normalized anatomical data. Our sample differed from the original one with respect to the proportion of aware subjects (48% vs. 35%, respectively). Moreover, mean explicit trait anxiety scores were somewhat higher in our sample than in the original one ($M = 35.61 \pm 7.48$ vs. $M = 32.7 \pm 1.5$ in the unaware group, and $M = 34.92 \pm 6.91$ vs. $M = 32.0 \pm 1.2$ in the aware group). But in correspondence with the original study, our mean values lie somewhat below the normative mean of the national (German) student population (see Laux et al., 1981).

5. Conclusion

Our close replication effort demonstrated that the original finding of a relationship between right basolateral amygdala responsiveness to non-consciously processed fearful faces and explicit trait anxiety (Etkin et al., 2004) is reproducible in a different cultural and linguistic context. By utilizing direct and indirect measures of anxiety, the present study extended previous research that illuminated associations between anxious tendencies and an automatic processing advantage for threat-related information in the amygdala. In our view, a central challenge of future research in the field is the prediction of actual anxiety reactions and experiences in experimental situations and everyday life based on amygdalar response characteristics during non-conscious threat processing.

CRediT authorship contribution statement

Vivien Günther: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing - original draft, Writing - review & editing. **Anja Hußlack:** Investigation, Data curation, Writing - review & editing. **Anna-Sophie Weil:** Investigation, Data curation, Writing - review & editing. **Anna Bujanow:** Investigation, Writing - review & editing. **Jeanette Henkelmann:** Investigation, Methodology, Writing - review & editing. **Anette Kersting:** Resources, Supervision, Writing - review & editing. **Markus Quirin:** Conceptualization, Methodology, Writing - review & editing. **Karl-Titus Hoffmann:** Resources, Supervision, Writing - review & editing. **Boris Egloff:** Conceptualization, Methodology, Writing - review & editing. **Donald Lobsien:** Conceptualization, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Thomas Suslow:** Conceptualization, Methodology, Project administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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