BEHAVIORAL AND PHYSIOLOGICAL BASES OF ATTENTIONAL BIASES: PARADIGMS, PARTICIPANTS, AND STIMULI

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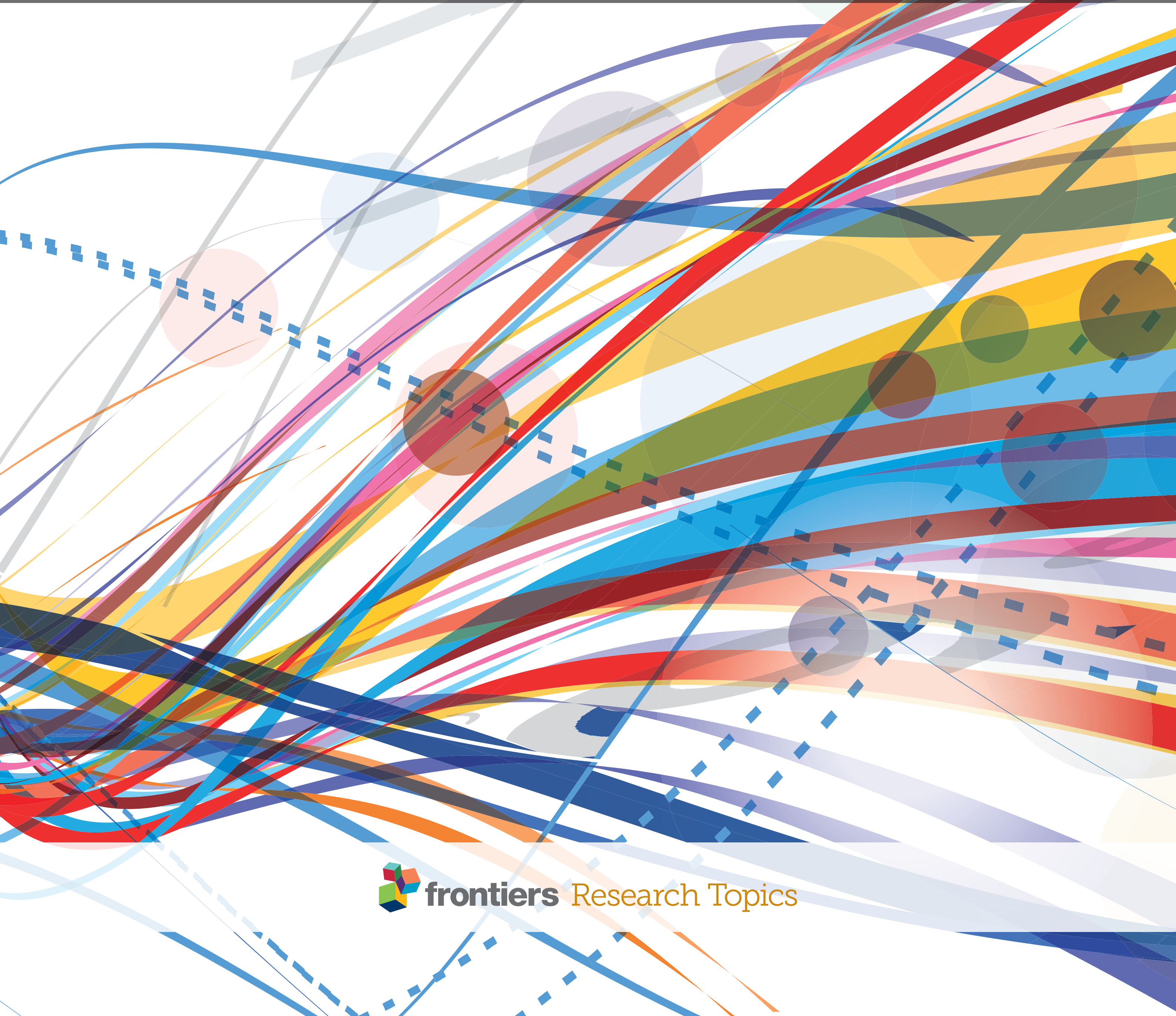

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BEHAVIORAL AND PHYSIOLOGICAL BASES OF ATTENTIONAL BIASES: PARADIGMS, PARTICIPANTS, AND STIMULI

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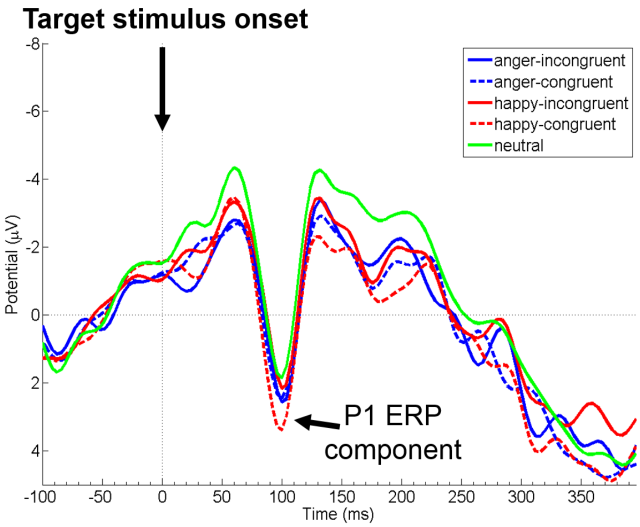

Event-related EEG amplitude courses in response to congruently and incongruently presented target stimuli to investigate attentional biases.
Image taken from: Pfabigan DM, Lamplmayr-Kragl E, Pintzinger NM, Sailer U and Tran US (2014) Sex differences in event-related potentials and attentional biases to emotional facial stimuli. Front. Psychol. 5:1477. doi: 10.3389/fpsyg.2014.01477

Attentional biases (ABs) play a prominent role in the development and maintenance of clinically relevant symptoms of, for example, anxiety and depression. In particular, increased attentional orienting and preoccupation with biologically relevant and mood-congruent stimuli has been observed, suggesting that the visual-attentional system is overly sensitive towards threat cues and avoidant of cues of reward in these disorders.

First, several experimental paradigms have been used to assess ABs, e.g., the dot-probe task, the emotional stroop task, and the spatial cueing task amongst others. Yet, these paradigms are based on different theoretical backgrounds and target different stages of the attentional process. Thus, different paradigms provided converging as well as diverging evidence with regard to ABs. However, it is often not entirely clear to what extent this reflects real differences and commonalities, or is caused by differences in methodology. For example, behavioral reaction time data can only provide a snapshot of selective attention. Measuring event-related potentials, eye movements, or functional brain imaging data enables exploring the exact temporal and spatial dynamics of attentional processes. Moreover, neuroimaging data reveal specific cortical networks involved in directing attention toward a stimulus or disengaging from it.

Second, ABs have been mainly discussed as symptoms of psychopathology, while results in healthy participants are still scarce; previous studies mostly compared extreme groups. However, a comprehensive theoretical and empirical account of ABs in psychopathology also requires a thorough account of ABs in the general healthy population. Moreover, the effect of gender, as an important contributing factor in processing of emotional stimuli, has also not been considered systematically in previous research.

Third, a variety of stimuli has been used in the assessment of ABs. So far, mostly facial or word stimuli have been applied. However, in everyday life not only facial emotion recognition but also a fast evaluation of complex social situations is important to be effective in social interactions. Recent research started using more complex stimuli to raise ecological validity. However, the use of ecologically valid stimuli poses some methodological challenges and needs to be applied more systematically.

The aim of this research topic is to integrate different paradigms and stimuli, addressing individuals from the whole range of the population continuum, and to apply different methodological approaches. It is intended to bring together expertise in stimulus selection, timing and implementing issues, advancing and broadening the overall understanding of ABs.

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EDITORIAL
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Editorial: Behavioral and physiological bases of attentional biases: paradigms, participants, and stimuli

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Keywords: attentional bias, bias indices, attentional ERPs, anxiety, depression, dot-probe task

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Processes of selective allocation of visual attention play a prominent role for survival, but also for development and maintenance of clinically relevant symptoms such as in anxiety or depression. Previous research provided evidence for increased attentional orienting and preoccupation with biologically relevant and mood-congruent stimuli, indicating tendencies of attentional biases. For instance, in anxiety, the visual-attentional system may be overly sensitive toward threat- and avoidant of reward cues.

The research articles appearing in the E-book *Behavioral and physiological bases of attentional biases: paradigms, participants, and stimuli* cover these topics and give a comprehensive overview on current directions and challenges in attentional bias research. Our driving motivation was to critically evaluate parameters that may directly or indirectly influence attentional biases and may thus be important for our understanding of attentional biases.

Our first aim was to demonstrate the variety of experimental paradigms and outcome measures used. So far, the *dot-probe task* (MacLeod et al., 1986) was the gold standard in attentional bias research. This was also reflected in the contributions to this research topic. Relying solely on behavioral measures such as response accuracy, response times, and bias indices, Hakamata et al. (2014) applied a dot-probe task whereas Sagliano et al. (2014) and Wittekind et al. (2015) applied (modified) versions of the Posner task (Posner, 1980), a cueing paradigm related to the dot-probe task. In contrast, Isomura et al. (2014) applied an innovative combination of two tasks—a search-recognition and a face-in-the-crowd task. Relying on physiological measures, Valuch et al. (2015) applied a gap-saccade and a dot-probe task while measuring saccadic reaction times. Focusing on the exact time course of neuronal activation, four studies investigated attentional biases using electroencephalography and measured attention-related event-related potentials (ERPs) in response to emotional and neutral stimuli. Sass et al. (2014) and Fisher et al. (2014) applied an emotion word Stroop task while Pfabigan et al. (2014) and Kappenman et al. (2014) administered versions of the dot-probe task. These studies investigated several attention-related ERPs time-locked to crucial events during the paradigms—pointing toward the huge diversity in measures used to assess attentional biases.

Our second aim was to demonstrate the variety of populations and stimuli, which may show, or elicit, differing attentional biases. Wittekind et al. (2015) examined individuals who had experienced displacement during World War II and their adult offspring. There was no evidence of attentional biases among formerly displaced individuals suffering from post-traumatic stress disorder (PTSD), and no evidence of transgenerational transmission of attentional biases in PTSD, utilizing pictorial stimuli. Only when utilizing word stimuli, evidence of trauma-related attentional biases among participants with PTSD could be replicated, highlighting that attentional biases may sometimes depend on the type of stimuli used. Fisher et al. (2014) examined subclinical samples, investigating the moderating effect of suspiciousness on attentional biases in anxiety and depression. Utilizing word stimuli, there was evidence of overlapping processes for suspiciousness and anxious apprehension, but not for suspiciousness and depression. Isomura et al. (2014) examined children with autism spectrum disorders (ASD), utilizing facial stimuli. Children with ASD showed quicker detection of angry faces than typically developed children, relying more on the extraction of local than configural features during face processing. Valuch et al. (2015) investigated the effects of perceived attractiveness in facial stimuli among healthy participants. Attractive faces captured attention more effectively than less attractive faces, and men showed a stronger bias toward attractive opposite-sex faces than women. Utilizing pictorial stimuli, Kappenman et al. (2014) examined healthy participants, whereas Sagliano et al. (2014) contrasted high and low anxious subclinical participants. Sass et al. (2014) examined subclinical samples, utilizing word stimuli, and found evidence of differential attentional biases among men and women and effects of co-occurring anxiety in attentional bias in depression. Sex differences were also evident examining a subclinical sample utilizing facial stimuli (Pfabigan et al., 2014).

Investigating the impact of participant sex on attentional biases, Pfabigan et al. (2014) applied a modified version of the dot-probe task which included also neutral-neutral stimulus pairs (Koster et al., 2004). These trials allowed a distinction of attentional allocation and disengagement processes. Importantly, this approach can also be applied to physiological data by calculating so-called ERP difference waves (i.e., subtracting neuronal activity evoked by neutral stimuli from the neuronal activity evoked by emotional ones), which allows the disentanglement of attentional processes also on the neuronal level. Despite limitations of the dot-probe task, this approach might be considerably useful in future attentional bias research.

Kappenman et al. (2014) investigated the reliability of reaction time measures, bias indices, and the N2pc ERP component in a standard picture-based dot-probe task. The authors reported poor reliability of behavioral measures, but moderate reliability of the N2pc component. They emphasize the need for experimental paradigms that are better suited for the assessment of attentional biases and they advocate including physiological measures to gain more reliable insight into the underlying processes.

Challenges in attentional bias research become quite clear in the course of the articles in this E-book. There is no common agreement as to whether particular stimuli or experimental set-ups are more reliable than others. For example, only a few studies addressed the topic of different presentation durations and their impact on attentional bias measures so far (e.g., Koster et al., 2007; Mingtian et al., 2011). Moreover, the dependent variables used to assess attentional biases vary considerably, in particular in physiological studies in which ERPs in varying time windows and electrode locations are reported. This limits the comparability of studies and does not allow generalizable conclusions.

Nevertheless, the current research topic also points toward future directions of attentional bias research. In particular, Kappenman et al. (2014) emphasize the need for task development to assess attentional biases in a more reliable way. The study by Isomura et al. (2014) should be considered pioneering in this regard. Moreover, using statistical methods that account for random variance due to stimulus variation (Judd et al., 2012) or applying difference measures also in physiological attentional bias research (Pfabigan et al., 2014) might be promising for research in this field.

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Does neurocognitive function affect cognitive bias toward an emotional stimulus? Association between general attentional ability and attentional bias toward threat

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Background: Although poorer cognitive performance has been found to be associated with anxiety, it remains unclear whether neurocognitive function affects biased cognitive processing toward emotional information. We investigated whether general cognitive function evaluated with a standard neuropsychological test predicts biased cognition, focusing on attentional bias toward threat.

Methods: One hundred and five healthy young adults completed a dot-probe task measuring attentional bias and the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) measuring general cognitive function, which consists of five domains: immediate memory, visuospatial/constructional, language, attention, and delayed memory. Stepwise multiple regression analysis was performed to examine the relationship between attentional bias and cognitive function.

Results: The attentional domain was the best predictor of attentional bias toward threat ($\beta = -0.26$, $p = 0.006$). Within the attentional domain, digit symbol coding was negatively correlated with attentional bias ($r = -0.28$, $p = 0.005$).

Conclusions: The present study provides the first evidence that general attentional ability, which was assessed with a standard neuropsychological test, affects attentional bias toward threatening information. Individual cognitive profiles might be important for the measurement and modification of cognitive biases.

Keywords: attentional bias, neurocognitive function, emotion, attention, cognitive bias

INTRODUCTION

Cognitive theories suggest that cognitive processing biased toward affective significance confers an increased risk for the development and exacerbation of emotional disorders (Beck, 1976; Mathews and MacLeod, 2005). Such bias is observed in multiple domains of cognition, including greater selective attention toward threats (MacLeod et al., 1986; Fox, 1996), enhanced memory recall of negative stimuli (Gilboa-Schechtman et al., 2002; Ridout et al., 2003), and distorted interpretations of ambiguous information (Lawson et al., 2002; Woud et al., 2014). Indeed, several meta-analytic studies have confirmed the presence of cognitive bias in anxious and depressive individuals (Bar-Haim et al., 2007; Mitte, 2008; Peckham et al., 2010; Phillips et al., 2010), although the exact mechanism for this has yet to be elucidated.

Biased attention toward threatening information is one of the most widely studied cognitive biases. Attentional bias refers to a tendency to quickly identify and easily dwell on emotional stimuli (MacLeod et al., 2002). This process includes visual perception of a stimulus and orientation to it (i.e., selective attention), the sensitivity of which varies among individuals (Posner, 1980; MacLeod et al., 1986). Our previous study found that years of education—a rough estimate of general cognitive function—had an inhibitory effect on attentional bias toward threat, suggesting that one's cognitive ability could affect attentional bias toward threatening stimuli (Hakamata et al., 2013). Despite the dearth of evidence that directly connects general cognitive ability and cognitive biases, several studies have suggested that these two variables might be associated, as they found that some cognitive abilities are generally different in high-anxiety individuals. For example, these individuals had their attention easily diverted by different distractors (Eysenck and Graydon, 1989; Mathews et al., 1990; Eysenck and Byrne, 1992) and showed reduced working memory capacity, even for non-emotional stimuli (Firetto and Davey, 1971; Eysenck, 1979; Darke, 1988; Eysenck et al., 2005; Hayes et al., 2008). Given that cognitive bias is known to be associated with emotional disturbances, such as anxiety (Beck, 1976; Mathews and MacLeod, 2005), these findings raise the possibility that compromised cognitive function facilitates biased cognitive processing toward emotional information. However, no study, to our knowledge, has directly examined the relationship between cognitive bias and cognitive function as assessed with a standard neuropsychological test.

Thus, in the present study, we investigated the relationship between neurocognitive function and cognitive bias, focusing on attentional bias toward threat. We hypothesized that individual attentional function would specifically predict attentional bias toward threat.

METHODS

ETHICAL CONSIDERATIONS

The Kitasato University Hospital Institutional Review Board approved the study, and all participants provided written informed consent. All the research procedures were conducted in accordance with the Declaration of Helsinki.

PARTICIPANTS

Participants were 113 individuals recruited via advertisements in a local magazine and billboards at Kitasato University. The eligibility criteria were as follows: no Axis-I psychiatric disorders or substance abuse history, which were determined using the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998), and no major medical/neurological illnesses. Eight subjects were excluded because they had epilepsy ($n = 2$), chronic subdural hematoma ($n = 1$), cerebral palsy ($n = 1$), Wilson disease ($n = 1$), histories of subarachnoid hemorrhage ($n = 1$) and hydrocephalus ($n = 1$), and strabismus ($n = 1$). Thus, data from 105 participants were included in the analyses (63 women, mean age: 22.3 years; range: 20–35, $SD = 3.2$).

PSYCHOLOGICAL ASSESSMENT

Anxiety

Anxiety levels were evaluated with the 20 items for trait anxiety from the Spielberger's State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970), a well-established self-report questionnaire measuring anxiety. STAI has been used in previous studies on attentional bias (see Bar-Haim et al., 2007). Each item is rated on a four-point scale (i.e., from 1: "Almost Never" to 4: "Almost Always"), with higher scores indicating greater anxiety. Internal consistency was Cronbach's alpha = 0.86 in the present sample.

Depression

Depressive symptoms were evaluated with the Beck Depression Inventory-II (BDI-II). BDI-II is a 21-item, self-report questionnaire to assess depressive symptoms experienced during the past 2 weeks (Beck et al., 1996). Each item is rated on a four-point scale (i.e., from 0 to 3, with higher scores indicating greater severity). Scoring ≤ 17 points on this scale is considered to indicate clinical depression. Internal consistency was Cronbach's alpha = 0.88 in the present sample.

Neurocognitive function

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was employed to assess multiple domains of cognitive function. The RBANS is a representative, clinician-administered neuropsychological test for adults aged between 20 and 89 years (Randolph, 1998). It includes 12 standard cognitive subtests, which are grouped into five domains as follows: immediate memory (list learning and story memory), visuospatial/constructional (figure copy and line orientation), language (picture naming and semantic fluency), attention (digit span and digit symbol coding), and delayed memory (list recall, list recognition, story recall, and figure recall). The Japanese version of the RBANS has well-established reliability and validity (Matsui et al., 2010).

To investigate attentional function, the Trail Making Test (TMT) Parts A and B (Reitan, 1992, 1955) were used. Part A requires participants to connect randomly distributed numbers consecutively with a line on paper, and Part B requires participants to connect numbers and letters in an alternating fashion. Response time (RT) indicates visuoperceptual speed and set-shifting ability (i.e., an ability to smoothly switch between different cognitive categories) in Parts A and B, respectively (Strauss et al., 2006).

Attentional bias

To measure attentional bias, we used the dot-probe task (DPT), the most commonly used and innovative program for attentional bias modification (MacLeod et al., 1986; MacLeod, 1995). The DPT was constructed on E-prime version 2.0 (Psychology Software Tools, Inc., Pittsburgh, PA). The DPT requires participants to identify a non-emotional probe, such as a letter or symbol (e.g., an asterisk), which can appear in one of two spatial locations. Immediately before probe presentation, threatening and nonthreatening stimuli appear simultaneously in two separate locations. Neutral and negative words were presented as stimuli. We used the word list from the original study by MacLeod et al. (1986). Each trial began with a centrally located fixation cross displayed for 500 ms, followed by a pair of words that appeared vertically on the screen for 500 ms. The words were replaced by an asterisk probe at either the top or bottom location that was just vacated by one of the words. Participants were instructed to press one of two buttons as quickly and accurately as possible to indicate the location of the probe. In total, 196 trials were presented to each participant. The probe replaced the neutral word in half of the trials, appearing on the top and bottom locations of the display with equal probability. The location of the probe was counterbalanced across the experiment. Trial-presentation order was randomized for each participant. Before performing the task, all participants received 32 practice trials on the DPT, using a different set of neutral words. The difference between RT toward neutral stimuli and RT toward negative stimuli serves as an index of attentional bias. Positive values indicate bias toward threat.

DATA ANALYSIS

To explore whether a specific cognitive domain affects attentional bias toward threat, we performed a stepwise multiple regression analysis predicting attentional bias, with the five cognitive domains of the RBANS and TMT indices as predictor variables. Effects of age, sex, and years of education were controlled for in the analysis. Inter-correlations were calculated between attentional bias, trait anxiety, depressive symptoms, TMT measures, and the five cognitive domain scores of the RBANS, controlling for age, sex, and years of education. Statistical analyses were performed with SPSS version 22.0J (IBM, Inc., Tokyo, Japan). The significance threshold was set at 0.05 (two-tailed).

RESULTS

Mean scores and standard deviations (SDs) of the RBANS, TMT, depressive symptoms, trait anxiety, and attentional bias are presented in **Table 1**. The RBANS and TMT scores in the present study were similar to those of the original studies, except for semantic fluency in the RBANS, the scores of which were relatively lower than those of the original study (Randolph, 1998; Strauss et al., 2006). Inter-correlations between the five cognitive domains of the RBANS, TMT indices, depressive symptoms, trait anxiety, and attentional bias are presented in **Table 2**. Attentional bias showed significant correlations with the attentional domain of the RBANS and the TMT Part A [$r_{(99)} = -0.27$, $p = 0.006$; $r_{(99)} = 0.20$, $p = 0.049$, respectively], indicating that lower performance in attention-related functions was associated with biased attention toward threatening information. Although attentional bias was not significantly correlated with trait anxiety in the current linear model, its relationship with depressive symptoms bordered on statistical significance ($p = 0.050$).

Next, stepwise multiple regression analysis revealed that the attentional domain of the RBANS was the best predictor of attentional bias toward threat [$F_{(4,\ 99)} = 6.06$, $R^2 = 0.20$, adjusted $R^2 = 0.16$, $p < 0.001$; **Table 3**]. The model explained 16% of the variance observed. Attentional function negatively affected attentional bias ($\beta = -0.26$, $p = 0.006$).

Table 1 | Mean scores and *SD*s of the RBANS subtests, TMT indices, attentional bias, trait anxiety, and depressive symptoms (*N* = 105).

	Mean	*SD*
Attentional bias	−0.06	13.16
Trait Anxiety (STAI)	43.83	9.10
Depressive symptoms (BDI-II)	7.79	6.72
RBANS		
Immediate memory		
List learning	32.10	3.79
Story memory	19.84	3.17
Visuospatial/constructional		
Figure copy	19.45	0.88
Line orientation	18.39	1.72
Language		
Picture naming	9.88	0.36
Semantic fluency	16.89	3.89
Attention		
Digit span	11.90	2.18
Digit symbol coding	63.84	9.01
Delayed memory		
List recall	8.17	1.73
List recognition	19.74	0.59
Story recall	10.98	1.56
Figure recall	17.50	2.58
Total index score	100.52	13.64
TMT		
Part A	25.18	7.43
Part B	51.02	12.21

RBANS, repeatable battery for the assessment of neuropsychological status; STAI, state-trait anxiety inventory; BDI-II, beck depression inventory II; TMT, trail making test.

As a reference analysis to specify which component of the attentional domain in the RBANS was more relevant to attentional bias, partial correlation analysis showed that the digit symbol coding of the attentional domain, not digit span [$r_{(99)} = -0.12$, $p = 0.236$], was negatively correlated with attentional bias toward threat [$r_{(99)} = -0.28$, $p = 0.005$].

Additionally, we performed a confirmatory analysis to examine whether there are gender differences in the RBANS. The ANCOVA, in which age and years of education were controlled for, showed that women had higher immediate memory scores (estimated marginal means: 104.3 ± 1.7 vs. 98.5 ± 2.1, $p = 0.041$) and delayed memory scores (105.7 ± 2.0 vs. 99.2 ± 2.5, $p = 0.053$), compared to men. This is partly consistent with a previous study that found a similar gender difference in the RBANS (Beatty et al., 2003). For the magnitude of the association between the attentional bias and the attentional domain, no significant difference was found between genders ($r = -0.24$ vs. -0.28, $p = 0.824$).

DISCUSSION

The present study was the first to examine the relationship between cognitive bias and neurocognitive function. The results showed that individuals with lower performance in the attentional domain, particularly in digit symbol coding, exhibited greater attentional bias toward threat, supporting our hypothesis. The significant correlation observed between attentional bias and the TMT Part A further supported the link between attentional function and attentional bias. These findings suggest that general attentional ability could affect biased attention toward threatening information.

It is important to note that the digit symbol coding test, which has been widely used to assess attentional resources as a part of working memory (Goldman-Rakic, 1994; Stratta et al., 1997; Pukrop et al., 2003), was significantly associated with attentional bias. Digit symbol performance is considered to be influenced by different cognitive components (Lezak et al., 2004), although recent evidence suggests that its primary component is visuoperceptual speed (Joy et al., 2004). Digit symbol coding—but not digit span, which specifically requires auditory inputs—was correlated with attentional bias. This suggests that the visuoperceptual facet of attention, some variance of which also overlaps with visuospatial working memory, might be more relevant to biased attention. This is in line with the significant correlation found between attentional bias and visuoperceptual speed as measured by the TMT Part A.

Recent neuroimaging research indicates that attentional bias toward threat is associated with activity in the dorsolateral prefrontal cortex (DLPFC) (Fani et al., 2012; Peers et al., 2013; Brunoni and Vanderhasselt, 2014). In accordance with this evidence, an fMRI study examining neutral activity during the digit symbol test observed DLPFC activation (Usui et al., 2009). The DLPFC has been implicated in the allocation of visuospatial attention (Makino et al., 2004; Anderson et al., 2007) as well as in working memory function (Wager and Smith, 2003; Brunoni and Vanderhasselt, 2014). These findings support the idea that

Table 2 | Correlations between attention bias score, trait anxiety, depressive symptoms, TMT and the RBANS ($N = 105$).

				RBANS					TMT	
	Attentional bias	**STAI-T**	**BDI-II**	**Immediate memory**	**Visuospatial/ constructional**	**Language**	**Attention**	**Delayed memory**	**Part A**	**Part B**
Attentional bias	–									
Trait anxiety (STAI)	0.09	–								
Depressive symptoms (BDI-II)	$0.20^{\P}$	0.78	–							
RBANS										
Immediate memory	−0.01	0.03	0.00	–						
Visuospatial/constructional	0.08	0.02	−0.03	0.09	–					
Language	−0.12	0.00	0.01	0.10	0.10	–				
Attention	-0.27^{**}	0.00	−0.05	0.28^{**}	0.18	0.10	–			
Delayed memory	0.07	0.01	−0.06	0.64^{***}	0.31^{***}	0.03	$0.19^{\P}$	–		
TMT										
Part A	0.20^{*}	0.10	0.11	−0.18	−0.01	−0.12	−0.18	0.03	–	
Part B	0.15	−0.08	−0.06	-0.31^{**}	0.04	-0.22^{*}	-0.35^{***}	−0.16	0.44^{***}	–

STAI, state-trait anxiety inventory; BDI-II, beck depression inventory-II;RBANS, repeatable battery for the assessment of neuropsychological status; TMT, trail making test.$^{\P}p = 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 3 | Stepwise regression analysis predicting attentional bias toward threat ($N = 105$).

Independent variables	Standardized β	t	p
CONTROLLED VARIABLES			
Age	−0.29	−2.34	0.021
Sex	−0.22	−2.30	0.024
Years of education	−0.08	−0.71	0.481
SELECTED VARIABLE			
Attentional domain (RBANS)	−0.26	−2.80	0.006

Five cognitive domains measured with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (i.e., immediate memory, visuospatial/constructional, language, attention, and delayed memory) and TMT (Trail Making Test) indices (i.e., Parts A and B) were incorporated into the model as predictor variables, controlling for age, sex, and years of education. $R^2 = 0.20$, *adjusted* $R^2 = 0.16$, $p < 0.001$. R^2 *change* $= 0.06$, $p = 0.006$ *after the attentional domain score of the RBANS was incorporated into the model.*

attentional bias might share neural underpinnings with visuoperceptual facets of attention, and that the two systems functionally interact with one another. Compromised attentional ability, particularly of visuoperceptual facets, might affect biased selective visual attention toward negative information.

Some limitations should be noted when interpreting the results. First, we used the RBANS to measure neurocognitive function. More detailed assessment tools for assessing neurocognitive function are necessary. Second, the semantic fluency scores in the present study were lower than the scores in the original study (Randolph, 1998; Strauss et al., 2006). Although this difference did not affect the main results, this point should be considered as a limitation. Third, a significant association between anxiety and attentional bias was not observed, whereas the association between depressive symptoms and attentional bias was partially demonstrated, as it was in previous meta-analytic studies (Peckham et al., 2010; Phillips et al., 2010). This might have been caused by insufficient statistical power to detect this association, given that the reported effect sizes for the relation between these two variables are not large and that they have been calculated from two-group comparisons between high-anxiety and low-anxiety participants, usually defined as scoring 1 *SD* above and below the mean on anxiety-related measures, respectively. In future studies, a prospective design using a larger sample is needed to determine whether poorer attentional function precedes greater attentional bias, while considering a variety of other potential intervening variables (e.g., gender).

In summary, we revealed that general attentional ability, assessed with a standard neuropsychological test, affects attentional bias toward threat. Compromised neurocognitive function in a specific domain might affect biased cognition toward an emotional stimulus therein. Consideration of individual differences in neurocognitive function might be important for the measurement and modification of cognitive bias.

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Attentional biases toward threat: the concomitant presence of difficulty of disengagement and attentional avoidance in low trait anxious individuals

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Attentional biases toward threats (ABTs) have been described in high anxious individuals and in clinical samples whereas they have been rarely reported in non-clinical samples (Bar-Haim et al., 2007; Cisler and Koster, 2010). Three kinds of ABTs have been identified (facilitation, difficulty of disengagement, and avoidance) but their mechanisms and time courses are still unclear. This study aimed to understand ABTs mechanisms and timing in low trait anxiety (LTA) and high trait anxiety (HTA) anxious individuals. In particular, in an exogenous cueing task we used threatening or neutral stimuli as peripheral cues with three presentation times (100, 200, or 500 ms). The main results showed that HTA individuals have an attentional facilitation bias at 100 ms (likely automatic in nature) whereas LTA individuals show attentional avoidance and difficulty to disengage from threatening stimuli at 200 ms (likely related to a strategic processing). Such findings demonstrate that threat biases attention with specific mechanisms and time courses, and that anxiety levels modulate attention allocation.

Keywords: attentional bias, threat, anxiety, spatial attention, avoidance, disengagement

INTRODUCTION

Different kinds of attentional biases toward threats (ABTs) have been described in high anxious individuals and in clinical samples, whereas they have been rarely reported in low anxiety individuals (Bar-Haim et al., 2007; Cisler and Koster, 2010). Indeed, when faced with fearful stimuli, high anxious individuals tend to detect them quickly (facilitation bias), at 100 and 200 ms of stimulus presentation times (PTs; Koster et al., 2006, 2007; Massar et al., 2011), and to remain anchored upon them (difficulty of disengagement), between 100 and 500 ms (Fox et al., 2001; Koster et al., 2004b, 2006; Massar et al., 2011), whereas non-anxious individuals seem to avoid the same stimuli (avoidance bias), after ~200 ms (Koster et al., 2006, 2007). The disengagement bias toward threat has been observed in both high state anxiety individuals at rapid PTs (Fox et al., 2001) and high trait anxiety individuals at a slower PT (Fox, 2002). Moreover, Koster et al. (2006) showed that, after an early facilitation bias, high anxious individuals show a subsequent tendency to shift their attention away from threatening stimuli (avoidance bias). Therefore, the facilitation and the disengagement biases have been found in high trait anxiety individuals only, whereas the avoidance bias has been reported in both high and low trait anxious individuals.

Mathews (1990) proposed that the ABTs play an important role in maintaining high anxiety levels, as anxious individuals would be more likely to detect potential threats in the environment, which would increase their anxiety levels. However, different, and often contrasting, hypotheses have been subsequently put forward to explain ABTs (for a review, see Cisler and Koster, 2010). For instance, Williams et al. (1997) suggested that high and low anxious individuals differ in their attention allocation mechanisms in presence of threatening stimuli: high anxiety individuals direct attention toward threat while low anxiety individuals direct attention away from threat. Similarly, Eysenck et al. (2007) proposed that high anxious individuals show an impairment in attentional control, enhancing vigilance for threatening stimuli and inducing difficulties in disengaging attention from threat. According to a different point of view, the so-called vigilance–avoidance hypothesis of ABTs (Mogg et al., 2004), high anxious individuals tend to overestimate the stimuli's threat value, and show an enhancement of automatic mechanisms detecting potential threats, but also tend to avoid further processing of stimuli closely matching their own phobic concerns.

More recently, Cisler and Koster (2010) proposed that, in high anxious individuals and in clinical samples, the three biases (facilitation, difficulty in disengagement, and avoidance) differ as regards: (i) type of processing (automatic or strategic), (ii) cognitive mechanisms (attentional control and emotion regulation goals), and (iii) neural bases (amygdala and prefrontal circuits). In detail, Cisler and Koster (2010) suggested that the automatic processing of attention, mediated by the amygdala, is responsible for detecting threatening stimuli and rapidly orienting attention toward them (facilitation bias). Strategic or conscious elaboration (mediated by the frontal cortex network) would be instead responsible for biased attention distribution (favoring allocation of attention on neutral stimuli: avoidance), and strategic attentional control (determining difficulties in disengagement). The model nicely explains findings (Koster et al., 2006) showing that high anxious (but not low anxious) individuals show different ABTs in response to aversive stimuli as a function of

PTs: in particular, facilitation bias and difficulty in disengagement with short PTs, and an avoidance bias with longer PTs. The time course of ABTs has been confirmed by Massar et al. (2011), who found an early attentional engagement for threatening stimuli in high anxious individuals; however, the authors also found a slower disengagement from threat cues in all participants, irrespective of their trait anxiety levels, in contrast with Koster et al.'s (2006) observations about the lack of ABTs in low anxious individuals. Therefore, Massar et al.'s (2011) findings would be compatible with the idea that the Cisler and Koster's (2010) model would also apply to non-anxious people, at least for threatening stimuli.

The idea that the same model can be applied to people with low and high anxiety is not consistent with recent data showing a differential modulation of hypervigilance (facilitation bias) and avoidance in high and low trait anxious individuals. Actually, using a conditioning procedure, Onnis et al. (2011) reported that high anxious participants showed an attentional facilitation when stimuli were presented for 200 ms and an attentional avoidance when stimuli were presented for 500 ms, whereas low anxious individuals showed an opposite attentional pattern, with an early tendency to divert attention from aversive stimuli (200 ms presentation) and a later orientation toward them (500 ms presentation). These data would confirm that facilitation and avoidance are characterized by distinct attentional mechanisms operating at different stages of information processing, but also would suggest that activation of such mechanisms is dependent on anxiety levels.

On the basis of the studies reviewed above, two questions are still open. First, Cisler and Koster's (2010) hypothesis, according to which the facilitation bias is related to an early automatic processing whereas avoidance and disengagement biases are driven by later strategic elaboration, has not been directly tested in a study tapping all the three ABTs in a comprehensive within-subject paradigm. Second, it is not clear whether the same cognitive mechanisms can account for ABTs in low and high trait anxious individuals.

The present study aimed to tackle these issues by an experimental paradigm combining within- and between-subjects observation, in which threatening or neutral stimuli modulated explicit allocation of spatial attention. By using three PTs we could systematically explore: (i) whether facilitation, difficulty of disengagement and avoidance are specifically related to early or late time windows, as foreseen by Cisler and Koster's (2010) model, within the same subjects, and (ii) whether the same pattern of ABTs can be observed in two groups of individuals with low or high trait anxiety, consistent with possible generalization of the model, independently from anxiety levels.

According to the original formulation of Cisler and Koster's (2010) model we could expect to find a facilitation bias at the shortest PTs, and difficulty of disengagement and avoidance bias at the longest PT in high anxious individuals, and no bias in low anxious participants. However, the present study would also make possible to find the same ABTs, with the same time course, in low anxious individuals too, thus suggesting that Cisler and Koster's (2010) model can apply irrespective of anxiety level, and can be considered as a general model of emotion-related modulation of attentional resources, reflecting adaptive (or maladaptive) response mechanisms. It is also possible to find partial discrepancies between high and low anxious individuals, compatible with the idea that high anxiety levels can affect deployment of attentional resources over environmental features, whereas the low anxious pattern of ABTs might reflect the most advantageous response modality to possible threats.

MATERIALS AND METHODS

PARTICIPANTS

Participants were 95 non-clinical female undergraduate students from the Second University of Naples, dwelling in South Italy (age range = 20–33 years, mean age = 23.85, SE = 3.2). As in previous studies on ABTs (e.g., Leyman et al., 2009; De Raedt et al., 2010), only female participants were included in the study to ensure maximum homogeneity of the sample, and because women are considered to show greater facility in decoding non-verbal messages and to rate their emotions more intensely than males (Killgore and Yurgelun-Todd, 2001).

The participants were assigned to one of two groups according to their anxiety scores on the Trait subscale of State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983): following previous studies (Onnis et al., 2011), participants with STAI-Trait score < 35 were included in the LTA group, and participants with a STAI-T score > 49 were included in the HTA group; individuals with intermediate scores (35–49) were excluded from the study. All subjects were right-handed, had normal or corrected-to-normal vision, and were naive to purposes and predictions of the experiment. Participants gave their written informed consent to take part in the experiment on a voluntary basis, without receiving any reward.

PROCEDURE

Participants completed state and trait versions of the STAI-Y and, then, were asked to perform a modified version of the Posner Task.

MATERIALS

State-Trait Anxiety Scale

The STAI-Y (Spielberger et al., 1983) consists of two 20-item scales aiming at measuring state and trait anxiety. The STAI-State subscale requires respondents to rate how they feel "right now... at this moment" using a 4-point scale (1 = not at all, 4 = very much so) in response to a series of self-descriptive statements. The STAI-Trait subscale, used here to allocate subjects to LTA or HTA groups, asks respondents to rate how they "generally" feel using a 4-point scale (1 = almost never, 4 = almost always) in response to a series of self-descriptive statements. These subscales have been demonstrated to be valid and to have solid psychometric properties (Spielberger et al., 1983).

Exogenous cueing task

Participants were presented with a dot detection task driven by an exogenous (threatening or neutral) spatial cue; this paradigm is a modified version of the Posner (1980). Each trial began with a fixation cross (+) flanked by two blank squares (340 × 340 pixel) on its right and left side. After 750 ms, a cue (a threatening or non-threatening image; 300 × 300 pixel) appeared in one of the two square for 100, 200, or 500 ms in randomized order, followed by a dot (1 cm) presented in one of the two squares, in the same (valid trial) or in the opposite (invalid trial) position as the cue.

Images used as cues were selected from a larger sample 150 images consisting of familiar scenes of animals, people or natural events in order to maximize ecological validity. In a preliminary phase, images were shown, one at a time, on a pc monitor to 30 undergraduate students (age range: 20–30), who were asked to judge threat degree of each stimulus on a scale from 0 (not threatening) to 4 (very threatening) by pressing a corresponding key on the pc keyboard. For the present experiment we used the 20 images judged as most threatening (mean score of threat degree = 2.9; range = 2.5–4), and the 20 images judged as least threatening (mean score of threat degree = 0.7; range = 0–1). Each stimulus appeared at least once in right and left squares.

Valid ($n = 192$, 80%; 96 threatening and 96 non-threatening) and invalid ($n = 48$, 20%; 24 threatening and 24 non-threatening) trials were presented in a randomized order for a total of 240 trials (**Figure 1**).

Participants were required to respond, as fast and accurately as possible, pressing a right key (m) on the keyboard when the target (dot) appeared on the right and a left key (z) when the target appears on the left. Both accuracy and response times (RTs) were recorded.

DATA ANALYSIS

A preliminary multivariate analysis of variance (MANOVA) with anxiety group (LTA, HTA) as the independent factor was conducted on age and anxiety levels, to characterize the two samples.

After removing outliers ($RT<150$ and >1000; Koster et al., 2004a), raw RTs for correct trials were analyzed by a mixed analysis of variance (ANOVA) 2X2X2X3 with one between-subject factor (anxiety group: LTA, HTA) and three within-subject factors (valence: threatening, non-threatening; validity: valid, invalid; PTs: 100, 200, 500 ms).

Planned contrasts with Bonferroni correction were used to compare RTs for threatening vs. non-threatening stimuli.

As suggested by Koster et al. (2006), for the analysis of single ABTs we calculated the facilitation score (RTvalid/non-threatening cue – RTvalid/threatening cue) and the disengagement score (RTinvalid/threatening cue – RTinvalid/non-threatening cue). A positive facilitation score indicates an early enhanced attentional capture by threatening cues compared with non-threatening cues (facilitation bias). A positive value on disengagement score indicates stronger attentional holding by threatening cues compared with non-threatening ones (disengagement bias). Negative values of both scores indicate a tendency to avoid threatening stimuli (avoidance bias). A value not different from zero at either score means lack of ABTs (i.e., no difference in processing of threatening vs. non-threatening cues).

A MANOVA with anxiety group (LTA, HTA) as independent factor was conducted on bias scores. Univariate analyses and planned comparisons with Bonferroni correction were then executed.

Single-sample t-test comparisons were used to evidence whether bias scores were significantly different from zero.

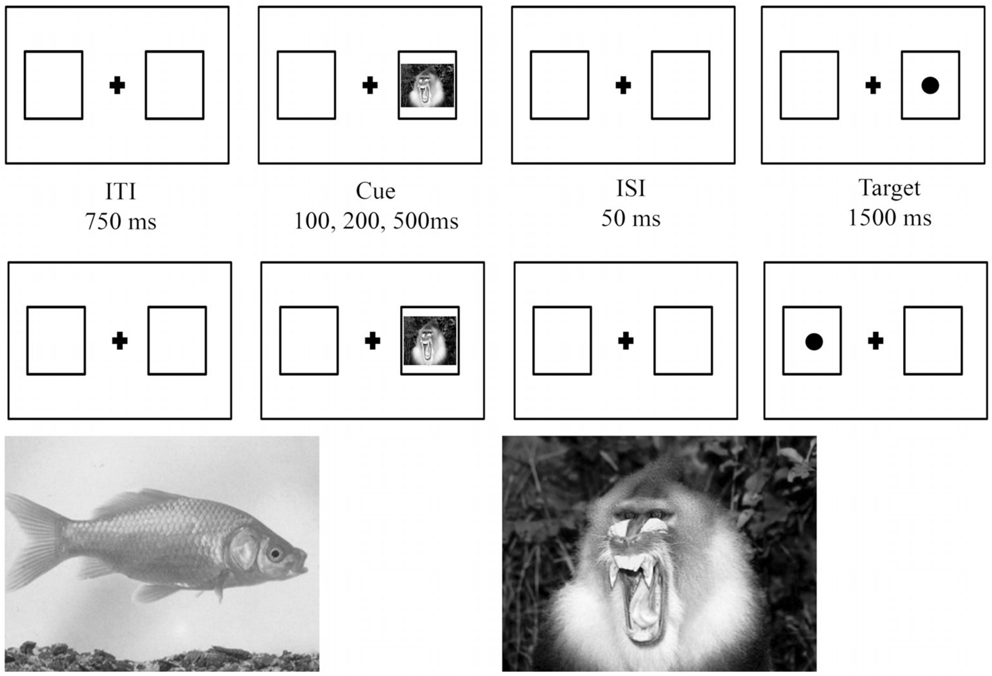

FIGURE 1 | Schematic overview of valid (first row) and invalid trials (middle row). Examples of non-treatening (left) or threatening (right) stimuli are depicted in the bottom row.

RESULTS

GROUP CHARACTERISTICS

On the basis of the results of the Trait subscale of the STAI, 27 participants were included in the LTA group and 28 in the HTA group, whereas 40 subjects were excluded from the study.

The MANOVA with trait anxiety group as the independent factor, and age, state and trait anxiety scores as outcome variables confirmed that the HTA group had significantly higher scores compared to the LTA group both in trait anxiety [HTA = 59.18; LTA = 31.30; $F(1,53) = 325.92$, $p < 0.001$, $\eta_p^2 = 0.86$] and in state anxiety [HTA = 46.68; LTA = 31.11; $F(1,53) = 40.06$, $p < 0.001$, $\eta_p^2 = 0.43$], whereas the two groups did not differ in age [HTA = 22.64; LTA = 23.26; $F(1,53) = 0.69$, $p = 0.40$, $\eta_p^2 = 0.01$].

DOT DETECTION TASK

Means and standard deviations for correct RTs are reported in **Table 1**.

The ANOVA on RTs showed that all within-subject main effects were significant. In particular, the effect of Validity [$F(1,53) = 293.98$, $p < 0.001$, $\eta_p^2 = 0.85$] was related to faster responses for valid ($M = 323.13$) than for invalid trials ($M = 382.18$; $p < 0.001$); the effect of Valence [$F(1,53) = 5.13$, $p = 0.03$, $\eta_p^2 = 0.09$] was due to faster responses for non-threatening ($M = 350.95$) than for threatening stimuli ($M = 354.36$; $p = 0.03$); last, the effect of PT [$F(2,106) = 83.42$, $p < 0.001$, $\eta_p^2 = 0.61$] was related to faster responses for longer PTs (100 ms = 370.40; 200 ms = 351.97; 500 ms = 335.59; all different from each other at $p < 0.001$). The main effect of Group was not significant [$F(1,53) = 0.78$, $p = 0.38$, $\eta_p^2 = 0.01$].

We also observed two significant interactions: Validity × PT interaction [$F(2,106) = 8.91$, $p < 0.01$, $\eta_p^2 = 0.14$], and Valence × PT × Validity interaction [$F(2,106) = 3.85$, $p = 0.02$, $\eta_p^2 = 0.07$], whereas all other interactions were not significant. Planned comparison on the Valence × PT × Validity interaction revealed significant shorter RTs for non-threatening ($M = 378.80$) compared to threatening stimuli ($M = 390.06$) only for invalid trials at 200 ms. No other significant difference emerged.

The MANOVA on bias scores showed a significant effect of Group factor on attentional facilitation at 200 ms [$F(1,53) = 4.75$, $p = 0.03$, $\eta_p^2 = 0.08$], as HTA individuals showed a positive facilitation bias ($M = 1.88$; SE = 3.45) and LTA showed a negative facilitation bias ($M = -8.84$; SE = 3.51).

Furthermore, one-sample t-tests on bias scores, in comparison to zero (Koster et al., 2006), revealed that LTA (**Figure 2**) showed attentional disengagement bias [$t(26) = 2.38$, $p = 0.02$] and avoidance [$t(26) = -2.27$, $p = 0.03$] at 200 ms; instead, HTA showed a significant facilitation bias at 100 ms [$t(27) = 2.06$, $p = 0.049$], whereas the difficulty in disengagement at 100 ms fell short of the significance level [$t(27) = 1.97$, $p = 0.059$].

DISCUSSION

Our study investigating ABTs in both low and high trait anxiety individuals, identified a significant facilitation bias at the shortest PT (100 ms) in HTA individuals, in line with previous studies (Koster et al., 2006, 2007). This bias is compatible with the idea that an automatic processing system is responsible for detecting

Table 1 | Mean and SE of the RTs in the Modified Posner Task as a function of anxiety group, validity, valence, and PT.

			LTA		HTA	
PT	**Validity**	**Valence**	**Mean**	**SE**	**Mean**	**SE**
100	Invalid	Threatening	402.59	13.37	394.44	13.13
		Non-threatening	401.63	12.75	380.79	12.52
	Valid	Threatening	354.45	11.53	334.52	11.32
		Non-threatening	354.69	11.62	340.12	11.41
200	Invalid	Threatening	395.60	12.58	384.54	12.35
		Non-threatening	378.81	13.50	378.78	13.26
	Valid	Threatening	332.88	11.76	309.64	11.54
		Non-threatening	324.04	11.07	311.52	10.87
500	Invalid	Threatening	373.15	12.67	357.73	12.44
		Non-threatening	373.17	12.64	364.94	12.41
	Valid	Threatening	313.83	10.87	299.00	10.67
		Non-threatening	309.84	9.97	293.04	9.79

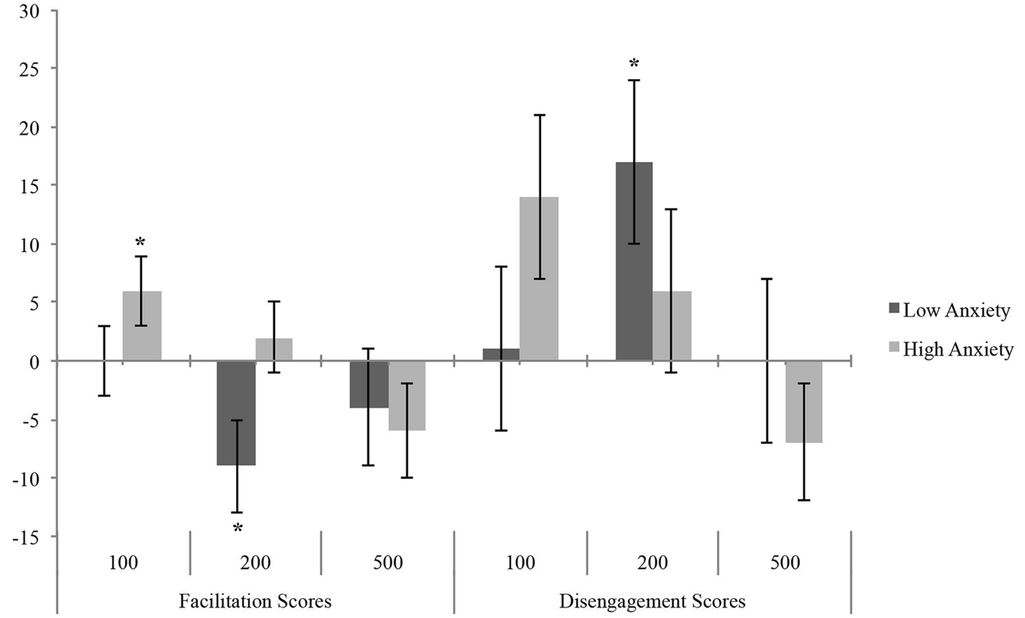

FIGURE 2 | Mean (and standard error) of Bias Score as a function of anxiety in LTA and HTA groups. Asterisks indicate significant differences between ABT and zero (*$p < 0.05$).

and orienting attention toward threat (Mathews and Mackintosh, 1998; Mogg and Bradley, 1998; Cisler and Koster, 2010).

The lack of attentional bias in HTA individuals at longer PTs (200 and 500 ms) is not consistent with findings reported by Koster et al. (2006) in HTA, and by Koster et al. (2007) in normal individuals with intermediate levels of trait anxiety. It should be remembered that ABTs at 200 ms PT have also been reported in spider-fearful individuals with high fear (Mogg and Bradley, 2006), when presented with fear congruent stimuli. It is important to underline that the discrepancies between the present results and those reported by Koster et al. (2006, 2007) and by Mogg and Bradley (2006) might be ascribed to the different characteristics of the stimuli employed in the experimental paradigms. In their studies Koster et al. (2006, 2007) used the International Affective Picture System (IAPS; Lang et al., 2005) scenes and intensive, high threat stimuli, as the authors themselves underlined (for instance, “mutilated face” with strong negative valence and high arousal value), whereas Mogg and Bradley (2006) used stimuli (spiders) with strong negative valence and high arousal value for the specific sample they assessed. Here, we employed familiar stimuli (such as animals, everyday scenes, and common objects), without selecting high intensity threatening items as in previous studies. It is therefore entirely plausible that the stimuli used in the present study were less liable to produce avoidance and disengagement biases in high anxious individuals. In fact, according to the dual competition framework (Pessoa, 2009), threat-related stimuli carry affective significance, which alters performance by strengthening sensory representations at the perceptual level and by prioritizing attention at the executive level. Although threat consistently leads to prioritize perceptual processing, its effect on executive control dramatically depends on the level of threat: high threat stimuli would enhance processing of the threat (hard prioritization), while low threat stimuli would determine a slight improvement of threat processing (soft prioritization). In line with this framework (Pessoa, 2009), the threat intensity of the stimuli used in the present study might have interacted with anxiety levels, determining a different prioritization in the HTA and LTA groups.

The main finding of the present study was indeed the divergence between the pattern of ABTs found in HTA and in LTA groups. A difference between high and low anxious individuals in attentional allocation mechanisms has been already hypothesized by Williams et al. (1997), who suggested that high anxiety would be characterized by a facilitation bias, whereas low anxiety individuals would be particularly characterized by avoidance bias. As recalled above, our experimental paradigm allowed us to detect only a significant facilitation bias in HTA individuals, whereas we found both difficulty in disengagement and attentional avoidance in the LTA group. Several previous studies on low anxiety individuals did report threat-related attentional biases (Mogg et al., 1994; Yiend and Mathews, 2001; Massar et al., 2011), whereas other studies only detected attentional avoidance (MacLeod and Mathews, 1988) or difficulty in disengagement (Massar et al., 2011). To the best of our knowledge, there is no previous evidence about co-occurrence of both biases in LTA at a specific time window (200 ms PT), but not at very rapid (100 ms) or longer (500 ms) PTs.

The specific time course of difficulty in disengagement and attentional avoidance, observed in LTA only, would exclude that these findings can be ascribed to a general slowing of responding to subsequent target stimuli caused by threat cues in exogenous cueing task (Mogg et al., 2008). According to an alternative interpretation, the simultaneous presence of difficulty of disengagement and avoidance at 200 ms in LTA would only reflect a form of cognitive freezing, as suggested by Fox et al. (2001) or non-attentional behavioral freezing, as suggested by Clarke et al. (2013). Freezing is an early response to detected danger throughout the animal kingdom that increase the chances of survival in threatening situations (LeDoux, 1996), but it has freezing-like responses have been also detected in normal human individuals engaged in concurrent cognitive tasks (Sagliano et al., 2014). It can be argued that the delayed responses to threatening stimuli in both valid and invalid trial could reflect a cognitive form of the freezing response, but it would remain to explain the reason why only LTA showed these biases, and only at 200 ms.

The finding of a specific disengagement bias at 200 ms is congruent with Cisler and Koster's (2010) model, positing that attentional facilitation is driven by automatic processing, while the disengagement bias and the attentional avoidance reflect strategic orienting of attention. In this perspective, the presence of such biases in LTA would support the idea that Cisler and Koster's (2010) model is not specific for the clinical sample but it can be applied to all individuals, independently from their anxiety level. However, as suggested by Koster et al. (2006), HTA individuals are characterized by an oversensitive threat appraisal system that leads to overestimate valence of threatening stimuli. This causes a shift of attention toward moderately threatening stimuli (facilitation bias) in these individuals, whereas LTA individuals do not show the same enhanced, rapid detection of threats, and are able to strategically avoid threatening stimuli and yet to take such stimuli under attentional control.

Disengagement bias may serve to maintain and enhance anxiety states (Fox et al., 2001). In contrast, avoidance bias, i.e., the ability to rapidly disengage from threat-related material once identified, may be a useful mechanism to keep anxiety levels under control.

Several studies (Beck and Clark, 1997) suggested that a top-down modification of attention allocation would reduce the risk of negative consequences from threat, thus resulting in an attentional avoidance of threatening stimuli. However, it should be underlined that LTA individuals showed at the same time window (200 ms) both attentional avoidance and difficulty to disengagement, an apparently paradoxical finding (see Cisler and Koster, 2010). Nevertheless, on the basis of the distinction between overt and covert attentional mechanisms (Posner, 1980). Weierich et al. (2008) argued that individuals might overtly avoid the threat and covertly maintain their attention on it. This might represent the most effective method to deal with potential threats, without activating strong emotion-related cognitive and neural processes. This ability to react to threat might reduce individual vulnerability to adverse events. Recently, Min et al. (2012) suggested that the ability to respond to stress and adversity, together with LTA levels, might reduce the risk to develop psychiatric disorder; Min et al. (2012) also suggested that evaluation and management of trait anxiety can enhance patient's resilience and improve treatment of depression and anxiety disorders. Our results are substantially in line with these statements. Indeed, the difference between high and low anxious individuals revealed in this study is compatible with the idea that the ability to simultaneously control and avoid threat showed by LTA might be considered the most advantageous response modality, likely allowing to minimize negative emotional responses to threats and, possibly, the risk of developing clinically relevant anxiety.

In other words, this specific pattern of ABTs might reflect the differences between HTA and LTA's behavior, and help comprehending why some individuals are characterized by low levels of anxiety.

The lack of analogous findings in LTA in previous studies might be ascribed to the specific methodological procedures adopted here, as regards the stimuli (we used familiar items to assess responses to plausible threats), the experimental paradigm (we used three randomized PTs to avoid participants prepare their responses), and the sample selection (we selected a gender-homogeneous sample, thus minimizing variability, on the basis of well defined cut-off values for low or high trait anxiety). These methodological choices likely contributed to put in evidence previously unreported findings in LTA, but also impose some caveats in generalizing the present results. Future studies will have to verify whether the same pattern is present in male individuals, and, above all, to take into account the possible effects of stimuli's valence also assessing physiological correlates of threat processing. Moreover, future studies might also take into account the possible interaction of depressive mood with ABTs, although available evidence would suggest that depressed individuals usually show ABTs at PT longer than those used in the present study (Mogg and Bradley, 2005).

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Depression, not PTSD, is associated with attentional biases for emotional visual cues in early traumatized individuals with PTSD

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Using variants of the emotional Stroop task (EST), a large number of studies demonstrated attentional biases in individuals with PTSD across different types of trauma. However, the specificity and robustness of the emotional Stroop effect in PTSD have been questioned recently. In particular, the paradigm cannot disentangle underlying cognitive mechanisms. Transgenerational studies provide evidence that consequences of trauma are not limited to the traumatized people, but extend to close relatives, especially the children. To further investigate attentional biases in PTSD and to shed light on the underlying cognitive mechanism(s), a spatial-cueing paradigm with pictures of different emotional valence (neutral, anxiety, depression, trauma) was administered to individuals displaced as children during World War II (WWII) with ($n = 22$) and without PTSD ($n = 26$) as well as to non-traumatized controls ($n = 22$). To assess whether parental PTSD is associated with biased information processing in children, each one adult offspring was also included in the study. PTSD was not associated with attentional biases for trauma-related stimuli. There was no evidence for a transgenerational transmission of biased information processing. However, when samples were regrouped based on current depression, a reduced inhibition of return (IOR) effect emerged for depression-related cues. IOR refers to the phenomenon that with longer intervals between cue and target the validity effect is reversed: uncued locations are associated with shorter and cued locations with longer RTs. The results diverge from EST studies and demonstrate that findings on attentional biases yield equivocal results across different paradigms. Attentional biases for trauma-related material may only appear for verbal but not for visual stimuli in an elderly population with childhood trauma with PTSD. Future studies should more closely investigate whether findings from younger trauma populations also manifest in older trauma survivors.

Keywords: attentional bias, trauma, depression, bias components, transmission

INTRODUCTION

In 2014, the United Nations Refugee Agency (UNHCR; United Nations Refugee Agency, 2014) reported that at the end of 2013, more than 50 million people were forcibly displaced due to war, conflicts, or human right violations. The negative consequences of forced displacement for the psychological and physical well-being are documented in many studies (e.g., Fazel et al., 2005; Porter and Haslam, 2005). Beyond that, forced displacement is associated with a highly increased risk for posttraumatic stress disorder (PTSD, e.g., Fazel et al., 2005; Steel et al., 2009; Freitag et al., 2013). Even decades later, a substantial proportion of those affected suffer from negative consequences (e.g., Muhtz et al., 2011; Freitag et al., 2013). In addition, the negative consequences of traumatic events are not restricted to those directly exposed, but seem to impact close relatives such as the children (Danieli, 1998; Leen-Feldner et al., 2013). For example, an increased risk for the development of PTSD was reported in offspring of individuals with PTSD (Baider et al., 2000, 2006; Yehuda et al., 2008). Nonetheless, the literature is not fully consistent and adequately designed population-based studies did not find clear-cut evidence for a transgenerational transmission (Van IJzendoorn et al., 2003; Levav et al., 2007; Fridman et al., 2011).

In order to study the long-term consequences of forced displacement, individuals displaced at the end of World War II (WWII) were increasingly investigated in recent years. Studies revealed a high rate of PTSD more than 60 years later (e.g., Teegen and Meister, 2000; Muhtz et al., 2011). According to DSM-IV (American Psychiatric Association [APA], 2000), PTSD is characterized by symptoms of re-experiencing, avoidance of trauma-related stimuli, and hyperarousal. Beyond that, PTSD is associated with different biases in information processing, for example, attentional biases for trauma-related stimuli (Buckley et al., 2000; Constans, 2005).

In experimental psychopathology, different paradigms have been adopted from cognitive psychology to investigate attentional biases in PTSD, most commonly the emotional Stroop task (EST, Williams et al., 1996) and the dot-probe paradigm (MacLeod et al., 1986). EST studies largely contributed to our knowledge of attentional biases in PTSD and the emotional Stroop effect (ESE) was considered a robust finding for many years as it was replicated across different trauma populations (for reviews see Buckley et al., 2000; Constans, 2005). However, the specificity and stability of the effect were critically discussed (Kimble et al., 2009; Cisler et al., 2011) and closer scrutiny reveals that interpretability of many studies is constrained by methodological limitations. For example, while some studies lack a trauma control group (Paunovic et al., 2002; El Khoury-Malhame et al., 2011a; Fleurkens et al., 2011), the interpretability of other studies is restricted because no negatively-valenced control stimuli were included (Harvey et al., 1996; Paunovic et al., 2002; Bremner et al., 2004). Thus, the question whether the effect is specific to PTSD and/or to trauma-related stimuli remains unresolved. Furthermore, the EST has several methodological problems (e.g., Algom et al., 2004) and does not allow to disentangle the different attentional bias components, that is, whether these are comprised of *attentional facilitation* (i.e., preferred processing of trauma-related compared to neutral stimuli), *attentional interference* (i.e., difficulties disengaging from trauma-related to other stimuli) or *attentional avoidance* (i.e., attention allocation toward the opposite location of trauma-related stimuli, cf. Cisler and Koster, 2010). The differentiation of these components can provide a better theoretical understanding and allows the development or improvement, respectively, of novel interventional techniques (Shipherd and Salters-Pedneault, 2008).

In dot-probe tasks, two stimuli of different valence are presented simultaneously for a set time (e.g., 500 ms). Subsequently, one of the two stimuli is replaced by a probe and participants are asked to respond to either its location or to classify the probe (e.g., * or **, Cisler et al., 2009). A faster reaction to probes that replace negatively-valenced (congruent trials) compared to neutral stimuli (incongruent trials) are interpreted as evidence for attentional facilitation as attention is already drawn to the spatial location of the threatening stimulus (Yiend, 2010). The opposite response pattern, that is, slower reactions to probes that replace negatively-valenced compared to neutral stimuli are indicative of attentional avoidance (Cisler and Koster, 2010; Yiend, 2010). The majority of studies administering variants of the dot-probe paradigm in PTSD do not provide evidence for attentional biases for trauma-related/negative material (Dalgleish et al., 2003; Elsesser et al., 2004, 2005; Fani et al., 2011); however, some studies demonstrated attentional biases (Bryant and Harvey, 1997; El Khoury-Malhame et al., 2011a,b). More recent studies suggest that acute stress (e.g., missile attacks) is associated with attentional avoidance of threat-related information (Bar-Haim et al., 2010; Wald et al., 2011a,b), which, in turn, predicted psychopathological symptoms in the short- (Bar-Haim et al., 2010; Wald et al., 2011a) and long-term (Wald et al., 2011b).

A paradigm that allows the differentiated assessment of attentional bias components represents the visual search task (VST, Öhmann et al., 2001). In VSTs, participants are asked to detect a discrepant target stimulus embedded in an array of identical stimuli. The VST was applied in two studies to differentially assess facilitation and interference in PTSD (Pineles et al., 2007, 2009). In the interference condition, a target (e.g., non-word) was presented in an array of experimental stimuli (e.g., trauma-related words) whereas in the facilitation condition, the arrangement was reversed (i.e., target experimental word embedded in an array of identical non-words). Facilitation to trauma-related words was inferred from faster reaction times to trauma-related compared to neutral targets in an array of non-word distracters. Interference to trauma-related words was inferred from slower reaction times to target stimuli embedded in arrays with trauma-related compared to neutral distracters (Pineles et al., 2009). In both studies, PTSD was associated with attentional interference to trauma-related stimuli (Pineles et al., 2007, 2009) and this effect was specific to trauma-related stimuli (Pineles et al., 2009). However, there was no evidence for attentional facilitation. This finding conflicts with the theoretical assumption of hypervigilance in PTSD (Pineles et al., 2009). However, the latter two paradigms are also plagued by interpretational problems (see Hauschildt et al., 2013, for a further discussion).

SPATIAL-CUEING TASK

One paradigm that enables the assessment of the precise underlying mechanism represents a modification of the *spatial-cueing paradigm* (Posner, 1980). A great advantage of cueing paradigms is the fact that the behavioral reaction is made in response to a neutral target, thus, response bias explanations can be ruled out (Yiend, 2010). Furthermore, by varying the stimulus-onset asynchrony (SOA), cueing paradigms allow the assessment of the temporal attention allocation. This is important when investigating attentional biases in PTSD as this disorder seems to be associated with delayed disengagement from trauma-related cues (e.g., Pineles et al., 2007) and disengagement is based upon controlled processes that need more time to take effect (cf. Yiend, 2010).

Attention is focused on a fixation point located between two rectangles. Subsequently, a cue is presented in one of the two rectangles, followed by a target that either appears in the same rectangle (valid trial) or in the opposite rectangle (invalid trial). In some trials, no cue appears (catch trials). The participants' task is to indicate (e.g., key press) in which rectangle the target was presented. For the assessment of attentional biases in psychopathology, the cue is varied as to its emotional valence (e.g., threatening, neutral). While facilitation is operationalized as faster responses to validly cued trials when the cue is threatening/disorder-specific compared to neutral, avoidance is characterized by slower RTs to threatening compared to neutral cues in valid trials. Slower RTs to threatening/disorder-specific compared to neutral stimuli in invalidly cued trials are interpreted as interference, faster RTs as avoidance (Koster et al., 2006). However, this pattern is only true for short SOAs (<300 ms, Posner and Cohen, 1984). With longer time intervals between cue and target (SOA), *inhibition of return* (IOR) occurs, that is, cued locations lose their attentional preference as attention is directed to uncued locations after a certain time (for a review see Klein, 2000). It is assumed that this effect is adaptive as redirecting attention to an already attended location does not provide additional information. As

PTSD seems to be associated with problems in disengaging and patients "stick" to trauma-related material, the IOR effect should be reduced or even absent as attention is not re-directed to new locations. Although some studies provide evidence for this assumption in anxiety (Nelson et al., 1993; Fox et al., 2002), other results speak for the stability of the IOR effect (Stoyanova et al., 2007; Lange et al., 2008). To the best of our knowledge, one study applied the spatial-cueing paradigm in PTSD (Hauschildt et al., 2013). A spatial-cueing paradigm with pictures of different emotional valence (trauma-related, negative control, general threat, neutral) and varying SOA (450, 1200 ms) was administered to 25 participants with PTSD, 22 non-PTSD and 24 healthy control participants. Although neither PTSD nor trauma exposition were associated with attentional biases, depressive symptomatology was linked with attentional avoidance of trauma-related and negative control stimuli.

TRANSGENERATIONAL INFORMATION PROCESSING STUDIES

First evidence that information processing biases can be transferred came from Motta and colleagues (Motta et al., 1994, 1997) who administered an EST to children of Vietnam veterans and non-veterans. In the first study (Motta et al., 1994) the mean difference between children of veterans and non-veterans for the PTSD-related card was 1.97 s, whereas the mean reaction time differences for all other cards varied between 0.21 and 0.81 s. In a replication study with a larger sample, children of veterans were significantly slower to color-name the war-related card compared to children of non-veterans, whereas RTs to all other cards (OCD-related, positive, neutral) did not differ between groups (Motta et al., 1997). Evidence for a transmission was also found when the children's group allocation was based on parental trauma exposure (Suozzi and Motta, 2004). These findings were replicated in a sample of children and adolescents (Moradi et al., 1999): children whose parents suffered from PTSD exhibited an ESE for threat-related compared to neutral words and compared to the children of healthy control participants. However, conflicting evidence stems from one study in which children of displaced individuals (with and without PTSD) were compared to children of non-traumatized healthy control participants regarding their color naming latencies in an EST (Wittekind et al., 2010). There was no evidence for attentional biases for trauma-related words in children of displaced individuals with PTSD. However, the sample differed from previous studies in several important aspects (e.g., time since parental traumatization, children's age, trauma type, parental trauma vs. PTSD) limiting comparability between studies.

To conclude, a substantial body of studies assert that PTSD is related to attentional biases for trauma-related material which seem to results from difficulties to disengage (Pineles et al., 2007, 2009). However, interpretability of many studies is constrained by methodological limitations and results need to be replicated across different paradigms and stimulus modalities (i.e., verbal vs. visual stimuli). Furthermore, prior research almost exclusively recruited younger trauma samples (average age in emotional Stroop studies: 36 years, Cisler et al., 2011), thus, it remains unclear whether attentional biases persist over the course of the disorder. Beyond that, essential influencing factors (e.g., SOA, stimulus type, comorbid depression) have been neglected in prior research on attentional biases in PTSD (also see Cisler et al., 2009). Transgenerational studies applied the EST to assess whether parental trauma or PTSD, respectively, is related to attentional biases in the second generation. Beside the fact that evidence is ambiguous, it is yet unclear whether findings translate to different paradigms and which attentional bias component drives the effect.

THE PRESENT STUDY

The aim of the present study was to replicate and extend the results by Hauschildt et al. (2013) in a sample of older individuals with chronic PTSD due to childhood trauma as well as their offspring. To meet this aim, we also administered a modified version of the spatial-cueing paradigm using visual instead of verbal stimuli that differed as to their emotional valence (trauma-related, depression-related, anxiety-related, neutral). As PTSD seems to be associated with delayed disengagement from trauma-related stimuli (Pineles et al., 2007, 2009), one would expect a diminished IOR effect for targets following trauma-related compared to other emotional or neutral pictures in individuals with PTSD. However, as the majority of previous studies do not provide evidence for a reduced IOR effect in PTSD (Hauschildt et al., 2013) and other anxiety disorders (Stoyanova et al., 2007; Lange et al., 2008), we assume that the IOR effect is not affected by cue valence. Regarding a transgenerational transmission, we hypothesized that offspring of PTSD participants demonstrate an attentional bias for trauma-related material; however, we did not have a directed hypothesis whether attentional biases result from facilitation, interference, or avoidance.

MATERIALS AND METHODS

PARTICIPANTS

Individuals displaced as children during or after WWII ($n = 50$) and one of their adult children were recruited by (a) a database built up in a previous study (for a detailed description of recruitment strategies see Muhtz et al., 2011), (b) contact to displacement networks and self-help groups, (c) word of mouth, and (d) personal contacts. Participants were born between 1932 and 1941 and experienced at least one traumatic event according to DSM-IV trauma criteria during their flight. Group allocation was based on the PTSD module of the Structured Clinical Interview for DSM-IV (SCID-I, First et al., 1997). To assure that diagnoses of PTSD were indeed due to forced displacement and not to a later trauma, we inquired whether participants experienced a traumatic event other than flight/displacement. If this was the case, participants had to indicate which of the traumatic events was worse. Subsequently, PTSD criteria for each traumatic event were assessed via the SCID and items were rephrased such that the relation to the respective event was stressed, e.g., instead of "traumatic event" we explicitly used "after displacement." Exclusion criteria for all groups were a lifetime history of psychotic, manic or bipolar symptoms, substance dependence within the last year or suicidal tendencies as assessed with the MINI Neuropsychiatric Interview (MINI, Sheehan et al., 1998). Of all traumatized participants who were assessed, two participants had to be excluded (manic disorder, trauma criteria A2 not fulfilled). Three adult children were excluded due to alcohol dependence, withdrawal of informed consent, and psychotic symptoms. Thus, the PTSD group comprised 22 traumatized participants of whom 12 fulfilled all PTSD criteria and 10 participants were diagnosed with subsyndromal PTSD as suggested by Blanchard et al. (1996, DSM-IV criteria A, B, E, F were fulfilled and either criterion C or D) and 21 of their adult children. The remaining 26 participants (and 24 of their children) were allocated to the non-PTSD group. Twenty-two non-traumatized (DSM-IV trauma criteria A1 and A2) participants who were not displaced during WWII, not married to an individual displaced during WWII, not meeting any current axis I disorder (based on the MINI) and one adult offspring formed the healthy control group. The latter group was recruited by means of advertisement in local media, notices in public places, and word of mouth. Written informed consent was obtained prior to the study from all participants. The study was approved by the local ethics committee.

MEASURES

Psychopathology

All participants were interviewed with the MINI interview (Sheehan et al., 1998) in order to determine (a) exclusion criteria for all participants, (b) (comorbid) psychiatric disorders in traumatized participants, and (c) absence of any current axis I disorder in non-traumatized controls. In order to quantify PTSD severity, the Posttraumatic Diagnostic Scale (PDS, Foa et al., 1997) was administered to all traumatized participants. The PDS is a self-report questionnaire showing high reliability and validity (Foa et al., 1997). All 17 items of the PDS were paraphrased such that "traumatic event" was replaced by "flight/displacement." Depression severity was quantified with the 17-item version of the Hamilton Depression Rating Scale (HDRS, Hamilton, 1960). Finally, verbal intelligence was estimated using a vocabulary test [Mehrfachwahl-Wortschatz-Intelligenztest B (MWT-B), Lehrl, 2005].

Stimulus selection

Pictorial stimuli of the present study captured five different conditions (Trauma, Depression, Anxiety, Neutral, Neutral old). Pictures were selected from the *International Affective Picture System* (IAPS, Lang et al., 2008), the internet or from books and media reports about displacement after WWII (trauma-related stimuli). Besides the emotional conditions and the neutral condition (IAPS pictures), we included a fifth condition (neutral old) containing pictures that came from the same time as the trauma- (i.e., displacement) related pictures. This was done to control for "age effects" as it is conceivable that trauma-related pictures are processed differentially due to their deviation from pictures taken from the internet or the IAPS. All stimuli were rated by 15 displaced individuals in a pilot study that was conducted via an online survey regarding (a) their relevance for (aa) flight/displacement after WWII, (ab) depression and (ac) anxiety (1 = very relevant, 2 = slightly relevant, 3 = not relevant), (b) neutrality (yes/no), and (c) personal relevance (yes/no). For the final picture set, trauma-related pictures had to be rated as highly displacement-relevant (rating = very relevant) by at least 80% and as personally relevant by at least 60% of displaced individuals. Furthermore, trauma-related pictures were rated as significantly more displacement-relevant than pictures from all other categories, all $ps < 0.001$. The final set of pictures comprised 10 trauma-related (e.g., refugee trek), 10 depression-related (e.g., sad person), 10 anxiety-related (e.g., snake), 10 neutral (IAPS, e.g., towel), and 10 neutral-old pictures (e.g., landscape). Pictures were presented in black-and-white.

Procedure and experimental task

Before the experimental paradigm started, demographic and psychopathological information (MINI, HDRS) were thoroughly inquired. Traumatized participants were also assessed with the PTSD module of the SCID.

The experimental paradigm was constructed using Superlab® software and was presented individually via a Macintosh computer in a dimly lit room to prevent reflections on the monitor. Participants were instructed in written and verbal form to classify via key press whether a target (black dot) was presented in the right or left rectangle ("m" and "y" [German keyboard], respectively, on the keyboard). They were told that each target would be preceded by a picture whose position was irrelevant for the task. To ensure that all participants understood the task, a practice trial with 10 items was administered to participants prior to the experimental task.

The procedure for each trial was as followed: to focus attention to a central point, a small fixation cross was presented between two rectangles (7 cm high by 9.4 cm wide) for 500 ms. The rectangles remained on the screen throughout a block of trials. Subsequently, a cue stimulus picture appeared with equal probability inside one of the two rectangles (400 ms). The cue varied as to its emotional valence (Trauma, Depression, Anxiety, Neutral-old, Neutral) and was followed by the fixation cross/rectangles for either 50 or 800 ms. Thus, SOA between cue and target varied between short (450 ms) and long (1200 ms) intervals (Moritz et al., 2009; Hauschildt et al., 2013). Then, the target was presented equally often in the center of one of the two rectangles and independent of the cue (i.e., the position of the cue had no predictive value for the position of the target). In valid trials, cue and target appeared in the same rectangle, whereas in invalid trials, cue and target appeared in opposite rectangles. The target remained on the screen until a response (i.e., key press) was made. In approximately 9% of trials, no target was presented (catch trials) and rectangles remained on the screen for 1500 ms before the next trial was automatically initiated. The inter-trial interval was 1000 ms. In total, the task comprised 450 trials with 10 practice, 40 catch and 400 experimental trials (5 conditions × 10 stimuli × 2 long/short × 2 valid/invalid × 2 right/left) presented in fully randomized order. The task was divided in two blocks (220 trials/block) with a short break in-between. Subsequently, participants rated all pictures as to their valence and personal relevance, respectively (1 = positive and personal relevant, 2 = positive, 3 = neutral, 4 = negative, 5 = negative and personal relevant). For the rating task, pictures were also presented in random order.

STRATEGY OF DATA ANALYSES

Dependent variables were reaction time (in ms) and accuracy (i.e., percentage of errors). According to a prior study (Hauschildt et al., 2013), only RTs between 150 and 2000 ms were considered for analyses. Furthermore, RTs of incorrect trials (i.e., wrong key) were omitted. For each participant, RTs for each combination of Cue Type, Validity, SOA, and Position were determined.

However, as position was not considered crucial for subsequent analyses, RTs were collapsed across position yielding six RTs per participant. Catch trials were not analyzed.

To test the main hypothesis, mixed-model analyses of variance (ANOVA) were conducted for each generation separately as we were especially interested in intra-generational effects. To facilitate interpretation, only relevant interactions including group are reported. Furthermore, an *IOR effect* was calculated by substracting mean RTs of valid trials from mean RTs of invalid trials (Moritz and Von Mühlenen, 2005). Positive values are indicative of a facilitation effect of the cue on the target, negative values for an inhibitory (i.e., IOR) effect. The alpha level for all statistical tests was 0.05 (two-tailed). Effect sizes were calculated with $\eta_p^2 \approx 0.01$ indicating a small, $\eta_p^2 \approx 0.06$ a medium, and $\eta_p^2 \approx 0.14$ a large effect (Kinnear and Gray, 2008). To break down significant interactions, One-Way ANOVAs were calculated. Greenhouse-Geisser correction for degrees of freedom was applied if assumption of sphericity was violated. Correlational analyses (Pearson) were conducted between IOR effects (i.e., $RT_{invalid}$ - RT_{valid}) for both SOA (450, 1200 ms) and depressive (parents: $n = 70$, offspring: $n = 66$) as well as posttraumatic symptomatology ($n = 48$).

RESULTS

SOCIODEMOGRAPHIC INFORMATION AND PSYCHOPATHOLOGY

As can be derived from **Table 1**, neither parental nor offspring groups differed regarding age, gender, or verbal intelligence (all *ps* > 0.09). As expected, traumatized participants with PTSD suffered from higher PTSD (PDS, $p < 0.001$) and depressive symptomatology (HDRS, $p < 0.001$). Offspring groups differed significantly on depressive symptom severity ($p = 0.003$); however, mean ratings were within the normal range in all offspring groups.

SUBJECTIVE VALENCE RATINGS

To verify stimuli allocation, participants' mean ratings were submitted to two Two-Way ANOVAs with Cue Type (Trauma, Anxiety, Depression, Neutral-old, Neutral) as within- and Group (PTSD, non-PTSD, non-Trauma) as between-subjects factor. Mean valence ratings (1 = positive and personally relevant, 2 = positive, 3 = neutral, 4 = negative, 5 = negative and personally relevant) served as dependent variables.

Parents

As expected, the main effect Cue Type was significant, $F_{(3.08,\ 206.63)} = 201.51$, $p < 0.001$, $\eta_p^2 = 0.75$. All emotional pictures were rated as more negative than neutral pictures (*ps* < 0.001). Furthermore, trauma-related pictures were perceived significantly more negative than anxiety- and depression-related pictures (*ps* < 0.001), however, the latter two conditions were not rated differently ($p > 0.99$). The main effect of Group also achieved significance, $F_{(2,\ 67)} = 12.53$, $p < 0.001$, $\eta_p^2 = 0.27$: the most negative ratings were obtained for the PTSD group which differed significantly from the non-Trauma ($p < 0.001$) and at trend level from the non-PTSD group ($p = 0.085$). The main effects were modified by a significant Cue Type × Group interaction, $F_{(6.17,\ 206.63)} = 4.64$, $p < 0.001$, $\eta_p^2 = 0.12$ (for *post-hoc* One-Way ANOVAs [$\alpha = 0.05$], see **Table 2**).

Offspring

Cue Type exerted a significant influence on valence ratings, $F_{(3.30,\ 210.88)} = 179.42$, $p < 0.001$, $\eta_p^2 = 0.74$. As in the parental generation, emotional pictures were rated as more negative than all other pictures (*ps* < 0.001) and trauma-related pictures as more negative than anxiety- and depression-related pictures (*ps* < 0.001, see **Table 2**). Furthermore, depression-related pictures were considered more negative than anxiety-related pictures

Table 1 | Background variables (sociodemography and psychopathology) for parental and offspring groups: means (standard deviation) or frequency.

Variable		PTSD (P) P: *n* = 22 O: *n* = 21	Non-PTSD (nP) P: *n* = 26 O: *n* = 24	Non-Trauma (nT) P: *n* = 22 O: *n* = 22	Statistics
Age (in years)	P	72.73 (2.27)	73.00 (2.00)	73.73 (2.98)	$F_{(2,\ 67)} = 1.18$, $p = 0.371$
	O	43.00 (7.40)	43.50 (4.74)	42.68 (5.28)	$F_{(2,\ 40.26)^b} = 0.15$, $p = 0.858$
Sex (female/male)	P	20/2	17/9	15/7	$\chi^2_{(2)} = 4.69$, $p = 0.096$
	O	15/6	15/9	15/7	$\chi^2_{(2)} = 0.42$, $p = 0.81$
Verbal intelligence	P	113.91 (10.81)	119.15 (11.64)	118.27 (11.36)	$F_{(2,\ 67)} = 1.42$, $p = 0.248$
	O	111.90 (11.42)	110.00 (12.39)	111.95 (13.30)	$F_{(2,\ 64)} = 0.19$, $p = 0.83$
Medication (yes/no)[a]	P	4/18	3/23	1/21	$\chi^2_{(2)} = 2.02$, $p = 0.364$
HDRS	P	11.73 (6.06)	5.14 (5.06)	2.37 (2.63)	$F_{(2,\ 39.98)^b} = 22.36$, $p < 0.001$, P > nP, nT[c]; nP > nT[c,d]
	O	4.10 (4.15)	4.13 (5.10)	1.27 (1.35)	$F_{(2,\ 32.98)^b} = 7.17$, $p = 0.003$, P, nP > nT[c]
PDS	P	15.50 (5.01)	6.31 (5.12)		$t_{(46)} = 6.26$, $p < 0.001$

PTSD, Posttraumatic Stress Disorder; P, parental generation; O, offspring generation.

[a] *Neuroleptics, antidepressents, soporifics, benzodiazepine.*

[b] *Corrected for unequal homogeneity of variances.*

[c] *Games-Howell corrected post-hoc tests were used.*

[d] *p = 0.051.*

Table 2 | Subjective valence ratings: means (standard deviation) and results of *post-hoc* ANOVAs.

	Picture type	PTSD (P, $n = 22$) Offspring PTSD ($n = 21$)	Non-PTSD (nP, $n = 26$) Offspring non-PTBS ($n = 24$)	Non-Trauma (nT, $n = 22$) Offspring non-Trauma ($n = 22$)	ANOVA (*post-hoc*)
P	Trauma	4.73^a (0.28)	4.61 (0.58)	3.98 (0.49)	$F_{(2,\ 67)} = 16.13$, $p < 0.001$, P > nT
	Anxiety	3.86 (0.53)	3.71 (0.63)	3.54 (0.60)	$F_{(2,\ 67)} = 1.64$, $p = 0.202$
	Depression	4.06 (0.48)	3.74 (0.41)	3.50 (0.42)	$F_{(2,\ 67)} = 9.30$, $p < 0.001$, P > nP, nT
	Neutral old	2.44 (0.40)	2.47 (0.41)	2.61 (0.35)	$F_{(2,\ 67)} = 1.17$, $p = 0.316$
	Neutral IAPS	3.00 (0.23)	2.91 (0.31)	2.95 (0.24)	$F_{(2,\ 67)} < 1$, $p = 0.518$
O	Trauma	4.11 (0.44)	4.10 (0.50)	3.73 (0.26)	
	Anxiety	3.47 (0.44)	3.58 (0.58)	3.25 (0.47)	
	Depression	3.71 (0.42)	3.80 (0.44)	3.65 (0.31)	
	Neutral old	2.51 (0.49)	2.67 (0.33)	2.49 (0.31)	
	Neutral IAPS	3.00 (0.18)	3.02 (0.13)	2.95 (0.18)	

PTSD, posttraumatic stress disorder; P, parental generation; O, offspring generation.

[a] *1 = positive and personally relevant; 2 = positive; 3 = neutral; 4 = negative; 5 = negative and personally relevant.*

($p = 0.001$). Groups also differed as to their overall ratings, $F_{(2,\ 64)} = 5.23$, $p = 0.008$, $\eta_p^2 = 0.14$. Children of the non-PTSD group rated pictures on average more negative than children of healthy controls ($p = 0.007$). However, the interaction Cue Type × Group did not reach significance, $F_{(6.59,\ 210.88)} = 1.29$, $p = 0.258$, $\eta_p^2 = 0.04$.

ACCURACY

One participant of the offspring non-Trauma group pressed the wrong keys, thus, these data could not be considered in all subsequent analyses. Accuracy was high (PTSD: 97.92%, non-PTSD: 97.88%, non-Trauma: 98.80%, offspring PTSD: 97.85%, offspring non-PTSD: 97.17%, offspring non-Trauma: 98.06%) and did not differ between groups, $F_{(5,\ 130)} < 1$, $p = 0.977$.

ATTENTIONAL BIASES

Parents

To test whether participants with PTSD exhibit attentional inference for trauma-related stimuli, a repeated measures Four-Way ANOVA with Group (PTSD, non-PTSD, non-Trauma) as between-subject factor and Cue Type (Trauma, Anxiety, Depression, Neutral-old, Neutral), SOA (450, 1200 ms), and Validity (Valid, Invalid) as within-subject factors was conducted. Mean RT served as dependent variable (see **Table 3**). A main effect of Cue Type emerged, $F_{(4,\ 268)} = 3.49$, $p = 0.008$, $\eta_p^2 = 0.05$: RTs for trauma- ($M = 491.57$ ms, $SE = 7.43$ ms) and anxiety-related pictures ($M = 491.67$ ms, $SE = 7.41$ ms) were significantly slower than RTs for neutral-old pictures ($M = 484.65$ ms, $SE = 7.07$ ms, $ps < 0.05$). The main effect of SOA was also significant, $F_{(1,\ 67)} = 124.32$, $p < 0.001$, $\eta_p^2 = 0.65$, due to faster RTs to long ($M = 468.33$ ms, $SE = 6.86$ ms) vs. short ($M = 509.48$ ms, $SE = 7.92$ ms) SOAs. Furthermore, RTs to invalid cues ($M = 469.22$ ms, $SE = 7.05$ ms) were faster than to valid cues ($M = 508.59$ ms, $SE = 7.77$ ms), $F_{(1,\ 67)} = 112.70$, $p < 0.001$, $\eta_p^2 = 0.63$, reflecting an IOR effect. Finally, groups differed as to their overall RT, $F_{(2,\ 67)} = 5.07$, $p = 0.009$, $\eta_p^2 = 0.13$, with the PTSD group being slower ($M = 521.41$ ms, $SE = 12.77$ ms) than both the non-PTSD and the non-Trauma group ($M = 470.53$ ms, $SE = 11.74$ ms and $M = 474.77$ ms, $SE = 12.77$ ms, respectively, $ps < 0.05$). However, neither the Three-Way interaction of Cue Type × Validity × Group, $F_{(8,\ 268)} < 1$, $p = 0.544$, $\eta_p^2 = 0.025$, nor the Four-Way interaction of Cue Type × SOA × Validity × Group were significant, $F_{(7.91,\ 264.92)} = 1.18$, $p = 0.314$, $\eta_p^2 = 0.03$. Thus, groups did not react differently to trauma-related stimuli.

Following Hauschildt et al. (2013), groups were dichotomized according to the presence of current depression (yes/no) as this disorder constitutes a common psychiatric comorbidity (Pietrzak et al., 2011) and is hardly considered as a confound in attentional bias research (Bar-Haim et al., 2007). We used a categorical MINI diagnosis to compose groups as we were interested in the impact of current depressive symptomatology. Sociodemographic and psychopatholgical characteristics are presented in **Table 5**. The mixed Four-Way ANOVA was repeated, this time with depressed ($n = 14$) vs. non-depressed ($n = 56$) as between-subject factor. As groups differed significantly regarding gender, the ANOVA was repeated with gender as an additional between-subject factor. Neither the main effect gender nor any interaction including gender was significant, $ps > 0.1$. Only relevant effects for group are reported. The depressed group ($M = 521.29$ ms, $SE = 16.43$ ms) was slowed compared to the non-depressed group ($M = 479.50$ ms, $SE = 8.22$ ms), $F_{(1,\ 68)} = 5.18$, $p = 0.026$, $\eta_p^2 = 0.07$. Whereas the Three-Way interaction of Cue Type × Validity × Group was not significant, $F_{(4,\ 272)} < 1$, $p = 0.715$, $\eta_p^2 = 0.01$, the Four-Way interaction of Cue Type × SOA × Validity × Group was, $F_{(3.94,\ 268.02)} = 4.02$, $p = 0.004$, $\eta_p^2 = 0.06$. To break down this interaction, Two-Way ANOVAs within SOAs (450 vs. 1200 ms) were calculated with Group (Depressed, non-Depressed) as between-subject and Cue Type (Trauma, Anxiety, Depression, Neutral-old, Neutral) as within-subject factors. IOR effects served as dependent variables. For short SOA, the relevant interaction of Group × Cue Type was significant, $F_{(4,\ 272)} = 2.52$, $p = 0.041$, $\eta_p^2 = 0.04$, whereas for the long SOA, significance was bordered, $F_{(4,\ 272)} = 2.38$, $p = 0.052$, $\eta_p^2 = 0.03$. As can be seen in **Figure 1**, the significant interaction (short SOA) reflected facilitated RTs (i.e., reduced IOR effect) for the depressed group in the depression- and trauma-related condition in comparison to the other conditions. For the non-depressed group,

Table 3 | Mean RTs (in ms), standard deviations and IOR effects for each combination of picture type, SOA, validity, and group.

SOA	Cue Type	Validity	PTBS ($n = 22$)			Non-PTBS ($n = 26$)			Non-Trauma ($n = 22$)		
			M	***SD***	**IOR**	***M***	***SD***	**IOR**	***M***	***SD***	**IOR**
450 ms	Neutral	Invalid	513.52	71.80	−45.57	467.85	48.91	−47.77	463.11	71.41	−53.56
		Valid	559.09	83.21		515.62	75.73		516.68	81.28	
	Neutral old	Invalid	513.48	75.01	−48.70	458.45	48.46	−42.88	459.62	72.92	−52.16
		Valid	562.18	92.82		501.33	57.75		511.78	82.29	
	Anxiety	Invalid	525.71	85.77	−55.51	467.15	54.73	−65.32	469.07	76.91	−65.86
		Valid	581.22	84.43		532.47	70.46		534.93	91.52	
	Depression	Invalid	524.89	68.32	−38.21	462.24	45.13	−61.96	459.82	79.76	−70.53
		Valid	563.11	74.93		524.20	70.44		530.36	88.45	
	Trauma	Invalid	521.67	69.33	−48.11	464.64	52.48	−57.68	456.22	69.16	−75.74
		Valid	569.78	79.52		522.32	74.43		531.96	101.34	
1200 ms	Neutral	Invalid	492.75	72.20	−10.69	439.99	59.03	−25.16	437.98	64.95	−34.75
		Valid	503.44	71.05		465.15	65.04		472.73	71.91	
	Neutral old	Invalid	497.81	73.32	−0.12	435.82	47.05	−27.46	447.33	68.71	−19.46
		Valid	497.93	57.28		463.28	45.92		466.79	63.38	
	Anxiety	Invalid	483.88	62.30	−29.38	434.49	47.64	−19.52	441.44	69.00	−20.99
		Valid	513.25	63.40		454.01	54.69		462.44	65.16	
	Depression	Invalid	489.36	66.05	−20.13	436.90	55.79	−27.44	439.13	65.83	−28.74
		Valid	509.48	78.04		464.34	56.26		467.87	61.96	
	Trauma	Invalid	491.07	69.83	−23.51	437.15	52.60	−26.13	444.12	62.56	−37.91
		Valid	514.58	61.91		463.27	47.85		482.03	78.08	

SOA, Stimulus onset asynchrony; IOR, Inhibition of return.

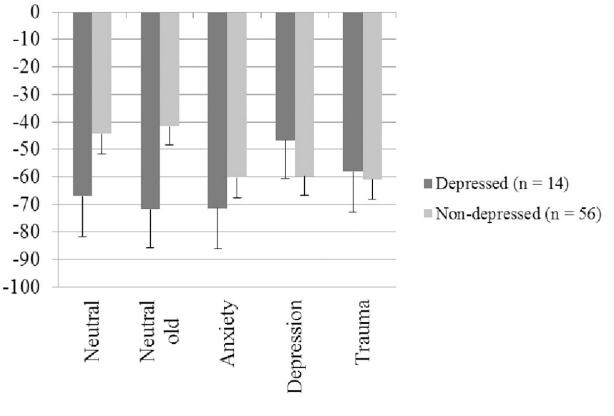

FIGURE 1 | IOR effects for short SOA (in ms, standard error) for each picture type. Negative values indicate an inhibitory of the cue (picture) on the target.

the reverse pattern emerged, that is, IOR effects for the neutral conditions were reduced compared to emotional conditions. For *post-hoc* conducted *t*-tests (short SOA), difference scores were calculated (i.e., IOR effect emotional condition - IOR effect neutral condition). For trauma-related cues, groups did not differ significantly, $t_{(68)} = 1.58$, $p = 0.118$, $d = 0.48$. However, for depression-related cues, the IOR effect was significantly reduced in depressed individuals, $t_{(68)} = 2.62$, $p = 0.011$, $d = 0.8$. Within group comparisons (depressed group) did not reveal a significant main effect of Cue Type, $F_{(4,\ 52)} < 1$, $p = 0.586$, $\eta_p^2 = 0.05$; however, numerically IOR effects were considerably smaller for depression-related compared to neutral cues ($M = -46.70$, $SE = 10.91$ vs. $M = -66.88$, $SE = 14.36$), $d = 0.42$.

Offspring

To examine whether children of individuals with PTSD show attentional biases for trauma-related stimuli, the mixed Four-Way ANOVA was repeated within the offspring generation (see **Table 4**). Cue Type did not influence RT, $F_{(4,\ 252)} = 1.07$, $p = 0.372$, $\eta_p^2 = 0.02$. However, RTs to long SOA ($M = 413.12$ ms, $SE = 8.23$ ms) were significantly faster than RTs to short SOA ($M = 455.89$ ms, $SE = 8.67$ ms), $F_{(1,\ 63)} = 192.45$, $p < 0.001$, $\eta_p^2 = 0.75$. Furthermore, the IOR effect occurred, $F_{(1,\ 63)} = 107.24$, $p < 0.001$, $\eta_p^2 = 0.63$, with shorter RTs to invalid ($M = 420.16$ ms, $SE = 8.54$ ms) than valid ($M = 448.85$ ms, $SE = 8.31$ ms) trials. Groups did not differ in their overall RT, $F_{(2,\ 63)} = 1.81$, $p = 0.171$, $\eta_p^2 = 0.05$. More critically, neither the Three-Way interaction of Cue Type × Validity × Group, $F_{(6.93,\ 219.35)} < 1$, $p = 0.520$, $\eta_p^2 = 0.03$, nor the Four-Way interaction of Cue Type × SOA × Validity × Group were significant, $F_{(7.67,\ 241.55)} < 1$, $p = 0.537$, $\eta_p^2 = 0.03$.

Relationship to psychopathology

For traumatized groups, there was a significant association between intrusions (assessed with the PDS) and IOR effects for anxiety-related ($r = 0.347$, $p = 0.016$) and depression-related cues ($r = 0.289$, $p = 0.046$) for short SOA. Furthermore, the IOR effect for anxiety-related cues and long SOA correlated with avoidance in the PDS ($r = -0.323$, $p = 0.025$). For the

Table 4 | Mean RTs (in ms), standard deviations and IOR effects for each combination of picture type, SOA, validity, and group (offspring).

SOA	Cue Type	Validity	Offspring PTBS($n = 21$)			Offspring non-PTBS($n = 24$)			Offspring non-Trauma($n = 21$)		
			M	*SD*	IOR	*M*	*SD*	IOR	*M*	*SD*	IOR
450 ms	Neutral	Invalid	462.48	114.38	−21.89	436.47	56.44	−35.79	416.29	48.21	−28.23
		Valid	484.37	78.15		472.26	55.04		444.52	58.25	
	Neutral old	Invalid	460.86	110.55	−19.17	439.33	63.09	−28.19	418.40	47.42	−24.50
		Valid	480.02	86.56		467.53	50.46		442.89	62.63	
	Anxiety	Invalid	469.68	106.88	−19.15	441.28	68.13	−33.75	421.91	54.76	−41.53
		Valid	488.82	94.57		475.03	55.97		463.44	66.04	
	Depression	Invalid	458.01	104.12	−40.57	444.36	57.00	−30.82	419.95	59.83	−29.70
		Valid	498.59	110.90		475.18	55.95		449.65	62.68	
	Trauma	Invalid	464.84	111.18	−21.95	435.84	66.16	−51.14	418.10	53.68	−34.67
		Valid	486.79	100.57		486.97	64.58		452.77	64.71	
1200 ms	Neutral	Invalid	422.49	103.65	−22.32	400.58	55.93	−37.81	377.56	44.93	−37.55
		Valid	444.81	84.31		438.39	70.44		415.11	65.21	
	Neutral old	Invalid	414.99	93.51	−28.97	402.51	63.78	−21.13	382.97	51.66	−20.85
		Valid	443.96	93.36		423.64	61.83		403.82	42.26	
	Anxiety	Invalid	424.80	94.99	−18.31	389.65	50.01	−33.86	381.90	53.53	−21.79
		Valid	443.11	94.60		423.51	59.90		403.68	52.68	
	Depression	Invalid	424.73	98.09	−18.07	394.97	58.37	−26.01	387.99	48.74	−17.50
		Valid	442.80	88.98		420.99	56.82		405.48	55.32	
	Trauma	Invalid	417.44	90.08	−34.99	392.85	56.72	−36.05	381.55	44.68	−24.31
		Valid	452.43	117.75		428.91	55.35		405.87	50.94	

SOA, Stimulus onset asynchrony; IOR, Inhibition of return.

Table 5 | Background variables (sociodemography and psychopathology) for the depressed and non-depressed group: means (standard deviations) or frequency.

Variable	Depressed ($n = 14$)	Non-depressed ($n = 56$)	Statistics
Former groups (PTSD/non-PTSD/non-Trauma)	11/3/0	11/23/22	
Age (in years)	73.07 (2.62)	73.16 (2.40)	$t_{(68)} < 1$, $p = 0.903$
Sex (female/male)	14/0	38/18	$\chi^2_{(1)} = 6.06$, $p = 0.014$
Verbal intelligence	114.71 (14.29)	117.86 (10.57)	$t_{(68)} < 1$, $p = 0.358$
HDRS	16.14 (4.44)	3.89 (3.42)	$t_{(68)} = 11.29$, $p < 0.001$
PDS	16.21 (5.47)	8.18 (5.93)	$t_{(46)} = 4.36$, $p < 0.001$

HDRS, Hamilton Depression Rating Scale; PDS, Posttraumatic Diagnostic Scale.

PTSD group, the association between intrusions and the IOR effect for anxiety-related cues and short SOA was even more pronounced ($r = 0.595$, $p = 0.004$). Interestingly, in the PTSD group avoidance in the PDS was correlated with an increased IOR effect for trauma-related cues (long SOA, $r = -0.522$, $p = 0.013$). Depression severity was not related to IOR effects for emotional cues, *ps* > 0.1. For offspring groups, depression did not correlate with any IOR effect, *ps* > 0.2.

DISCUSSION

SUMMARY OF THE MAIN RESULTS

The first aim of the present study was to differentially assess attentional bias components (i.e., facilitation, interference, avoidance) in older trauma survivors (with and without chronic PTSD) using a spatial-cueing paradigm with pictorial stimuli of varying emotional valence. Secondly, we wanted to investigate whether children of traumatized participants would exhibit attentional biases for trauma-related material and whether this effect was attributable to parental trauma vs. PTSD.

Parents

Traumatized participants with PTSD did not show attentional interference for trauma-related stimuli (i.e., no reduction of the IOR effect), nor did they react with attentional facilitation or avoidance. However, correlational analyses revealed that the magnitude of the IOR effect for anxiety- and depression-related cues and short SOA was influenced by symptom severity. Specifically, more intrusions were related to smaller IOR effects. In contrast, for long SOA, avoidance negatively correlated with IOR effects for anxiety-related cues in the traumatized groups, that is, more self-reported avoidance was associated with larger inhibitory effects. For the PTSD group, self-reported avoidance was related to larger IOR effects for trauma-related cues which is indicative of attentional avoidance. Interestingly, when groups were dichotomized based on current depression status, a reduced IOR effect emerged for depression-related cues in depressed compared to non-depressed individuals for short SOA (i.e., 450 ms). This finding speaks to impaired disengagement in depression and was specific to depression-related material.

Our results diverge from previous studies claiming impaired disengagement in PTSD (Pineles et al., 2007, 2009) and from studies administering an EST in which—despite all methodological limitations—attentional biases for trauma-related or threatening stimuli were rather consistently found. In the present study, we also administered an EST with different emotional word conditions (Trauma, Anxiety, Depression, Neutral) and found evidence for an attentional bias for trauma-related words (Wittekind et al., in preparation). Thus, although traumatized participants with PTSD showed some kind of attentional bias, it did not become apparent using a different paradigm and stimulus modality. Due to the discrepant findings, it seems unlikely that our null findings result from a lack of power or the overall low symptom severity. Rather, different paradigms and stimulus modalities might explain the equivocal evidence and the inconsistent findings highlight the necessity to replicate results across different paradigms and modalities, respectively, before firm conclusions can be drawn. In general, attentional biases in PTSD were more consistently found under conditions in which disorder-related stimuli are present during the task (interference tasks, e.g., EST and VST) and when verbal material is applied. Divergence of findings pinpoint that different paradigms cannot self-evidently be used interchangeable as they might capture different aspects of attention (e.g., cueing: spatial attention vs. EST: selective attention, e.g., Shalev and Algom, 2000). Results suggest that PTSD is related to deficits in selective (but not in spatial) attention, possibly due to deficits in attentional control and consequently an inability to inhibit the impact of emotional distracters (Derryberry and Reed, 2002; Bardeen and Orcutt, 2011).

Another interpretation of the null findings is that the IOR effect is unaffected by emotional cues in PTSD. This interpretation is in line with previous studies that investigated IOR effects using emotional cues and found no evidence for any effects of these cues on the magnitude of the IOR effect (Stoyanova et al., 2007; Lange et al., 2008). Whereas in the study of Lange et al. IOR effects were not related to symptom severity, we found a significant relation between severity of intrusive symptomatology and the magnitude of the IOR effect indicating that IOR effects are not totally unaffected by emotionality in traumatized participants.

Regarding stimulus modality, in the spatial-cueing paradigm we used pictorial instead of verbal stimuli. Our results converge with the findings of other studies (Elsesser et al., 2005; Hauschildt et al., 2013) in which trauma or PTSD, respectively, was not associated with attentional biases for pictorial trauma-related stimuli. Furthermore, in a meta-analysis on attentional biases in anxiety disorders, attentional biases in clinical participants were only found for words, but not for pictures (Bar-Haim et al., 2007). Although it has been assumed that visual stimuli might be more attention-grabbing (Moritz et al., 2008), an advantage of verbal stimuli is that they might capture a wider range of traumatic experiences (Pineles et al., 2009). For example, in the present sample participants experienced a wide range of traumatic experiences that can more easily be grasped by broader expressions such as *flight, hunger* or *loss* than by pictures of single events.

Our finding that depressed participants exhibited interference for depression-related stimuli is noteworthy as it replicates and extends previous studies that investigated attentional biases in depression. That depression is associated with difficulties to disengage from depression-related pictorial cues corroborates prior studies that showed attentional biases for sad facial expressions in acutely (Gotlib et al., 2004; Fritzsche et al., 2010; for a review see Bistricky et al., 2011) and formerly depressed individuals (e.g., Fritzsche et al., 2010). Whereas most of the forerunner studies administered variants of the dot-probe task, in the present study results could be replicated with a different paradigm. Interestingly, although the IOR effect was immune to emotionality in anxiety, it was reduced in depression suggesting that it doesn't represent a stable phenomenon *per se*. We can only speculate why this discrepancy occurred. One possibility is that whereas emotional facial expressions are more salient stimuli (e.g., Bradley et al., 1997; Bistricky et al., 2011), anxiety-related stimuli in this study did not contain biologically relevant information. Rather, trauma-related cues become associated with threat during the traumatic event but are not inherently dangerous (Ehlers and Clark, 2000). Difficulties to disengage from depression-related stimuli might constitute a risk and maintaining factor for depression as attention remains on mood-congruent stimuli and this in turn might potentiate processes such as rumination, negative thinking or a negative emotional state (e.g., Beck, 1967; Ingram, 1984).

The finding that reduced IOR effects were only found for short (i.e., 450 ms) but not long (i.e., 1250 ms) SOA is unexpected as attentional biases in depression are assumed to occur at later stages of information processing that need more time to take effect (i.e., strategic processing, see for example, Yiend, 2010). However, neuroscience studies provide evidence that biases might also affect automatic (early) processes (Suslow et al., 2010).

Offspring

Regarding our second question, there was no evidence for a transgenerational transmission of attentional biases, that is, offspring of traumatized participants (with PTSD) did not react differently to trauma-related cues (i.e., no reduced IOR effect). Our findings diverge from studies with Vietnam veterans in which children of veterans exhibited attentional biases for trauma-related material compared to children of non-veterans (Motta et al., 1994, 1997). However, our results are in line with a forerunner study in which children of displaced individuals (with and without PTSD) did not show attentional biases for trauma-related material in an EST (Wittekind et al., 2010). Furthermore, in an EST, which was also administered in the present study (Wittekind et al., in preparation), there was no evidence for attentional biases in children of traumatized participants. Comparability between studies is constrained by methodological differences, for example, age of children at assessment or attentional paradigm (EST vs. spatial-cueing). Beyond that, group differences in the studies of Motta et al. might be attributable to differences in personal relevance as PTSD (i.e., Vietnam)-related words might be more personally relevant for children of veterans compared to non-veterans and personal relevance is associated with longer color naming latencies (e.g., Williams et al., 1996). However, personal relevance was not controlled for, neither by obtaining ratings of the stimuli nor by the inclusion of traumatized parents with and without PTSD. Taken together, results of the present and previous studies argue for the conclusion that parental trauma or PTSD due to forced displacement is not related to attentional biases for trauma-related material in their children. These findings are in line with the broader literature on secondary traumatization which provides evidence that children of traumatized individuals are well adjusted (e.g., Van IJzendoorn et al., 2003; Fridman et al., 2011).

LIMITATIONS

Results of the present study need to be interpreted against the background of several limitations. First, as more than 65 years passed between initial traumatization and assessment, we cannot answer the question whether attentional biases had occurred earlier in time. Second, sample size was rather small making it more difficult to detect subtle differences. However, as traumatization dates back more than 65 years, many of the individuals affected might not be available for assessment as traumatization and PTSD in particular are associated with higher morbidity and mortality (Boscarino, 2006; Glaesmer et al., 2011). In consequence, only the more resilient individuals might been reached for assessment. Thus, the sample under investigation represents a specific population and it remains to be tested whether findings can be transferred to other trauma populations. Second, Mogg et al. (2008) argue that findings from spatial-cueing paradigms might represent a generic response slowing for threat-related stimuli rather than a "pure index of disengagement processes" (p. 665). However, this problem also applies to other attentional paradigms (e.g., Algom et al., 2004). Furthermore, as we applied a localization instead of a categorization task, it is conceivable that attentional effects were confounded by motor preparation effects. To circumvent these latter confounds, future studies should combine attentional paradigms with paradigms that allow the assessment of visuospatial attention allocation, for example eye-tracking. Third, the applied cut off for RTs represent a limitation as we did not apply standard cut off values, for example, two standard deviations. However, as our aim was to extend and replicate findings of Hauschildt et al. (2013), we decided to keep the same strategy of data analysis to provide better comparability. Fourth, although trauma-related pictures were on average rated as negative and personally relevant by participants with PTSD, it is still conceivable that depicted trauma-related events (e.g., refugee treks) were not experienced by all individuals as flight histories differed substantially among traumatized participants. Finally, within both the PTSD and the depressed group comorbidity with depression or PTDS, respectively, was the norm rather than exception. In consequence, it remains unresolved whether attentional biases in the depressed group were indeed attributable to depression or related to comorbidity. Studies are needed that compare pure depression- and anxiety samples with a mixed depression-anxiety sample.

CONCLUSIONS

Attentional biases for emotional visual cues were related to depression, not PTSD, in an older trauma sample (with and without PTSD). Specifically, depression was associated with attentional interference for depression-related stimuli. Results of the present study do not support the assumption that PTSD in older adults is associated with difficulties to disengage from pictorial trauma-related stimuli. Rather, it seems that attentional biases in PTSD are specific to verbal stimuli and that selective, but not spatial, attention is affected. Future studies should directly compare visual and verbal stimuli within one paradigm and assess both selective and spatial attention. Beyond that, future studies should assess whether information processing biases in older trauma survivors resemble the ones found for younger trauma samples, for example, by directly comparing acute and chronic PTSD samples. Furthermore, there was no evidence for a transgenerational transmission of biased information processing. However, as offspring of the current study was rather old, it would be interesting to investigate information processing biases in younger children of parents with PTSD. One promising means to treat attentional biases represent attentional bias modification paradigms (ABM-trainings, Browning et al., 2010; Hakamata et al., 2010).

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Preliminary evidence that different mechanisms underlie the anger superiority effect in children with and without Autism Spectrum Disorders

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Previous studies have demonstrated that angry faces capture humans' attention more rapidly than emotionally positive faces. This phenomenon is referred to as the anger superiority effect (ASE). Despite atypical emotional processing, adults and children with Autism Spectrum Disorders (ASD) have been reported to show ASE as well as typically developed (TD) individuals. So far, however, few studies have clarified whether or not the mechanisms underlying ASE are the same for both TD and ASD individuals. Here, we tested how TD and ASD children process schematic emotional faces during detection by employing a recognition task in combination with a face-in-the-crowd task. Results of the face-in-the-crowd task revealed the prevalence of ASE both in TD and ASD children. However, the results of the recognition task revealed group differences: In TD children, detection of angry faces required more configural face processing and disrupted the processing of local features. In ASD children, on the other hand, it required more feature-based processing rather than configural processing. Despite the small sample sizes, these findings provide preliminary evidence that children with ASD, in contrast to TD children, show quick detection of angry faces by extracting local features in faces.

Keywords: Autism Spectrum Disorders, anger superiority effect, children, face-in-the-crowd effect, visual search, emotion, facial expressions, attention

INTRODUCTION

The ability to detect threatening social stimuli quickly and modify our behaviors according to the context is beneficial for avoiding social conflict. Our visual system is, therefore, thought to have evolved to be more sensitive to threatening faces than to other facial expressions (Ohman and Soares, 1993; Ohman et al., 2001). Angry faces are universally treated as signals of potential threat. They are processed rapidly and efficiently, and are particularly efficient in capturing attention (Vuilleumier and Schwartz, 2001). This phenomenon is defined as the anger superiority effect (ASE). ASE has been studied using a visual search paradigm in which participants searched for discrepant angry or happy faces in a crowd of distractor faces (i.e., Face-in-the-crowd task; Hansen and Hansen, 1988; Horstmann and Bauland, 2006; Pinkham et al., 2010). Several studies have confirmed that ASE can also be observed with schematic-faces (Fox et al., 2000; Eastwood et al., 2001; Ohman et al., 2001; Horstmann, 2009). By using schematic faces it is possible to eliminate many low-level perceptual variations found in photographs of emotional expressions, and to better control experiment variables.

ASE has recently been tested in participants with Autism Spectrum Disorders (ASD) using the face-in-the-crowd paradigm in adults (Ashwin et al., 2006; Krysko and Rutherford, 2009), as well as children and adolescents (Rosset et al., 2011; Isomura et al., submitted). ASD are neurodevelopmental disorders characterized by social communicative difficulties and restricted behaviors and interests (American Psychiatric Association, 2013). Previous studies have reported that individuals with ASD show specific difficulties in social and emotional information processing (Dawson et al., 2005). In particular an atypical pattern of face processing has often been reported: while TD individuals tend to use a configural style for face processing (Tanaka and Farah, 1993), individuals with ASD have been shown to have difficulties in configural processing and to focus more on local features in faces (Behrmann et al., 2006). Also, recent studies revealed that individuals with ASD showed atypical emotional responses to faces, in which undifferentiated affective responses were observed to different facial emotions in event-related potentials (ERPs) responses (Wagner et al., 2013) as well as facial electromyography (EMG) activities (McIntosh et al., 2006; Beall et al., 2008; Rozga et al., 2013).

Contrary to their atypical cognitive processing and emotional responses to facial emotions, however, recent studies have revealed that ASE exists in most of the population with ASD as well as TD individuals (Ashwin et al., 2006; Krysko and Rutherford, 2009; Rosset et al., 2011; Isomura et al., submitted). Interestingly though, it has been consistently reported that ASE in ASD was not as robust as that in TD individuals. Individuals with ASD did not show the effect when a large number of distractor faces (crowd size) was presented. (Ashwin et al., 2006; Krysko and Rutherford, 2009; Isomura et al., submitted). In addition, Isomura et al. (submitted) found age differences in ASE only in ASD but not in TD. These findings suggest that individuals with ASD employed compensatory but less-effective mechanisms that might be learned/acquired in their development.

Previous studies using schematic face stimuli in TD individuals have suggested that ASE requires configural/holistic level of face-processing, because the effect was not seen when threatening single features were presented in isolation (Fox et al., 2000; Tipples et al., 2002; Weymar et al., 2011). There are, however, no studies on individuals with ASD that examined the cognitive mechanisms underlying ASE. Therefore, we aimed to directly examine the cognitive mechanisms underlying ASE in ASD in order to understand how individuals with ASD compensatorily develop/acquire the mechanisms to process social threat rapidly. Given the atypical pattern of face processing in individuals with ASD (Behrmann et al., 2006), they may extract facial information from local features, rather than using higher level configural processing in detecting emotional faces (Ashwin et al., 2006; Behrmann et al., 2006; Krysko and Rutherford, 2009).

In the current study, we examined whether ASD and TD participants employed a configural processing or a feature-based processing during a face-in-the-crowd task. Here we employed a recognition task in combination with the face-in-the-crowd task. A recent study revealed that humans' cognitive tendency toward configural processing of faces reduces their ability to recognize differences of local features (Wilford and Wells, 2010). In our study, therefore, we had expected that the cognitive pattern that participants employ during a face-in-the-crowd task would be reflected in their performance of the recognition task. We used whole faces, local features with outline of a face, and inverted faces for recognition. Inverted faces are well known to disrupt configural processing (Yin, 1969) while they include same volume of physical information as the whole (upright) faces. Thus, we had expected that participants would show poorer performance on recognition of both local features and inverted faces if they relied on the configural processing when searching.

Given that the previous study showed that children with ASD aged 9–10 years old started to show ASE (Isomura et al., submitted), we focused on children with an average age of about 10 years old in the current study. We hypothesized that TD children would show better performance in recognizing the whole face rather than local features or inverted faces based on previous studies (Tanaka and Farah, 1993; Wilford and Wells, 2010). In addition, TD children would show better performance in recognizing local features in *happy* faces then those in *angry* faces according to a previous study showing that negative facial expressions disrupt the processing of local features (Eastwood et al., 2008). In ASD children, on the other hand, we hypothesized that they would show similar performance in recognizing whole faces, local features, and inverted faces because individuals with ASD may focus on local features during the face-in-the-crowd task.

METHODS

ETHICS NOTE

This study was ethically reviewed by the institutional ethics committee of experiments for human participants prior to the study (permission number, #H2012-05). We adhered to the Declaration of Helsinki and the institutional guidelines for experiments with human participants.

PARTICIPANTS

Twenty children with ASD (16 male and 4 female) and 22 typically developing children (18 male and 4 female) participated in this study. The participants in the ASD group were diagnosed either with Pervasive Developmental Disorder (3 children), Autism Spectrum Disorder (9), Asperger's syndrome (5), High-functioning Autism (2), or Pervasive Developmental Disorder—Not Otherwise Specified (1) by child psychiatrists based on either DSM-IV or ICD10. Subjects have been participating in the Developmental Disorders and Support for Acquiring Reading and Writing Skills project at the Kokoro Research Center in Kyoto University. Children with no history of any psychiatric condition were recruited via the local community as a control group.

Intelligence Quotient (IQ) was measured using the Japanese version of the Wechsler Intelligence Scale for Children (either WISC-III or WISC-IV). Subjects' parents answered the Japanese version of the Autism Spectrum Quotient (AQ) (Wakabayashi et al., 2006). To be included in the ASD group, participants had to meet the criteria of AQ with a score more than 20, and to be included in the TD group, they had to meet the criteria of AQ with a score less than 20, according to the cut-off criteria established by Wakabayashi et al. (2006). Additionally, participants had to meet the criteria of IQ with a score of 70 or higher for both groups.

One individual in the ASD group and 4 individuals in the TD group were excluded from analysis because they did not meet the criteria of AQ. Consequently, 19 children (15 male and 4 female; 2 left-handed children) with ASD (mean age = 10.15; $SD = 1.09$; range = 8:6–12:2) and 18 TD children (14 male and 4 female; 2 left-handed children) (mean age = 10.03; $SD = 1.15$; range = 8:5–12:0) were included in analysis. Mean age, AQ scores, and IQ scores are listed in the left column of **Table 1**. Independent samples t-tests showed that the groups were matched for age [$t_{(35)} = -0.309$, $p = 0.759$], and Full scale IQ [$t_{(35)} = 0.740$, $p = 0.464$]. AQ scores showed a significant difference between groups [$t_{(35)} = -11.49$, $p < 0.001$]. The parents of all the participants gave written informed consent to participate in this study, which was conducted in accordance with the institutional ethics provisions.

APPARATUS

Visual stimuli were presented on a 15-inch touch-sensitive monitor with a resolution of 1024 by 768 pixels (Mitsubishi, RDT151TU or TSD-AT1515-CN), controlled by custom-written software under Visual Basic 2010 (Microsoft Corporation, Redmond, Washington, USA) running on a personal computer (HP Compaq 6730b/CT or Panasonic CF-SX2).

STIMULI

Warming-up trials

Each trial included the presentation of a self-start key, a fixation picture, and face stimuli. A light-blue-colored rectangle (179 (W) × 136 (H) pixels: 5.3 cm × 4.1 cm on screen (7.6° × 5.9° of visual angle) was used as the self-start key, which was presented at 1.5 cm (2.1° of visual angle) above the bottom of the screen. In the middle of the rectangle, a trial number was presented so that participants could know how many trials they had completed. Fixation pictures were presented at the center of the

Table 1 | Mean (SD; range) chronological age, IQ scores, and AQ scores from all participants (left column) and from the participants who were included in analysis in the recognition task (right column) for each ASD and TD group.

	All participants			Participants analyzed in the recognition task		
	TD	**ASD**	***t*-value; *p*-value**	**TD**	**ASD**	***t*-value; *p*-value**
Sex	Male = 14; Female = 4	Male = 15; Female = 4	–	Male = 11; Female = 3	Male = 8; Female = 2	–
Handedness	Left-handed = 2	Left-handed = 2	–	Left-handed = 2	Left-handed = 1	–
Age	10.03 (1.15)	10.15 (1.09)	$t_{(35)} = -0.309$	10.09 (1.30)	10.47 (1.10)	$t_{(22)} = -0.775$
	(8:5–12:0)	(8:6–12:2)	$p = 0.759$	(8:5–12:0)	(8:7–12:2)	$p = 0.447$
Full-scale	105.7 (13.37)	102.3 (15.06)	$t_{(35)} = 0.740$	103.3 (9.28)	103.4 (13.85)	$t_{(22)} = -0.023$
IQ	(89–148)	(73–124)	$p = 0.464$	(89–118)	(88–121)	$p = 0.982$
AQ	13.06 (3.33)	29.58 (5.16)	$t_{(35)} = -11.49$	12.5 (3.50)	28.0 (4.92)	$t_{(22)} = -8.53$
	(7–17)	(22–40)	$p < 0.001$	(7–17)	(22–35)	$p < 0.001$

screen and covering the whole stimulus area of faces. Twenty-four types of pictures of popular cartoon characters were used for the fixation pictures. The face stimuli were schematic pictures portraying angry, happy, and neutral facial expressions. They were created with reference to previous studies (Ashwin et al., 2006; Horstmann, 2007; Isomura et al., submitted). The faces were drawn in black against a white background. All lines in the face drawings were of 2 pixel width. The individual faces were 48 (W) by 54 (H) pixels (1.4 × 1.6 cm on the screen (2.0° × 2.3° of visual angle). Each emotion had two types of faces which were different in the angle of eyebrows and flatness of mouth (Each type was named Emotion-degree1, and Emotion-degree2, respectively) **Figures 1(A–E)**. The face stimuli were presented inside a stimulus area of 268 × 218 pixels (8.0 × 6.5 cm on the screen (11.4 × 9.3° of visual angle). The stimulus area was divided into 4 × 3 grids. We randomized positions of face stimuli for each trial. First we randomly chose a grid for each face stimulus and then altered its position within a grid in a range of ± 8 pixels from the center of the grid in both vertical and horizontal dimensions. This procedure resulted in a moderately irregular arrangement of the stimuli, intended to eliminate possible suprastimulus cues to the target's position (Duncan and Humphreys, 1989; Horstmann, 2007). An example of stimulus displays is shown in **Figure 1F**.

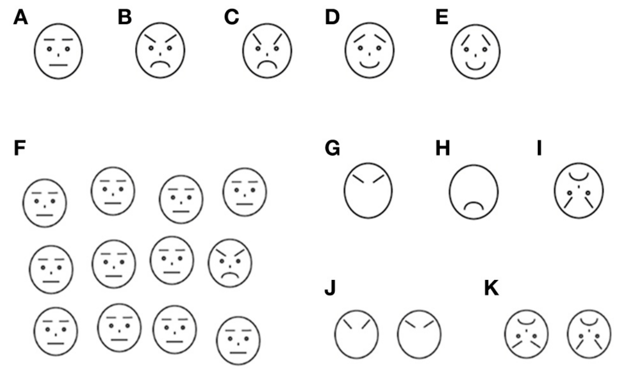

FIGURE 1 | Stimuli used in this study. (A) Neutral face **(B)** Angry face with Emotion-degree1 **(C)** Angry face with Emotion-degree2 **(D)** Happy face with Emotion-degree1 **(E)** Happy face with Emotion-degree2 **(F)** Examples of matrix in the search: one angry face with Emotion-degree1 and 11 distractor neutral faces **(G–I)** Examples of the stimuli used in the recognition task; **(G)** Eyebrows of angry face with Emotion-degree1 **(H)** Mouth of angry face with Emotion-degree2 **(I)** Inverted angry face with Emotion-degree2 **(J), (K)** Examples of the choices in the recognition task.

Search-Recognition task

In the main task, named the Search-Recognition task (see procedure for details), recognition task was added to the search task. Stimuli to be recognized were varied in whole faces (identical with the faces used in the search task), local features with outline of face, and inverted faces. Examples of the recognition stimuli were shown in **Figures 1G–I**.

PROCEDURE

Warming-up trials

First, participants were given 36 trials of a face-in-the-crowd task (i.e., visual search task). This was conducted to calculate individual's mean response time in detection of target faces which would be used in the subsequent Search-Recognition task. Participants were seated approximately 40 cm from the monitor with eye level at the center of the screen, and instructed to touch a discrepant object as quickly and accurately as possible. Each trial started when participants touched the self-start key, after which a fixation picture was presented for 500 ms to keep the children's attention on the screen, and then the face stimuli were displayed. Face stimuli consisted of one emotional face (target) and 11 neutral faces (distractors). The face stimuli were presented until a response was made. When the participants responded correctly, a high tone sounded and a cartoon picture which indicated a correct response was presented, whereas a low tone sounded and a cartoon picture which indicated an incorrect response was presented when they made an incorrect response. Emotion-type (Angry/Happy) and Emotion-degree (1/2) were varied with a pseudorandom sequence. Target position was also controlled by pseudorandom sequences. It took participants approximately 2–3 min to complete all trials.

Search-Recognition task

After the warming-up trials, participants were given 6 blocks of the Search-Recognition task. Each block consisted of 36 trials, 12 trials of which were test trials (search-recognition trials) and the rest of the trials were baseline trials (only search trials). In the test trials, the search task was immediately followed by a recognition task where the participants were additionally required to recognize the target face that they had detected in the preceded search task. The recognition task was given only when the participants made a correct choice in the search trial. In the recognition task, whole faces, local-features of faces (i.e., eyebrows or mouth), or inverted faces were presented randomly, and two Emotion-degrees of faces from the same emotion and same Recognition-type [whole faces, local-features of faces (i.e., eyebrows or mouth), or inverted faces] were given as choices. Examples of the display on the recognition trials are shown in **Figures 1J,K**. They were told that there was a time-limit during the search and thus solve the task as quickly as possible. The time-limit was, however, set only in the baseline trials, and it was not applied to test trials. The time-limit was set individually, with the time calculated by the mean response time in the warming-up trials multiplied by 1.25. If participants could not respond within a given time in the baseline trials, the trial was terminated and visual and auditory feedback which indicated time-out was given. Otherwise, the same visual and auditory feedback as the warming-up trials was given according to their response. The test trials were presented once in 3 trials in average to prevent participants from expecting the presentation of test trials. This less-frequent and random presentation of test trials and time-limit in the baseline trials was employed to avoid participants using the intentional strategy of spending more time to perform better in the recognition task. Some children took a rest between blocks, and in total it took 20–30 min for children to complete all trials.

DATA ANALYSIS

Participants' performance on the test trials in the Search-Recognition task was analyzed. Relative accuracy (percentage of correct response to all trials) and median response time on correct trials were calculated individually at each condition separately and used for statistical analysis. Because outliers do not affect the median value as strongly as mean, we did not exclude any values obtained from each participant. All statistical analysis was performed using SPSS 22 (IBM Japan, Ltd).

RESULTS

First, we analyzed their performance on the search task that preceded the recognition task. As our tasks were designed to produce no or very low numbers of errors, the response times were used for analyses (Results of accuracy were shown in Figure S1). We conducted a general linear model (GLM) repeated measures on the response times with three factors: Emotion-type (Angry vs. Happy), Emotion-degree (Degree1 vs. Degree2), and Group (TD vs. ASD). The results revealed that there was a main effect of Emotion-type [$F_{(1,\,35)} = 26.80$, $p < 0.001$, $\eta_p^2 = 0.434$], a main effect of Emotion-degree [$F_{(1,\,35)} = 40.10$, $p < 0.001$, $\eta_p^2 = 0.534$], and an interaction between Emotion-type and Emotion-degree [$F_{(1,\,35)} = 9.35$, $p = 0.004$, $\eta_p^2 = 0.211$]. Neither main effect of Group [$F_{(1,\,35)} = 2.78$, $p = 0.105$, $\eta_p^2 = 0.074$] nor interactions involving Group [Group × Emotion-type: $F_{(1,\,35)} = 3.49$, $p = 0.070$, $\eta_p^2 = 0.091$; Group × Emotion-degree: $F_{(1,\,35)} = 0.114$, $p = 0.737$, $\eta_p^2 = 0.003$; Group × Emotion-type × Emotion-degree: $F_{(1,\,35)} = 3.63$, $p = 0.065$, $\eta_p^2 = 0.094$] reached statistical significance, but trends of group difference was found in interaction with Emotion-type, as well as with the other two factors. Subsequent analysis (Bonferroni correction) for the interaction between Emotion-type and Emotion-degree revealed that angry faces were detected more quickly than happy faces both in the faces of Emotion-degree1 (i.e., less exaggerated), $F_{(1,\,35)} = 24.82$, $p < 0.001$, $\eta_p^2 = 0.415$, and in faces of Emotion-degree2 (i.e., more exaggerated), $F_{(1,\,35)} = 8.85$, $p = 0.005$, $\eta_p^2 = 0.202$. This indicated that ASE existed in children of both groups, but it was less significant when the faces included more exaggerated features (**Figure 2**). These results would be explained by exaggerated emotional faces being physically and emotionally more salient among neutral faces compared to the less exaggerated ones, and that it resulted in showing some floor effect on response times for the detection of exaggerated angry faces and exaggerated happy faces. However, the robust phenomenon of faster detection of angry faces than happy faces (i.e., ASE) was observed both in TD and ASD.

Next, we focused on participants' performance in the recognition task that followed the search task to examine their cognitive pattern employed during the search. In analyzing this, participants who could not perform better than expected by chance (a binomial test with significance level of 0.1) in both whole recognition and in features recognition, which were our main focus, were excluded from further analyses because we could not confirm that they understood the task requirement properly. Consequently, 4 individuals from the TD group and 9 individuals from the ASD group were excluded from the subsequent analysis (Mean accuracy and mean response time at each condition from all participants are shown in Figure S2). Information from the participants who were included in this analysis was listed in the right column of **Table 1**. Participants' performance (mean values, SDs, and 95%CIs for accuracy and response times in each group) was described in **Table 2**. We conducted a GLM analysis with repeated measures on the accuracy data with Recognition-type (Whole, Features vs. Inverted), Emotion (Angry vs. Happy), as the within-subjects

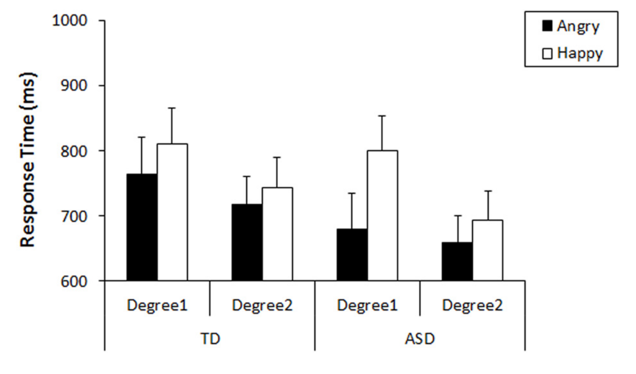

FIGURE 2 | Mean response times in the detection of angry/happy targets with each Emotion-degree in the search. Error bars: 95% CI.

Table 2 | Accuracy and response times (means, standard deviations, and 95% CIs) for each group at each condition in recognition.

		Angry									Happy								
		Whole			Features			Inverted			Whole			Features			Inverted		
		Mean	**SD**	**95%CI**	**Mean**	**SD**	**95%CI**	**Mean**	**SD**	**95%CI**	**Mean**	**SD**	**95%CI**	**Mean**	**SD**	**95%CI**	**Mean**	**SD**	**95%CI**
TD	Accuracy	68.9%	3.9%	60.9% ~77%	65.8%	2.7%	60.2% ~71.5%	63.7%	4.4%	54.5% ~72.9%	58.4%	4.5%	49.0% ~67.8%	68.0%	3.4%	61.0% ~74.9%	64.3%	4.8%	54.4% ~74.2%
	Response Time (ms)	910.1	53.6	799.0 ~1021.3	1158.3	78.5	995.4 ~1321.1	1039.4	47.5	941.0 ~1137.9	1006.2	54.7	892.8 ~1119.7	1090.6	77.3	930.3 ~1250.8	1071.1	57.4	952.1 ~1190.0
ASD	Accuracy	70.8%	4.6%	61.3% ~80.2%	62.6%	3.2%	55.9% ~69.3%	64.3%	5.3%	53.4% ~75.2%	66.2%	5.4%	55.0% ~77.3%	66.0%	4.0%	57.8% ~74.2%	63.6%	5.6%	51.8% ~75.3%
	Response Time (ms)	879.6	63.4	748.0 ~1011.1	920.4	92.9	727.7 ~1113.0	947.3	56.2	830.8 ~1063.7	878.3	64.7	744.0 ~1012.5	981.3	91.4	791.7 ~1170.9	969.0	67.9	828.2 ~1109.7

factor, and Group (ASD vs. TD) as the between-subjects factor. The results revealed neither main effects nor significant interactions [Recognition-type: $F_{(2,\ 44)} = 0.321, p = 0.727, \eta_p^2 = 0.014$; Emotion: $F_{(1,\ 22)} = 0.398$, $p = 0.535$, $\eta_p^2 = 0.018$; Group: $F_{(1,\ 22)} = 0.037$, $p = 0.849$, $\eta_p^2 = 0.002$; Recognition-type × Emotion: $F_{(2,\ 44)} = 2.35$, $p = 0.108$, $\eta_p^2 = 0.096$; Recognition-type × Group: $F_{(2,\ 44)} = 0.929, p = 0.403, \eta_p^2 = 0.040$; Emotion × Group: $F_{(1,\ 22)} = 0.141$, $p = 0.711$, $\eta_p^2 = 0.006$; Recognition-type × Emotion × Group: $F_{(2,\ 44)} = 0.281$, $p = 0.756$, $\eta_p^2 = 0.013$] (**Figure 3A**). We further conducted a GLM analysis with repeated measures on response times with the same three factors above: Recognition-type, Emotion, and Group. The results revealed a main effect of Recognition-type [$F_{(2,\ 44)} = 9.701$, $p < 0.001$, $\eta_p^2 = 0.306$], and a three-way interaction among all factors [$F_{(2,\ 44)} = 3.94$, $p = 0.027$, $\eta_p^2 = 0.152$]. Other factors did not show any statistically significant effect [Emotion: $F_{(2,\ 44)} = 1.59$, $p = 0.215$, $\eta_p^2 = 0.068$; Group: $F_{(1,\ 22)} = 1.83$, $p = 0.190$, $\eta_p^2 = 0.077$; Recognition-type × Group: $F_{(2,\ 44)} = 1.59, p = 0.215, \eta_p^2 = 0.068$; Emotion × Group: $F_{(1,\ 22)} = 0.032$, $p = 0.860$, $\eta_p^2 = 0.001$; Recognition-type × Emotion: $F_{(2,\ 44)} = 0.791, p = 0.460, \eta_p^2 = 0.035$]. Subsequent analysis for three-way interaction showed that there was a significant simple interaction between Group and Recognition-type when the Emotion was Angry [$F_{(2,\ 44)} = 4.61$, $p = 0.015$, $\eta_p^2 = 0.173$], but no simple interaction was found when the Emotion was Happy [$F_{(2,\ 44)} = 0.076, p = 0.927, \eta_p^2 = 0.003$]. This indicated that the Group difference was observed only for recognition of angry faces, but not for recognition of happy faces. Further analysis in the recognition of angry faces revealed a simple simple main effect of the Recognition-type in TD [$F_{(2,\ 26)} = 13.03$, $p < 0.001$, $\eta_p^2 = 0.501$], but not in ASD [$F_{(2,\ 18)} = 1.04, p = 0.373, \eta_p^2 = 0.104$]. Subsequent multiple comparisons (Bonferroni correction) in the TD group revealed that recognition of whole faces showed shorter response time than recognition of inverted faces or recognition of local features ($p = 0.001$, $p = 0.002$, respectively), but there was no significant difference on the response times between the recognition of inverted faces and the recognition of local features ($p = 0.183$) (**Figure 3B**). These results indicated that TD children showed better performance in the recognition of angry whole faces than in the recognition of local features in angry faces or angry inverted faces, whereas ASD children showed similar performance among them. Even though the sample size may not be sufficient to clearly reveal the group differences, consistent tendency was observed in the most of individuals within each group. The comparison between response times on recognition of angry whole faces and recognition of local features in angry faces are shown in **Figure 3C**.

In addition, the results of ANOVA showed another simple interaction between Recognition-type and Emotion [$F_{(2,\ 26)} = 4.05$, $p = 0.030$, $\eta_p^2 = 0.237$] in TD. In ASD, no simple interaction was found [$F_{(2,\ 18)} = 0.763, p = 0.481, \eta_p^2 = 0.078$]. Further analysis (Bonferroni correction) in the TD group showed that the recognition of angry whole faces showed faster response time than the recognition of happy whole faces [$F_{(1,\ 13)} = 5.01, p = 0.043$]. On the other hand, the recognition of local features in angry faces

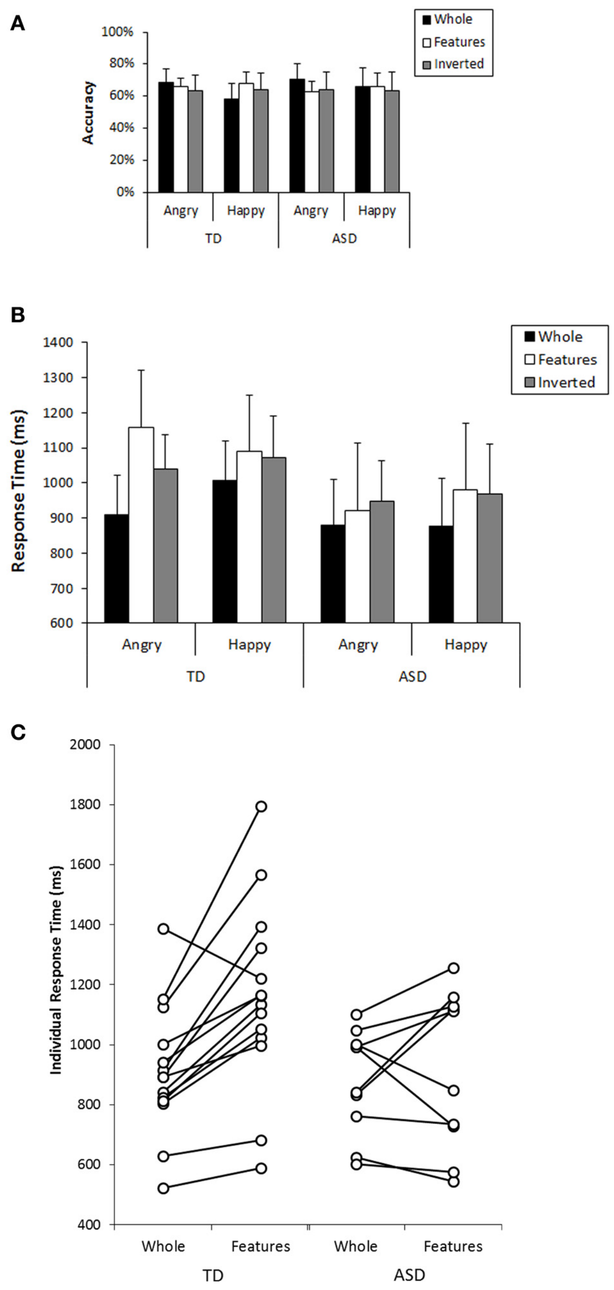

FIGURE 3 | (A) Mean accuracy in the recognition task for each condition in TD and ASD group. Error bars: 95% CI. **(B)** Mean response times in the recognition task for each condition in TD and ASD group. Error bars: 95% CI. **(C)** Comparison in the response times between recognition of *angry whole faces* and recognition of *local features in angry faces* in individual subjects in TD and ASD.

showed marginally significant longer response time than that in happy faces [$F_{(1,\ 13)} = 3.21$, $p = 0.097$] (**Figure 3B**). These results indicated that TD children showed better performance in recognizing angry faces than in recognizing happy faces when they were presented as whole faces, but the opposite tendency was observed when they were presented as local features. In ASD, such tendency was not observed.

DISCUSSION

The current study revealed that ASE exists in individuals with ASD as well as the TD individuals, consistent with previous studies (Ashwin et al., 2006; Krysko and Rutherford, 2009; Rosset et al., 2011; Isomura et al., submitted). More importantly, we obtained evidence that different mechanisms may underlie ASE between ASD children and TD children. The results of the recognition task revealed that TD children and ASD children processed particularly angry faces in different manners. TD children took more time to recognize local features in angry faces and angry inverted faces than to recognize angry whole faces. Furthermore, shorter time was required for the recognition of angry whole faces than in the recognition of happy whole faces, on the other hand, longer time was required in the recognition of local features in angry faces than in happy faces. These results suggest that detection of angry faces required more configural face processing and disrupted the processing of local features in TD children, as we had hypothesized. This is consistent with previous studies that revealed negative facial expressions capture attention and disrupt the processing of local features (Eastwood et al., 2003, 2008). In ASD, however, they showed similar response times among the recognition of whole faces, local features and inverted faces in angry faces. This suggests that detection of angry faces in ASD was processed in a feature-based manner rather than configural processing. Although the sample sizes were small, our results provide the preliminary evidence that they may, in contrast to TD children, extract facial information from local features, but still showed rapid processing of angry faces over happy faces similarly to TD children. This suggested the possibility that local features in angry faces by themselves may work as emotion-evoking stimuli that elicit rapid processing for children with ASD, in contrast to TD individuals where rapid processing of angry faces does not emerge from single feature detection (Fox et al., 2000; Tipples et al., 2002; Weymar et al., 2011).

Previous studies on facial emotion recognition have reported that individuals with ASD use local, feature-based processing, in contrast to the global, configural-based strategy used by TD individuals (Tantam et al., 1989; Behrmann et al., 2006; Harms et al., 2010). Furthermore, some evidence suggests that individuals with ASD may interpret emotional faces by memorizing the specific features associated with each emotion (i.e., rule-based strategy; Rutherford and McIntosh, 2007; Harms et al., 2010). The results in the current study revealed that the feature-based processing in ASD caused faster detection of angry faces over happy faces, even if they compensatorily learn how to interpret emotional faces. Taken together with the previous finding that revealed the age-related acquisition of ASE in individuals with ASD (Isomura et al., submitted), we propose the following hypothesis on mechanisms behind ASE in individuals with ASD. Individuals with ASD may not show innate mechanisms to orient toward angry faces rapidly, because they failed to treat angry faces as threatening stimuli. However, as they compensatorily learn the way to interpret facial emotions and become able to connect angry facial expressions to threat, they may start to show proper emotional responses that were observed in ASE. Further studies are required to examine this possibility.

Finally, several limitations of our study should be acknowledged. First of all, because of the small sample sizes, it would be still early to draw definitive conclusion, especially on the processing style in children with ASD. However, the findings in the current study are in line with previous studies that have suggested feature-based face processing in ASD (Behrmann et al., 2006), and that have reported the less robust effect of the anger superiority, suggesting different processing mechanisms underlie in individuals with ASD (Ashwin et al., 2006; Krysko and Rutherford, 2009). We believe that our exploratory results here have paved a road for future investigations with larger sample sizes. Physiological measurements in addition to behavioral measures would provide more in-depth insight. Second, in the current study, we used schematic faces as stimuli to control low-level perceptual variations. At the same time, however, schematic faces reduced ecological validity. Especially for people with ASD, ecological validity is important because they may develop and apply rules to schematic face stimuli to compensate for their difficulties with emotional detection (Rutherford and McIntosh, 2007). Also, the use of schematic stimuli may have facilitated children with ASD to focus on local features. To confirm the results of the current study, and to examine whether there are differences from the results obtained here using schematic stimuli, we should examine the effect in children with and without ASD using photographic faces. Third, in the current study, we could not examine sex differences because of the small sample size of female participants. As a previous study reported attentional bias toward facial emotions to be different between male and female (Tran et al., 2013), we need to examine the effect of sex in future studies. Moreover, we have only included participants who have normal-range intelligence. To better understand the ASD population as a whole, it is necessary to examine ASE in lower-functioning ASD, which may provide important cues for identifying subtypes of ASD.

CONCLUSIONS

This study demonstrated that the detection of angry faces required more configural face processing and disrupted the processing of local features than the detection of happy faces in TD children, according to the response times in the recognition of faces. In ASD children, on the other hand, the detection of angry faces required feature-based processing rather than configural processing. Despite the small sample sizes, these findings provide the preliminary evidence that different mechanisms underlie both TD and ASD children though they similarly showed faster detection of angry faces over happy faces. In contrast to TD children, children with ASD may extract emotional information from local features in angry faces (i.e., v-shaped eyebrows and downward mouth) and showed the proper emotional response of detecting angry faces over happy faces.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fpsyg.2014.00461/abstract

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Using eye tracking to test for individual differences in attention to attractive faces

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We assessed individual differences in visual attention toward faces in relation to their attractiveness via saccadic reaction times. Motivated by the aim to understand individual differences in attention to faces, we tested three hypotheses: (a) Attractive faces hold or capture attention more effectively than less attractive faces; (b) men show a stronger bias toward attractive opposite-sex faces than women; and (c) blue-eyed men show a stronger bias toward blue-eyed than brown-eyed feminine faces. The latter test was included because prior research suggested a high effect size. Our data supported hypotheses (a) and (b) but not (c). By conducting separate tests for disengagement of attention and attention capture, we found that individual differences exist at distinct stages of attentional processing but these differences are of varying robustness and importance. In our conclusion, we also advocate the use of linear mixed effects models as the most appropriate statistical approach for studying inter-individual differences in visual attention with naturalistic stimuli.

Keywords: attention, faces, gender, eye color, attractiveness, gap effect, dot probe, linear mixed effects models

INTRODUCTION

At all times, humans are capable of processing only a limited amount of their visual environment. This selectivity is called visual attention and because of its widespread involvement in cognitive tasks, ranging from reading and communication to scene perception and navigation, experimental psychology has aimed to understand the principles governing attention. Although many models of visual attention have been advocated, many open questions remain. One open question concerns the origins of inter-individual differences in visual attention. Here, we tested whether principles suggested by evolutionary psychology explain some of the inter-individual differences in attention.

Previous research has demonstrated that human faces are among the most interesting stimuli for visual attention (e.g., Bindemann et al., 2005; Ro et al., 2007; Langton et al., 2008; Thoma and Lavie, 2013). However, the degree to which a particular face receives attention might differ between individuals. First, evolutionary psychology suggests that certain phenotypical features in faces are perceived as particularly attractive (because they signal health or reproductive quality; Fink and Penton-Voak, 2002; Rhodes, 2006) but the relevance of such visual cues differs between individuals according to their sexes (Buss, 2003). For example, one study has shown that blue-eyed males find blue-eyed females particularly attractive and are more likely to choose them as partners while a comparably specific preference was not observed with blue- or brown-eyed women, or brown-eyed men (Laeng et al., 2007). According to an evolutionary explanation, this could be due to the recessive inheritance of genes for blue-eyes: when both partners have blue eyes, the common offspring will have blue eyes as well and this might serve as an additional assurance of paternity for the blue-eyed male. Second, research has demonstrated that humans spend more time looking at faces that are considered attractive than at less attractive faces (e.g., Aharon et al., 2001; Shimojo et al., 2003; Sui and Liu, 2009; Leder et al., 2010; Chen et al., 2012). However, these effects are not the same in all individuals: compared to women, men exhibit a higher motivation to view attractive opposite-sex faces (Levy et al., 2008; Hahn et al., 2013) and are more likely to show attentional biases toward attractive opposite-sex stimuli (Maner et al., 2003, 2007).

The two observations mentioned above were the point of departure for our study. We aimed at addressing the following open questions in this area. First, it is not known which kind of attentional sub-process differs between individuals when they are confronted with faces of varying attractiveness. For example, Leder et al. (2010) have embedded highly and less attractive faces in photographs of natural scenes and reported increased looking times at the attractive faces. Similarly, Maner et al. (2003) recorded participants' eye movements while looking at displays containing four highly attractive and four less attractive faces that were either male or female. They found that male participants looked significantly longer at attractive female faces than at less attractive female faces. This bias was not found with male faces. In contrast, women looked longer at highly attractive female as well as male faces. However, none of the published studies provides conclusive evidence whether these results are due to more robust capture of attention by the highly attractive faces, or to the stronger holding of attention once it has been captured. Hence, in the present study we disentangle these processes by directly measuring the participants' ability to disengage attention from attractive versus less attractive faces in Experiment 1, as well as the relative potential of different classes of faces for attentional capture in Experiment 2. Second, previous research has remained inconclusive about whether individual preferences for particular face features, as measured by attractiveness ratings, result in equivalent inter-individual differences in attention measures. Hence, we compared our measures of disengagement and capture of attention to attractiveness ratings collected from the same participants. We focused our investigation on the variables of gender and eye color because prior research suggested a high statistical power of these effects. To that end, we tested whether interactions between participant's sex and eye color and the respective facial characteristics co-determine any of the two attentional sub-processes in response to faces.

In the current study, we also aimed to establish new statistical benchmarks for conclusions on attentional processes in response to faces. Our review of the literature showed that all prior studies in this area based their conclusions on conventional statistics. However, these methods are not optimally suited for studying inter-individual attentional differences as a function of naturalistic stimuli, such as faces. Faces vary on a variety of unknown characteristics ranging from low-level features, such as lightness, and feature combinations, such as lip versus eye curvature, to holistic characteristics (e.g., the overall facial silhouette, the eyes' distance, and the proportional size of the nose). Because some of these features might not even be known, it is almost impossible to control for all of them. However, one statistical approach is particularly suited for studying inter-individual differences with such less controlled stimuli, namely linear mixed effects models (LMMs). This approach allows incorporating the variance explained by particular stimuli (here: faces) into a model (here: of predicting looking behavior through participant sex and/or eye color; cf. Baayen et al., 2008; Baayen and Milin, 2010; Kliegl et al., 2010). Our study should therefore also be regarded as an appeal for the use of LMM approaches when naturalistic stimuli are used to test for individual differences in attentional processing.

EXPERIMENT 1: DISENGAGEMENT OF ATTENTION FROM FACES

To collect a direct measure of attentional disengagement, we employed a *gap saccade task* (Saslow, 1967) in which participants are instructed to make a saccade away from a centrally fixated stimulus to a second stimulus that appears in the visual periphery. The main manipulation concerns the centrally fixated stimulus, which is either extinguished (typically) 200 ms prior to the onset of the peripheral stimulus (in 'gap' trials), or remains visible until after the onset of the peripheral stimulus (in 'overlap' trials). Here, the often-replicated gap effect consists in saccades to peripheral stimuli having shorter latencies or saccadic reaction times (SRTs) in gap than overlap trials. Although the effect depends on properties of the oculomotor system (Dorris and Munoz, 1995; Walker et al., 1995) recent evidence corroborated a causal role of attention in the gap effect (Pratt et al., 2006; Jin and Reeves, 2009).

We adapted the gap-saccade task to directly measure the influence of (a) overall facial attractiveness, (b) face gender, and (c) face eye color on disengagement of attention from a centrally presented face image. Our participants were required to disengage their attention from a fixated face and make a saccade to an abruptly appearing peripheral dot target. Consistent with the classical task, the face was either switched off 200 ms prior to the onset of the peripheral target (in gap trials) or remained visible until after the onset of the peripheral target (in overlap trials). In addition to the gap effect we predicted that attractive faces would delay disengagement more effectively than less attractive faces, resulting in higher SRTs. Motivated by the research outlined in the Introduction, we also tested for an interaction between participant sex and face gender, henceforth referred to as gender interaction (GI) as well as the more complex interaction between participant sex and eye color and face gender and eye color, henceforth named eye color and gender interaction (EGI).

METHODS

Participants

Forty participants with a mean age of 24 years ($SD = 3.7$) were assigned to four groups of ten that resulted from crossing the variables participant eye color (blue/brown) and sex (male/female). We chose a group sample size of ten based on a previous study (Laeng et al., 2007) which reported an effect size of Cohen's $d = 1.11$ for the critical difference in the group of blue-eyed men using a sample size of 22 participants in a rating study. Assuming an effect of the same size in the present population, a group sample size of 10 would imply a statistical power of $(1 - \beta) = 0.84$ to reveal this difference in a two-tailed test (for $\beta/\alpha = 1$ in a compromise analysis as implemented in Faul et al., 2007). Here and in Experiment 2, participants were undergraduate Psychology students, recruited at University of Vienna that participated voluntarily (in exchange for partial course credit). Upon arrival in the lab, they were inspected for their eye color. Participants with eye colors not clearly recognizable as blue or brown were assigned to a different experiment unrelated to the present study. All participants had normal or corrected-to-normal visual acuity and intact color vision. All participants were Caucasian and naïve with respect to the research hypotheses. The experiment was conducted in accordance with the Declaration of Helsinki and APA ethical standards in the conduct of research. Written informed consent was obtained from all participants.

Face stimuli

Two different sets ('natural faces' vs. 'morphed faces') of feminine and masculine faces with blue or brown eyes served as stimuli. Each set comprised 24 face images with six different faces per eye color and gender combination. Both sets were derived from the same source photographs. The source photographs were full frontal face portraits of male and female Caucasian adults aged 20–30 years with neutral expressions. For clarity, we henceforth refer with the words 'male,' 'female,' and 'sex' to our study participants and with the words 'masculine,' 'feminine,' and 'gender' to the face images. Photographs were taken under constant lighting conditions, exposure settings, shooting distance, and viewing angle. The digital camera (Canon EOS 550D with a Canon 50 mm f/1.8 lens) was calibrated using a ColorChecker Passport (X-Rite Inc., Grand Rapids, MI, USA) color standard. Exposure settings were set to f/20, 1/20 s, ISO 100 and lighting was delivered by two Bowens

GM500 Digital (Bowens International Ltd., Essex, UK) flashes with softboxes mounted on tripods. The set of morphed faces was created using FantaMorph 5.0 (Abrosoft Co., Beijing, China) by averaging three different (either only masculine or only feminine) source photographs to one masculine or feminine face. None of the source photographs was used in more than one morphed face.

All natural and morphed faces used for the present study were selected from a larger pool of candidate stimuli that underwent pretests for attractiveness ratings on a 7-point scale (1 = ‘very unattractive’; 7 = ‘very attractive’) by an independent sample of participants from the same undergraduate student population ($n = 60$ for natural; $n = 24$ for morphed faces). In line with previous literature (e.g., Langlois and Roggman, 1990), average rated attractiveness of morphed faces ($M = 4.07$, $SD = 0.78$) was significantly higher than of natural faces [$M = 3.57$, $SD = 0.94$, $t(135.7) = 3.47$, $p < 0.001$]. Because of a higher variance in attractiveness judgments in natural faces than in morphed faces, the final set of natural faces comprised a broader variation in (pre-rated) attractiveness but approximately equal numbers of attractive and less attractive faces.

Each face stimulus was presented in two variations (which differed only by eye color) to the same participants. All face stimuli were processed in Adobe Camera RAW and Adobe Photoshop CS5 (Adobe, Inc.) to standardize their appearance and to exclude possibly confounded influences on perceived attractiveness and/or attention. For that, a standardized mask was placed on hair, clothes, and background regions of the images and they were presented on a 50% gray background in the experiment (see **Figure 1**). The irises within all faces were retouched by inserting a standardized blue or brown iris with constant pupil size and iris color.

Apparatus

The experiment was programmed in MATLAB (MathWorks, Natick, MA, USA) using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) and the Eyelink toolbox (Cornelissen et al., 2002) running on a personal computer under Windows XP (Microsoft, Inc.). Stimuli were displayed on a 19-in. color CRT monitor (Sony Multiscan G400) with the screen resolution set to 1,280 × 1,024 pixels at a vertical refresh rate of 85 Hz and 32 bit color depth. The monitor was calibrated using an i1Pro spectrophotometer (X-Rite Inc., Grand Rapids, MI, USA). Viewing distance was held constant at 64 cm with chin and forehead rests. Gaze data were recorded monocularly using an EyeLink 1000 Desktop Mount (SR Research Ltd., Kanata, ON, Canada) video-based eye tracker sampling at 1000 Hz. Prior to the start of the experiment, the system was calibrated on the participants' dominant eye using a standard 5-point calibration sequence. In the course of the experiment, a manual drift check was conducted prior to every 12th trial. If the recorded gaze position differed by more than 1° from a central fixation target, recalibrations were performed (other trials started automatically after the participant had fixated on the central target for 1.5 s).

Experimental procedure

Participants were informed that the purpose of the experiment was to study the effect of human faces on visual attention and were given basic task instructions (i.e., ‘fixate on the face until a dot appears; as soon as you see a dot, look at it as quickly as possible’). They were not informed about the specific hypotheses and experimental manipulations. The experiment comprised 384 trials in randomized order which were presented in four blocks of 96 trials (between blocks, participants were allowed to rest briefly). Morphed and natural faces were presented randomly intermixed across all trials. The sequence within a trial is depicted in **Figure 2**. Each trial started with a central fixation (or drift check). Next, a face image (2.7 × 3.2°) was presented at screen center together with four equidistant dark gray circular placeholders marking the possible target locations. Placeholders had diameters of 1.7°, line strengths of 0.1°, and were placed above, below, left, and right, all at center-to-center distances of 6.8° from the face. In every trial, a black circular target dot with a diameter of 0.85° appeared 1 s after the onset of the face, randomly in one of the four placeholders.

FIGURE 1 | Morphed faces served as stimuli in Experiments 1 and 2. Each face was a composite produced by averaging three natural faces using image processing software. There were two versions of each face, one with blue eyes and one with brown eyes (only one version is depicted). Natural faces (which were the basis for the morphs but also served as a separate set of stimuli in Experiment 1) are not depicted due to privacy reasons.

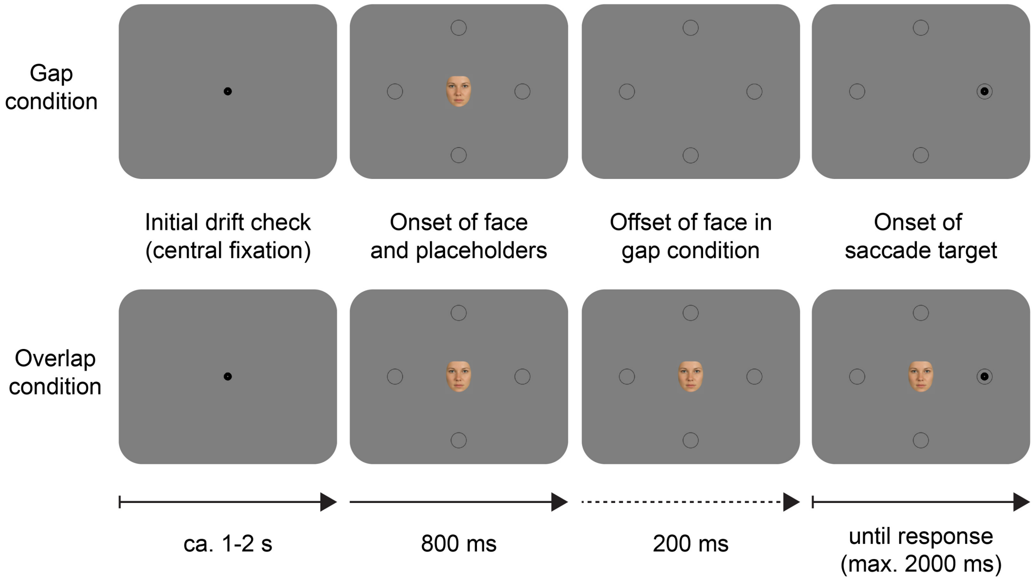

FIGURE 2 | Example schematic trials in the gap saccade task used in Experiment 1. The saccade target appeared randomly in one of the four placeholders around the central face stimulus. In the gap condition, the face disappeared 200 ms prior to the onset of the saccade target whereas in the overlap condition the face remained on screen until the end of the trial. Arrows indicate the flow of time.

Participants executed a saccade away from the face to the peripheral target and the trial ended automatically once a target fixation was registered (fixations were detected online in an invisible square region of 2.5 × 2.5° around the target). At the start of each experimental session, participants completed 16 training trials. Training trials were identical to experimental trials except that participants were given positive feedback, whenever their saccade was correct (‘okay,’ displayed at screen center) and negative feedback if they aborted fixation prior to target onset (‘too fast’) or if no target fixation was registered within 2 s after target onset (‘timeout’). In the experimental blocks only negative feedback was provided, and these trials were automatically repeated at the end of the block.

Rating procedure

After completing the four experimental blocks, participants were presented a final rating block, in which each face was shown one by one in randomized order at screen center (just as in the experimental procedure) together with a 7-point rating scale (ranging from 1 = ‘very unattractive’ to 7 = ‘very attractive’). Participants judged the attractiveness of each face by pressing the according number button on a standard PC keyboard. The rating task here (and in Experiment 2) was self-paced and participants could freely choose how long they wanted to view each face before giving their rating. In total, a complete run (including setup, experiment, rating, and participant debriefing) lasted about 70 min.

Data analysis

Raw gaze data was parsed into sequences of saccades and fixations using the SR Research algorithm (SR Research Ltd., Kanata, ON, Canada). Saccades were determined by criteria of change in gaze position (>0.1°), eye velocity (>30°/s), and acceleration (>8,000°/s^2). Gaze data were pre-processed in MATLAB. We analyzed SRTs, defined as the difference between the onset time of the saccade target and the onset time of the first saccade that landed on the target. In total, we recorded SRTs from 15,360 trials (384 trials from each of the 40 participants). Out of these, 557 trials (3.6%) were excluded because SRT was faster than 50 ms (most likely due to anticipations or measurement artifacts or because of blinks ahead of the saccade or during it). SRTs and ratings were analyzed in *R version 3.1.1* (R Core Team, 2014) using the *lme4 package version 1.1-7* (Bates et al., 2014) for fitting and analyzing LMMs. We applied the Satterthwaite (1946) approximation for computing *p*-values for *t*-statistics and the Kenward and Roger (1997) approximation for *F*-statistics, as implemented in the *lmerTest package version 2.0-6* (Kuznetsova et al., 2014). Preliminary analyses of SRTs and inspections of Q–Q plots and histograms revealed that a Log-transformation (natural logarithm) of SRTs was necessary to approximate a normal distribution and to achieve normally distributed model residuals. This is a common transformation for distributions of reaction times (RTs) which are often positively skewed (Baayen and Milin, 2010). In the analysis of SRTs and ratings, we included random intercepts for subjects and stimuli (individual face images).

RESULTS

For each of the two datasets (‘natural faces’ vs. ‘morphed faces’) we selected an appropriate model using the Akaike information criterion (AIC; Akaike, 1974; Stephens et al., 2005) and significance tests based on the χ^2-distributed likelihood-ratio between two models (cf. Glover and Dixon, 2004; Baayen and Milin, 2010). For all datasets, the general approach was the same: first, we defined a null model, which included only the random effects (as well as a fixed effect for the gap manipulation, for the SRT data). Then, we defined a sequence of nested models by step-wise increasing the complexity of the fixed effects structure. Interactions between phenotypic features of the face stimuli and the participants' traits were coded within single variables (i.e., a four-step variable GI for the four possible combinations of participant sex and face gender; and EGI for the 16 possible combinations of participant sex and eye color and face gender and eye color). We ordered these variables and applied a successive differences contrast coding scheme. Each more complex model was a special case of the previous, simpler model. After each step, we checked whether the change in model fit justifies a decision in favor of the more complex model instead of the simpler model. The fixed effects of the best model were subsequently analyzed in more detail.

Subjective attractiveness ratings

Model selection: evidence for gender interaction in morphed faces. Results are presented in **Table 1**. For natural faces the data yielded no evidence for any of the interaction effects (as evident by the non-significant χ^2-statistic in the likelihood-ratio test and the increasing AIC). However, for morphed faces the data suggested an interaction between participant sex and face gender (reflected in the decreasing AIC and the significant χ^2-statistic in the likelihood-ratio test). Noteworthy, there was no evidence for the more complex gender and eye color interaction in this data either. Hence, we concluded that (at least for the highly attractive morphed faces) a model including a gender-based interaction explained subjective attractiveness ratings best (cf. **Figure 3**). For this model, we examined the gender-based individual differences in attractiveness ratings more closely.

Model results: stronger preference for highly attractive feminine faces in men. Tests of the fixed effects part of the model mor$_1$ confirmed the significant interaction between participant sex and face gender, $F(3,67.1) = 4.82$, $p = 0.004$. Model coefficients suggested that feminine faces were rated as more attractive than masculine faces by male as well as female participants. However, this bias toward feminine faces was stronger in male than in female participants (see **Table 2**).

Table 1 | Comparison of nested linear mixed effects models (LMMs) fitted to subjective attractiveness ratings in Experiment 1.

			Model comparison			
Model	***df***	**Formula**	**AIC**	**χ^2**	***df***	***p***
Natural faces						
nat$_0$	4	Attractiveness ~ 1 + RE	2956			
nat$_1$	7	Attractiveness ~ 1 + GI + RE	2962	0.05	3	0.997
nat$_2$	19	Attractiveness ~ 1 + EGI + RE	2979	6.54	12	0.886
Morphed faces						
mor$_0$	4	Attractiveness ~ 1 + RE	2756			
mor$_1$	7	Attractiveness ~ 1 + GI + RE	2748	14.1	3	0.003
mor$_2$	19	Attractiveness ~ 1 + EGI + RE	2755	17.5	12	0.133

Attractiveness, individual subjective attractiveness judgments; Formula, model definition for the lme4 software package; RE, random effects structure [(1| Participant) + (1| Stimulus)]; GI = [Participant Sex × Face Gender]; EGI = [Participant Sex × Participant Eye Color × Face Gender × Face Eye Color].

Table 2 | Fixed effect estimates for a rating bias toward feminine morphed faces in Experiment 1.

Fixed effect	***B (SE)***	***t***	***p***
(Intercept)	4.67 (0.144)		
Female participants (feminine – masculine)	0.47 (0.219)	2.15	0.041
Male participants (feminine – masculine)	0.72 (0.219)	3.29	0.003

Results based on individual ratings on a 7-point scale. Masculine, ratings of masculine morphed faces. Feminine, ratings of feminine morphed faces. Positive B and t values indicate that feminine faces received higher ratings than masculine faces.

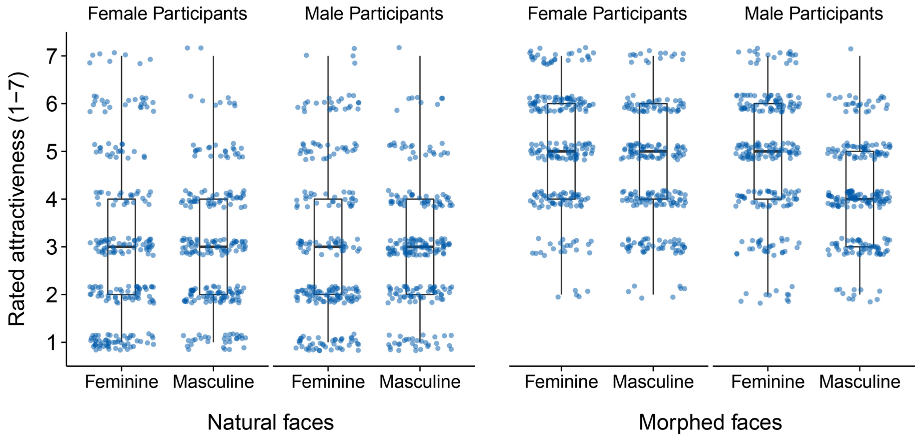

FIGURE 3 | Distribution of all subjective attractiveness judgments collected in Experiment 1. Dots represent raw data points ($n = 960$ in each of the two stimulus sets) and are displayed jittered on the discrete rating scale for better visualization.

Saccadic reaction times

Model selection: facial attractiveness affects saccadic reaction times. Log-transformed SRTs (logSRTs) were analyzed separately for natural faces and morphed faces. Baseline models included the fixed effect of the gap manipulation, random intercepts for participants and stimuli as well as a random slope for the gap manipulation. Due to the generally larger variation in attractiveness judgments for the natural faces we included overall facial attractiveness (as a two-step variable, 'attractive' vs. 'unattractive'). This categorization was based on z-transformed ratings given by each participant at the end of the experiment and computing a mean attractiveness score for each face. Faces with mean attractiveness scores above the median of all values were assigned to the 'attractive' group while the other faces were assigned to the 'unattractive' group.

Results are presented in **Table 3**. For natural faces, the data yielded evidence for an effect of overall facial attractiveness. Apart from that, there were no indications for gender-based (or eye color and gender based) interactions in either of the datasets.

Model results: slower disengagement from attractive faces. Estimated SRTs per condition are depicted in **Figure 4**. The model for the morphed faces (intercept $B = 5.29$, $SE = 1.78 \times 10^{-2}$) yielded a significant gap effect with logSRTs being significantly shorter in gap trials as compared to overlap trials, $B = -1.78 \times 10^{-1}$ ($SE = 1.48 \times 10^{-2}$), $t = -12.0$, $p < 0.001$. Similarly, the model for natural faces (intercept $B = 5.29$, $SE = 1.89 \times 10^{-2}$) resulted in the expected gap effect, with logSRTs being shorter in gap trials as compared to overlap trials, $B = -2.03 \times 10^{-1}$ ($SE = 1.53 \times 10^{-2}$), $t = -13.29$, $p < 0.001$. Importantly, this model also yielded a significant effect of facial attractiveness: logSRTs were significantly shorter with unattractive compared to attractive faces, $B = -1.87 \times 10^{-2}$ ($SE = 6.94 \times 10^{-3}$), $t = -2.69$, $p = 0.007$. The interaction between the fixed effects of gap and facial attractiveness was not statistically significant, $t = 0.35$, $p = 0.726$.

DISCUSSION

Experiment 1 tested whether (a) attentional disengagement from attractive faces is slower than from less attractive faces, (b) women and men differ in how they attend to opposite-gender versus same-gender faces, and (c) there are specific eye color preferences in opposite-gender faces in men (Laeng et al., 2007; Bovet et al., 2012). Our eye tracking experiment resulted in evidence for hypothesis (a) but did not support claims (b) and (c), i.e., we found no evidence that interactions between participants and face traits co-determine attentional disengagement from faces. In addition to the eye tracking experiment we also collected subjective attractiveness ratings for natural and morphed faces. Only for the highly attractive morphed faces, the data yielded evidence for a gender-based interaction: men as well as women gave higher ratings to feminine faces than to masculine faces but this bias was much larger in men than in women. This begs the question of why we were unable to detect the gender-based interaction at least in the morphed faces with our eye tracking experiment. In principle, it is possible that specific individual preferences for attractive facial features affect attentional processes but not necessarily the process of disengagement. Hence, in Experiment 2 we created an *attentional capture test*, asking participants to make a saccade *toward* one of two faces.

EXPERIMENT 2: ATTENTIONAL CAPTURE BY FACES

Instead of presenting only one face at screen center, we presented two faces at different locations in the periphery and asked participants to make a saccade toward one of these locations (and ignore the other). When multiple stimuli are in the visual field they compete for attention and cognitive processing by activating their respective neural representations (Desimone and Duncan, 1995). This competition for attentional priority is determined by stimulus-driven influences, such as the strength of the visual signal and by observer-based top–down influences (e.g., expectations, goals, or memory). A suitable task to measure these early processes of attentional capture is the so called '*dot probe*' or '*double*

Table 3 | Comparison of nested LMMs fitted to log-transformed SRTs in Experiment 1.

			Model comparison			
Model	***df***	**Formula**	**AIC**	**χ^2**	***df***	***p***
Natural faces						
nat$_0$	7	logSRT ∼ 1 + Gap + RE	3315			
nat$_1$	9	logSRT ∼ 1 + Gap × Attractiveness + RE	3311	7.40	2	0.025
nat$_2$	21	logSRT ∼ 1 + Gap × Attractiveness × GI + RE	3324	11.68	12	0.472
nat$_3$	69	logSRT ∼ 1 + Gap × Attractiveness × EGI + RE	3368	52.06	48	0.319
Morphed faces						
mor$_0$	7	logSRT ∼ 1 + Gap + RE	3679			
mor$_1$	13	logSRT ∼ 1 + Gap × GI + RE	3687	4.34	6	0.631
mor$_2$	37	logSRT ∼ 1 + Gap × EGI + RE	3714	20.75	24	0.652

logSRT, individual log-transformed SRTs in the gap-saccade task; attractiveness, two-staged categorization (attractive vs. unattractive) of natural faces based on subjective attractiveness judgments; formula, model definition for the lme4 software package; RE, random effects structure [(1+Gap| Participant) + (1| Stimulus)]. GI = [Participant Sex × Face Gender]. EGI = [Participant Sex × Participant Eye Color × Face Gender × Face Eye Color].

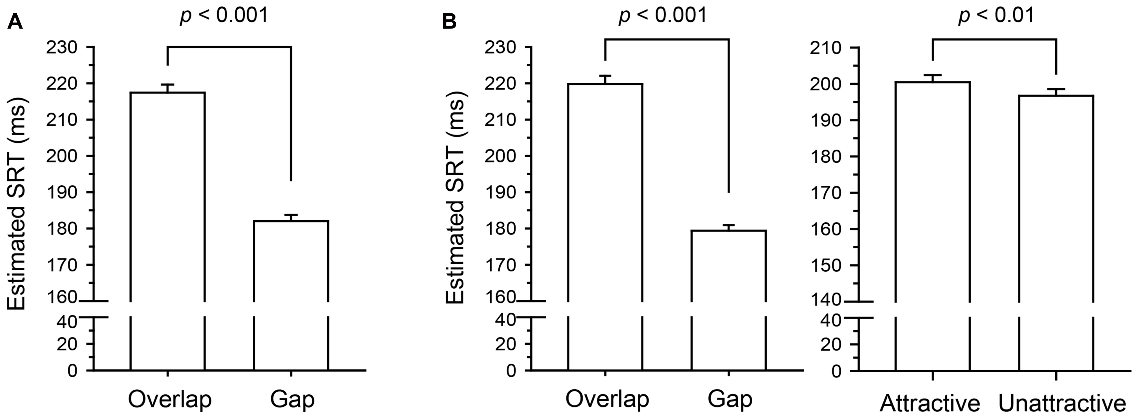

FIGURE 4 | Saccadic reaction times (SRTs) per factor level as estimated by the final linear mixed effects models (LMMs) in Experiment 1. For better interpretability, the estimated logSRT for each factor level was transformed back to the original millisecond scale for plotting. Error bars represent +1.96 *SEM* after removing random effect variances. **(A)** Model results for the morphed faces. The data yielded a significant effect of the gap manipulation with lower SRTs in the Gap than in the Overlap condition. **(B)** Model results for the natural faces. The gap effect was qualitatively identical to the results from the morphed faces. In addition, the data yielded an effect of facial attractiveness, with longer SRTs with attractive than with unattractive faces.

cueing' task (MacLeod et al., 1986) which has been often used in experimental psychopathology studies (e.g., Frewen et al., 2008; Yiend, 2010). The basic procedure implies a brief presentation of two task-irrelevant images (the cues) at a certain eccentricity left and right of screen center. Directly afterward, an unrelated target stimulus – often called the 'dot probe' – is presented either at the left or at the right position and subjects are asked to (usually) manually report the presence, identity, or position of the dot. If the dot probe appears at an attended location, RTs to the dot should be significantly faster than if the dot appears at an unattended location. This procedure has been adapted in various ways to study different questions about individually varying attention.

Here, we adapted a version of the dot-probe task which was used to study attentional biases in eating disorders (Blechert et al., 2010). We used two photographs of faces as cues. Instead of extinguishing both photos and replacing one of them with a probe that was unrelated to the images, we kept the faces visible and presented two differently colored frames around them. The participants were instructed to make a saccade to one pre-defined target color frame and ignore the differently colored distractor frame. Keeping the photos on screen and requiring participants to make a saccade toward one of the cued locations allows insight into the process of attentional capture. More specifically, it allows inferring which of the two concurrently presented faces captures attention more readily. Compared to the classical version of the dot probe task which requires manual button presses, saccades are a more ecological response when studying attentional capture (Kowler et al., 1995; Deubel and Schneider, 1996). Additionally, this procedure enables us to gage the temporal properties of attentional capture by varying the stimulus onset asynchrony (SOA) which is the interval between the appearance of the face cues and the target/distractor rectangles. We used two short intervals (150, 250 ms) and one long interval (1 s). These values are based on the literature on the so-called '*inhibition of return*' (IOR) effect, which describes the often observed finding that attention is first captured by a particular stimulus location but if no target is presented soon afterward, this particular location is actively inhibited. This results in prolonged RTs when responding to a target presented at a cued location after about 300 ms (Posner and Cohen, 1984; Taylor and Klein, 1998; Klein, 2000). Using this procedure, we studied whether interactions between participant's sex and eye color and the respective facial characteristics co-determine the capture of attention by attractive faces.

METHODS

Participants

Forty new participants with a mean age of 22 years ($SD = 2.7$) were recruited from the same student population as in Experiment 1 to four groups of ten, resulting from crossing the variables participant sex (female vs. male) and eye color (blue vs. brown).

Apparatus

Setup and recording were identical to Experiment 1, with the exception that a drift check was conducted ahead of every trial.

Stimuli and procedure

In Experiment 2 only the morphed faces served as stimuli (see **Figure 1**). We used eight different feminine and eight different masculine faces. Each face was presented with two different eye colors (blue and brown) to the same participants, resulting in 16 feminine and 16 masculine face images altogether. At the start of each session, participants were informed that the purpose of the experiment was to study the effect of human faces on visual attention and were given basic task instructions (e.g., 'fixate on the screen center until two colored boxes appear; as soon as you see the boxes, look at the yellow box as quickly as possible'). The experiment comprised 576 trials which were randomly assigned to six blocks of 96 trials (between blocks, participants were allowed to rest briefly). In 384 trials the two simultaneous face cues showed the exact opposite phenotypic traits (e.g., the face at the target's location was feminine with blue eyes, and the face at the distractor location was masculine with brown eyes). In the remaining 192 trials, the two faces shared either their eye color (e.g., blue-eyed feminine face and blue-eyed masculine face) or gender (e.g., blue-eyed feminine face and brown-eyed feminine face). Face identity, gender, and eye color were uncorrelated with target and distractor positions, hence face features where uninformative about target location. **Figure 5** illustrates two example trials.

Every trial started with the presentation of a central fixation target for a drift check. Then, two faces were presented to the left and to the right of the central fixation. After a variable SOA (150 ms/250 ms/1 s) two colored rectangles appeared concomitantly to frame the faces. The task of the participants was to make a saccade to the target rectangle, which was defined by color. The target color could be either green (CIE $L^* = 94.5$, $a^* = -77.3$, $b^* = 79.4$) or yellow (CIE $L^* = 94.6$, $a^* = -4.7$, $b^* = 85.3$), while the other color was used for the distractor. The target and distractor colors were counterbalanced across participants, announced in the initial instructions, and retained throughout the experiment. At the start of each session, participants practiced some trials to become familiar with the task and the stimuli.

Because of lower acuity in the visual periphery, the faces had to be displayed at a larger size than in Experiment 1. The appropriate size was determined by pre-tests using various stimulus sizes until a quick and reliable discrimination of gender and eye color at peripheral locations was secured. Hence, faces were shown at a size of 4 × 5° and the target and distractor rectangles had a size of 5.6 × 6.8° with line strengths of 0.25°. Faces and rectangles were presented at an eccentricity of 7.5° from the central fixation. Following the experimental blocks, participants subjectively rated the attractiveness of each face in a separate rating block where the procedure was identical to Experiment 1. In total, the data collection lasted about 80 min per participant (including setup, experiment, rating, and participant debriefing).

Data analysis

Data processing was done using the same software packages and settings as in Experiment 1. In total, we recorded SRTs from 23,040 trials (576 trials from each of the 40 participants). Out of the complete dataset, 876 trials (3.8%) were excluded due to the same criteria as in Experiment 1 in addition to trials in which the first saccade was erroneously directed to the distractor instead of the target rectangle.

RESULTS

Subjective attractiveness ratings

Model selection: evidence for gender interaction in morphed faces. Results were similar to Experiment 1 and are presented in **Table 4**. According to the (decreasing) AIC and the significant likelihood-ratio test, an appropriate model for our rating data was mor$_1$, a model that included the interaction of participant sex and face gender (GI). In contrast, including the full interaction of participant sex and eye color and face gender and eye color (EGI) was not corroborated by the data.

Model results: preference for highly attractive feminine faces in men. Testing the fixed effects part of the final model mor$_1$ confirmed the significant interaction of participant sex and face gender, $F(3,97.1) = 3.73$, $p < 0.014$. **Table 5** shows the fixed effect estimates reflecting the rating bias toward feminine faces for men and women separately. The tendency for rating feminine faces as more attractive than masculine faces was present in female as well as male participants, but this bias was only significant in male participants.

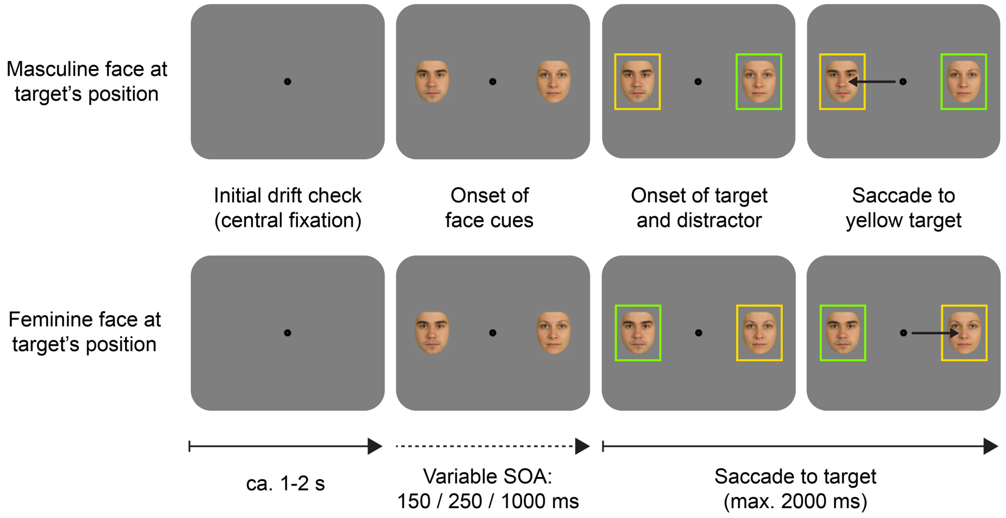

FIGURE 5 | Example schematic trials in the cueing task of Experiment 2. In this example, the target color is yellow, and the distractor color is green (this was counterbalanced across participants). Phenotypic features of the face cues were non-predictive of the upcoming target's location. SRT to the target was analyzed as a function of the face cue presented at the target, and the distractor location, as well as the stimulus onset asynchrony (SOA). Arrows in the rightmost pictures indicate saccades. Arrows below the pictures indicate the flow of time.

Table 4 | Comparison of nested LMMs fitted to ratings of morphed faces in Experiment 2.

			Model comparison			
Model	***df***	**Formula**	**AIC**	**χ^2**	***df***	***p***
mor_0	4	Attractiveness ∼ 1 + RE	5438			
mor_1	7	Attractiveness ∼ 1 + GI + RE	5433	11.16	3	0.011
mor_2	19	Attractiveness ∼ 1 + EGI + RE	5452	5.23	12	0.950

Attractiveness, individual subjective attractiveness judgments; formula, model definition for the lme4 software package; RE, random effects structure [(1| Participant) + (1| Stimulus)]. GI = [Participant Sex × Face Gender]. EGI = [Participant Sex × Participant Eye Color × Face Gender × Face Eye Color].

Table 5 | Fixed effect estimates for a rating bias toward feminine morphed faces in Experiment 2.

Fixed effect	***B (SE)***	***t***	***p***
(Intercept)	4.10 (0.125)		
Female participants (feminine – masculine)	0.31 (0.178)	1.74	0.088
Male participants (feminine – masculine)	0.53 (0.178)	2.99	0.004

Results based on individual ratings on a 7-point scale. Masculine, ratings of masculine morphed faces; feminine, ratings of feminine morphed faces. Positive B and t values indicate that feminine faces received higher ratings than masculine faces.

Table 6 | Comparison of nested LMMs fitted to log-transformed SRTs in Experiment 2.

			Model comparison			
Model	***df***	**Formula**	**AIC**	**χ^2**	***df***	***p***
Target face cues						
targ_0	6	logSRT ∼ 1 + SOA + RE	–1576			
targ_1	15	logSRT ∼ 1 + SOA × TGI + RE	–1577	18.88	9	0.026
targ_2	51	logSRT ∼ 1 + SOA × TEGI + RE	–1544	38.21	36	0.369
Distractor face cues						
dist_0	6	logSRT ∼ 1 + SOA + RE	–1576			
dist_1	15	logSRT ∼ 1 + SOA × DGI + RE	–1583	24.63	9	0.003
dist_2	51	logSRT ∼ 1 + SOA × DEGI + RE	–1540	28.79	36	0.798

logSRT, individual log-transformed SRTs; formula, model definition for the lme4 software package; SOA, stimulus onset asynchrony; RE, random effects structure [(1| Participant) + (1| Stimulus)]. TGI = [Participant Sex × Target Face Cue Gender]. TEGI = [Participant Sex × Participant Eye Color × Target Face Cue Gender × Target Face Cue Eye Color]. DGI = [Participant Sex × Distractor Face Cue Gender]. DEGI = [Participant Sex × Participant Eye Color × Distractor Face Cue Gender × Distractor Face Cue Eye Color].

Saccadic reaction times

Model selection: evidence for gender interaction in attentional capture. To determine whether participants' attention was biased toward a particular face gender and eye color depending on their own expression of these traits, we analyzed the obtained SRTs separately with respect to (a) the target face cue properties, and (b) the distractor face cue properties. In all analyses of SRTs, we also modeled the effect of SOA, as we hypothesized that any individual preferences might show a different pattern with the shorter as compared to longer SOAs. In all models, we included random intercepts for combinations of particular target and distractor faces. **Table 6** presents the results.

The model comparison showed that including the interaction between participant sex and target's face cue gender (TGI), or the interaction between participant sex and distractor's face cue gender (DGI) improved goodness of fit over the respective baseline models. However, the present data yielded no evidence for EGIs (TEGI/DEGI) in any of the tested models. Hence, our data suggest that male and female participants differed in how quickly they made saccades to the target rectangle depending on whether the face shown at the target location was masculine or feminine (and whether the face at the distractor location was masculine or feminine, respectively). To further scrutinize this interaction we looked at the estimates of these models in more detail.

Model results: men's attention is more effectively captured by feminine faces. Estimated SRTs are depicted in **Figure 6**. Testing the fixed effects in the final target face cue model (targ_1) confirmed our conclusions from the model comparisons. The SOA effect, $F(2,40) = 40.17$, $p < 0.001$, as well as the TGI interaction effect, $F(3,104) = 4.77$, $p = 0.004$, were significant. The interaction between these two was not significant, $F(6,178) = 0.77$, $p = 0.594$. Results for the corresponding distractor face cue model (dist_1) followed the same pattern: the overall SOA effect, $F(2,40) = 40.25$, $p < 0.001$, and the overall DGI effect, $F(3,104) = 5.28$, $p = 0.002$, were significant. Again, the interaction between them was not significant, $F(6,165) = 1.47$, $p = 0.184$. To scrutinize these effects (in particular the gender based differences) we looked at the models' contrast estimates which are listed in **Table 7**.

For both models, the estimated SOA effects showed that saccades were initiated significantly faster after the (long) 1 s SOA than after the (short) 250 or 150 ms SOAs, while there was no difference between the latter two. Interestingly, our results confirmed an attentional bias for feminine faces, exclusively for male participants: saccades of male participants toward the target rectangle were initiated significantly faster if a feminine face was presented at the target location, and significantly slower if a feminine face was presented at the opposite, distractor location. In contrast, SRTs of female participants were not affected by the gender of the face stimulus at either of the locations.

DISCUSSION

Experiment 2 followed up on the aim to investigate interactions between participant traits and face stimulus characteristics (eye color and gender) in the attentional bias for attractive faces. There was one important conceptual difference to Experiment 1: we measured attentional capture *toward* one out of two simultaneously presented faces and not *disengagement* from one fixated face. Hence, Experiment 2 targeted a sub-process of attention that precedes disengagement (cf. Posner and Petersen, 1990). In addition, by presenting two faces simultaneously in the visual periphery we created the necessary preconditions for biased competition between faces – a viewing situation that more closely mirrors attentional orienting toward particular individuals in a social world.

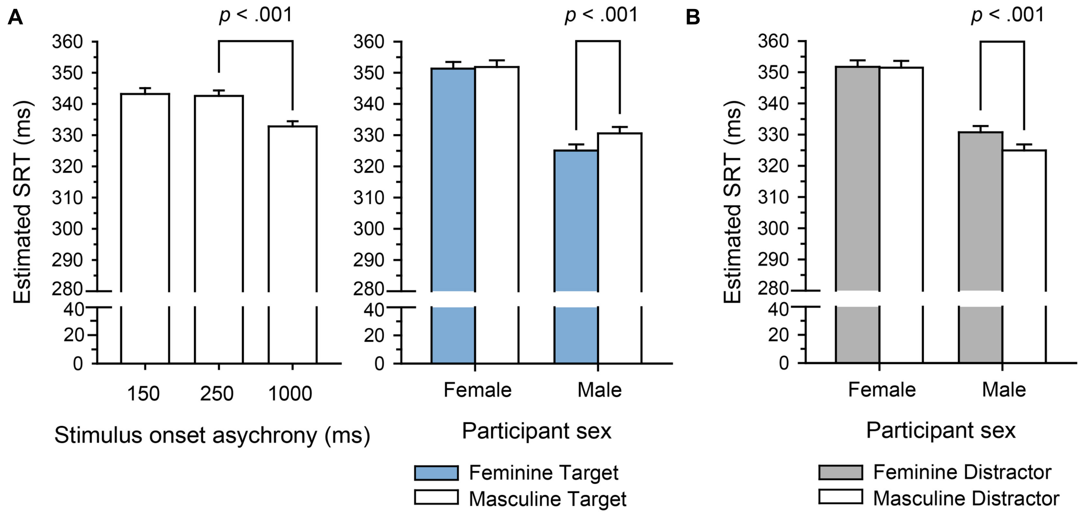

FIGURE 6 | Saccadic reaction times per factor level as estimated by the final LMMs in Experiment 2. For better interpretability, the estimated logSRT for each factor level was transformed back to the original millisecond scale for plotting. Error bars represent +1.96 *SEM* after removing random effect variances. **(A)** Model results for the target face cues. The data yielded a significant effect of the SOA manipulation and a significant interaction of participant sex and target face cue gender. For male participants, SRTs were shorter when the face at the target location was feminine. **(B)** Model results for the distractor face cues. The model yielded a significant interaction of participant sex and distractor face cue gender. For male participants, SRTs were longer when there was a feminine face at the distractor location (the SOA effect is not depicted for the distractor model as it was qualitatively identical to the target model).

Table 7 | Fixed effect estimates for SOA and GI parameters in the final LMMs of log-transformed SRTs in Experiment 2.

Fixed effect	*B (SE)*	*t*	*p*
Target model			
(Intercept)	5.83 (0.034)		
SOA_{250}–SOA_{150}	-1.8×10^{-3} (3.83×10^{-3})	–0.46	0.642
SOA_{1000}–SOA_{250}	-2.9×10^{-2} (3.84×10^{-3})	–7.50	<0.001
$Female_{mas}$ – $Female_{fem}$	1.5×10^{-3} (4.59×10^{-3})	0.32	0.749
$Male_{mas}$ – $Male_{fem}$	1.7×10^{-2} (4.62×10^{-3})	3.64	<0.001
Distractor model			
(Intercept)	5.83 (0.034)		
SOA_{250}–SOA_{150}	-1.8×10^{-3} (3.83×10^{-3})	–0.47	0.641
SOA_{1000}–SOA_{250}	-2.9×10^{-2} (3.83×10^{-3})	–7.51	<0.001
$Female_{mas}$ – $Female_{fem}$	-7.5×10^{-4} (4.59×10^{-3})	–0.16	0.870
$Male_{mas}$ – $Male_{fem}$	-1.8×10^{-2} (4.62×10^{-3})	–3.84	<0.001

SOA, stimulus onset asynchrony (150/250/1,000 ms); female, female participants; male, male participants; mas, masculine faces; fem, feminine faces.

This ultimately enabled us to establish the hypothesized interaction of participant sex and face stimulus gender, reflecting an attentional bias toward attractive feminine faces in men. Again, we supplemented our eye tracking data with subjective attractiveness judgments. The latter revealed that men judged feminine faces as more attractive than masculine faces while this bias toward feminine faces was much weaker (and non-significant) in women.

In addition, we manipulated the interval between the onset of the face cues and the onset of the targets in order to measure the temporal properties of attentional deployment to the face cues. We expected an IOR effect as usually observed with SOAs exceeding 300 ms (e.g., Klein, 2000): an interaction between cueing (by preferred faces) and the length of the interval reflecting that attention is initially captured by an attractive face and later inhibited at the same location. This should have resulted in prolonged RTs to the location where attention was captured by an attractive face with the 1 s SOA. Also, because only men showed a preference for feminine faces, the IOR effect was expected to be present in men only. This IOR should then be reflected in an interaction between the GI and SOA's fixed effects. However, this interaction was not observed. Rather, we found a general acceleration of SRTs after the longest SOA. One possible explanation for this finding could be that the longest SOA implicated a better temporal warning signal of the target due to the large temporal gap between the middle (250 ms) and long SOA (1 s) which could modulate SRTs independently and in addition to any face-based spatial attention effects (e.g., Walker et al., 1995).

Our main findings are in line with previous literature showing that men exhibit stronger preferences for attractive opposite-sex faces than women. Yet, even with our modified experimental procedure our data did not support the hypothesis that eye color additionally interacts with individual preferences – as predicted by Laeng et al. (2007) who found that blue-eyed men rated blue-eyed women as more attractive than brown-eyed women and who explained this with an evolutionary adaptive strategy of blue-eyed men to maximize their subjective assurance of paternity. It is worth pointing out that the present study had a smaller number of participants (per eye color and sex group, respectively) than the original Laeng et al. (2007) study. In principle, one might suspect that the original finding was not corroborated because of low statistical power. However, taking the originally reported effect size into account, it is unlikely that our study would fail to capture such a large effect in all datasets that we collected throughout this study, if it is present.

GENERAL DISCUSSION

The current study tested potential contributions of gender and eye color to individual differences in preferences for attractive faces and the respective effects on two distinct attentional processes: disengagement of attention (Experiment 1) and capture of attention (Experiment 2). In prior studies of looking times at attractive faces, these phases were generally confounded and it could not be decided which sub-process was responsible for the effects. For our tests, we chose SRTs rather than looking times, because saccades are tightly coupled to the engagement of attention (Kowler et al., 1995; Deubel and Schneider, 1996). Also we conducted our tests with a focus on two sources of inter-individual differences in attention to faces. First, we studied if men and women showed distinct patterns of attentional processes in response to same-sex and opposite-sex faces, respectively. This was confirmed for attentional capture (in Experiment 2) but not for the disengagement of attention (in Experiment 1). Second, we studied whether eye color could explain additional differences between participants, such as an attentional bias of blue-eyed men toward blue-eyed feminine faces. The latter could not be confirmed in any of our experiments. Thus, our results show that individual preferences for attractive faces are partly reflected in respective differences in visual attention but not all of these inter-individual differences are equally robust.

This brings us to an important related point. Our results differ from previous reports because we were unable to replicate the finding that blue-eyed men consider blue-eyed women more attractive than brown-eyed women (Laeng et al., 2007). One reason for the failure to replicate the original finding could be that the stimuli of Laeng et al. (2007) were more naturalistic portrait photographs including features beyond the face itself (such as hairstyles and clothing). For the present study, we constructed new stimuli and minimized any potentially confounded features that could attract attention independently of face gender and eye color. However, it is possible that contextual features are necessary to induce stronger individual evaluative and behavioral preferences so that the use of the stronger constrained stimuli in the present experiments prevented us from replicating the original finding. Another possibility is that the eye color may have been more salient and easier to discriminate in the original study of Laeng et al. (2007) where faces were shown full-screen on an 11.4″ monitor whereas their size was smaller in the present study (Experiment 1: $2.7 \times 3.2°$; Experiment 2: $4 \times 5°$).

What is even more important in our view, the result of a study also hinges on the specific statistical procedures applied to the collected data. Inconsistencies across studies could stem from distorted data due to averaging across subjects, stimuli, or conditions without accounting for random variance that is not generalizable to the independent variables of the design (e.g., Wells and Windschitl, 1999; Judd et al., 2012). This is particularly problematic for studies addressing interactions between groups of participants and experimental stimuli. While this problem was taken care of with the present LMM analyses, spurious interactions might become significant with more traditional statistics. Also, the exclusive use of the classic approach of null hypothesis significance testing (NHST) has been often criticized (e.g., Bakan, 1966; Greenwald, 1975; Cohen, 1994; Loftus, 1996; Sohn, 1998; Nickerson, 2000) and more informative statistical tools, such as model comparisons and measures of Information Theory and Bayesian statistics have been advocated recently (e.g., Glover and Dixon, 2004; Stephens et al., 2005; Wagenmakers, 2007).

CONCLUSION

In the present paper we linked research on individual preferences for attractive faces to inter-individual differences in visual attention toward faces of varying attractiveness. Using a combination of well-controlled experimental approaches and linear mixed effects modeling, we replicated previous results showing that attractive faces lead to longer dwell times. In addition, we found evidence for gender-based differences in attentional capture. We could not replicate a previously reported EGIs and close with a recommendation for the statistical analysis of inter-individual differences in general.

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Electrophysiological evidence of the time course of attentional bias in non-patients reporting symptoms of depression with and without co-occurring anxiety

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Anxiety is characterized by attentional biases to threat, but findings are inconsistent for depression. To address this inconsistency, the present study systematically assessed the role of co-occurring anxiety in attentional bias in depression. In addition, the role of emotional valence, arousal, and gender was explored. Ninety-two non-patients completed the Penn State Worry Questionnaire (Meyer et al., 1990; Molina and Borkovec, 1994) and portions of the Mood and Anxiety Symptom Questionnaire (Watson et al., 1995a,b). Individuals reporting high levels of depression and low levels of anxiety (depression only), high levels of depression and anxiety (combined), or low levels of both (control) completed an emotion-word Stroop task during event-related brain potential recording. Pleasant and unpleasant words were matched on emotional arousal level. An attentional bias was not evident in the depression-only group. Women in the combined group had larger N200 amplitude for pleasant than unpleasant stimuli, and the combined group as a whole had larger right-lateralized P300 amplitude for pleasant than unpleasant stimuli, consistent with an early and later attentional bias that is specific to unpleasant valence in the combined group. Men in the control group had larger N200 amplitude for pleasant than unpleasant stimuli, consistent with an early attentional bias that is specific to pleasant valence. The present study indicates that the nature and time course of attention prompted by emotional valence and not arousal differentiates depression with and without anxiety, with some evidence of gender moderating early effects. Overall, results suggest that co-occurring anxiety is more important than previously acknowledged in demonstrating evidence of attentional biases in depression.

Keywords: attentional bias, anxiety, depression, emotion, event-related brain potentials

INTRODUCTION

An impressive body of research has demonstrated that depression and anxiety are characterized by cognitive biases, including attentional bias or preferential attentional processing of unpleasant or threatening information. Attentional bias has been argued to contribute to the etiology and maintenance of anxiety and depression (e.g., Beck, 1976; Beck et al., 2005; Bar-Haim et al., 2007; Levin et al., 2007; Gotlib and Joormann, 2010). Whereas anxiety has been consistently associated with attentional biases to threatening or emotionally arousing stimuli (Williams et al., 1996; McNally, 1998; Becker et al., 2001; Fox et al., 2002), evidence of attentional biases in depression has been mixed (Mogg and Bradley, 2005; Gotlib and Joormann, 2010), with some studies finding preferential processing of unpleasant stimuli (Gotlib and Cane, 1987; Bradley et al., 1997), others insufficient attention to pleasant stimuli (Gotlib et al., 1988; McCabe and Gotlib, 1995; Gilboa and Gotlib, 1997; McCabe et al., 2000), and others a lack of differentiation between pleasant and unpleasant stimuli (e.g., McCabe and Gotlib, 1995; Deldin et al., 2001).

Along with inconsistent evidence regarding the nature of attentional biases in depression, evidence regarding the time course of bias has also been inconsistent. Understanding the time course of attentional processing is critical in elucidating the degree to which early registration and vigilance, relying primarily on early sensory processing involving brain areas such as visual cortex and amygdala, or later, more elaborative attentional and cognitive-control mechanisms, relying primarily on cortical and prefrontal regions (for reviews, see Bishop, 2007; Gotlib and Joormann, 2010), are involved in abnormal attention to emotional information. Some evidence exists for an early attentional bias in favor of unpleasant information in depression, especially from electrophysiological studies (e.g., Williams et al., 2007; Jaworska et al., 2010). Evidence of a later bias in favor of unpleasant information in depression also exists (e.g., Gotlib and Joormann, 2010), supporting the hypothesis that later attentional processes (such as difficulty disengaging from or inhibiting unpleasant information) are involved in attentional biases in depression (e.g., Leyman et al., 2007; Joormann and D'Avanzato, 2010).

Depression and anxiety are frequently co-occurring and share considerable conceptual and measurement overlap (Clark and Watson, 1991; Heller et al., 1998; Keller et al., 2000; Nitschke et al., 2001; Watson, 2009). Surprisingly few studies account for anxiety in attentional bias investigations of depression (Bar-Haim et al., 2007), despite the potential for co-occurring anxiety to affect the nature or timing of bias in depression. A large literature documents evidence of both early (e.g., Williams et al., 1996; Bar-Haim et al., 2007; Eysenck et al., 2007; Li et al., 2007; Sass et al., 2010) and later (e.g., Fox et al., 2002; Li et al., 2007) attentional biases in anxiety, with evidence of attention being captured quickly by threatening stimuli (e.g., Williams et al., 1996), difficulty disengaging from threatening stimuli once attended (e.g., Fox et al., 2002), initial engagement with threatening stimuli followed by avoidance (e.g., Mogg et al., 2004), and preferential engagement with both pleasant and threatening compared to neutral information (e.g., Martin et al., 1991; Sass et al., 2010). In samples with depression and co-occurring anxiety, evidence of attentional bias is sometimes found (e.g., Rossignol et al., 2008; Markela-Larenc et al., 2011) and sometimes not (Bradley et al., 1995).

Co-occurring anxiety can also affect patterns of brain activity in regions implementing attentional control (e.g., Heller, 1990, 1993; Heller et al., 1995; Heller and Nitschke, 1997; Keller et al., 2000). Although anxiety is a broad construct that is often treated monolithically, anxious apprehension (worry) and anxious arousal (panic or sympathetic arousal), are accompanied by distinct patterns of abnormal activity in brain regions implementing attentional control (e.g., Heller et al., 1997; Heller and Nitschke, 1998; Engels et al., 2007, 2010) and are associated with a dissociable time course of attentional bias to emotionally arousing stimuli (Sass et al., 2010). In order to examine the neural mechanisms involved in the time course of attentional disruption in depression with and without co-occurring anxiety, it is important to investigate these dimensions of anxiety in conjunction with depression.

Different patterns of attentional biases depending on depression and anxiety co-occurrence could have substantial implications for treatment. Unique cognitive characteristics of depression with and without anxiety (and potential targets for intervention) could be associated with different neural mechanisms. Co-occurring anxiety can affect patterns of brain activity that are related to attentional processing (e.g., Heller, 1990, 1993; Heller et al., 1995; Heller and Nitschke, 1997; Keller et al., 2000; Engels et al., 2010). Resting EEG, functional magnetic resonance imaging (fMRI), and event-related brain potential (ERP) studies provide evidence of lateralization patterns in depression with less right than left activity over parieto-temporal regions (e.g., Deldin et al., 2000; Engels et al., 2010; Stewart et al., 2011). These posterior brain regions are associated with vigilance and autonomic arousal, and less activity in these areas in depressive states is presumably due to less arousal characterized by symptoms such as anhedonia (e.g., Heller and Nitschke, 1998; Engels et al., 2010). For example, ERP studies demonstrate evidence of reduced right parietal N200 amplitude (Deldin et al., 2000) and P300 amplitude (Sumich et al., 2006) in depressed individuals. Conversely, depression with co-occurring anxious apprehension has been associated with greater right inferior occipital cortex fMRI activity and co-occurring anxious arousal with greater right inferior temporal gyrus fMRI activity in response to unpleasant vs. neutral information in the context of an emotion-word Stroop task (Engels et al., 2010). Examining lateralized neural mechanisms reflecting the time course of processing of emotional stimuli may provide critical insights in understanding biased processing of emotional stimuli in depression with and without co-occurring anxiety.

In addition to co-occurring anxiety, emotional valence and emotional arousal are important to systematically investigate in attentional biases in depression. Pleasant stimuli are inconsistently included in attentional bias studies and when they are included, are not consistently matched to unpleasant stimuli on emotional arousal level (e.g., see Mogg and Bradley, 2005, **Table 1**; Williams et al., 1996). It is possible that a general emotional arousal confound contributes to variance in findings. That is, it may be the high emotional arousal value of unpleasant stimuli and not unpleasant valence *per se* that drives attentional biases in depression. In order to assess this issue, the present study matched unpleasant and pleasant stimuli on emotional arousal level.

Gender is also important to investigate systematically in attentional bias research. Women are estimated to suffer from depression and anxiety twice as often as men (Weissman et al., 1996; Nolen-Hoeksema, 2001; Craske, 2003). Several studies indicate that gender moderates emotional information processing in depressed (e.g., Wright et al., 2009) and anxious (e.g., Sass et al., 2010) participants. For example, depressed women took longer to categorize negative faces than did control women, whereas depressed men performed no differently than control men (Wright et al., 2009). Failing to include gender may further contribute to inconsistency regarding the nature and timing of

Table 1 | Means and standard deviations for questionnaire scores used to form groups.

	Group Questionnaire Scores		
	PSWQ	**MASQ-AA**	**MASQ-AD**
Group			
Depression-only	36 (9.4)	22 (2.5)	25 (2.8)
Combined	71 (5.4)	71 (5.4)	27 (3.9)
Control	38 (8.6)	20 (2.2)	13 (2.4)

PSWQ refers to the Penn State Worry Questionnaire. MASQ-AA and MASQ-AD refer to the anxious arousal and anhedonic depression subscales of the Mood and Symptom Questionnaire, respectively.

attentional biases in depression and may unnecessarily limit understanding of how these biases contribute to and maintain depression. Gender also moderates processing of emotional stimuli in control participants, with women sometimes showing evidence of preferential processing of unpleasant stimuli (e.g., Lang et al., 1998), and men tending to show the opposite pattern, prioritizing pleasant information (Lang et al., 1998; Bradley and Lang, 2007). A more comprehensive understanding of the nature and time course of the processing of emotional stimuli in control participants may provide useful information regarding the higher prevalence rates of depression and anxiety in women.

In examining attention to emotional stimuli in depression and anxiety, many studies have used an emotion-word variant of the Stroop task. Distracter word content is unpleasant ("assault"), neutral ("cabinet"), or pleasant ("festive"), and participants are asked to ignore the content or meaning of the word while responding to the color of the word. A recent meta-analysis indicated that clinically depressed individuals show slower color naming in the emotion-word Stroop task for unpleasant than for neutral words, consistent with biased processing of unpleasant information (Epp et al., 2012). Similarly, in anxiety, a large literature demonstrates that color naming is slowed in anxious participants when the distracter word is unpleasant or threatening, with larger effects in individuals diagnosed with anxiety disorders and smaller or inconsistent effects in individuals with self-reported trait or state anxiety (e.g., Williams et al., 1996; Koven et al., 2003; Bar-Haim et al., 2007). Reaction time (RT) alone may not be a precise indicator of attentional bias given that delayed RT can be interpreted as avoidance instead of heightened attention toward negative stimuli (e.g., De Raedt and Koster, 2010). In contrast, ERP methodology offers high temporal resolution that can differentiate early sensory from later more elaborative processing prior to response selection and execution. In general, early sensory processing occurs prior to 300 ms (e.g., Luck et al., 2000), and later conflict detection processes occur beginning approximately 300–600 ms (e.g., Donchin and Coles, 1988; Coles et al., 2000; West, 2003).

The present study focused on P100 and posterior visual N200 amplitude as indices of earlier, more automatic stimulus processing, and P300 (sometimes called P3b, late positive potential (LPP), or late positive complex (LPC) as an index of later, more elaborative stimulus processing. P100 amplitude peaks approximately 100 ms after stimulus onset and grows larger as more extrastriate cortex resources are devoted to processing stimuli (Luck et al., 2000). P100 was larger for sad than for joyful facial expressions (Jaworska et al., 2010) and smaller for positive words in depressed than in control participants (Dai and Feng, 2011). In anxious participants in the emotion-word Stroop task, P100 was larger for unpleasant than neutral stimuli (e.g., Li et al., 2007) and for unpleasant and pleasant than neutral stimuli (Sass et al., 2010).

Posterior visual N200 (what is sometimes called N100) immediately follows P100 over occipito-parietal sensors (e.g., Allison et al., 2002; Ruz and Nobre, 2008; Sass et al., 2010), but peaks later (approximately 200 ms) than classical N100 elicited in visual attention tasks (e.g., Gonzalez et al., 1994; Anllo-Vento and Hillyard, 1996), especially those using short intertrial intervals (ITIs). N200 likely originates in extrastriate cortex and is maximal over bilateral occipital-posterior regions [sometimes called early posterior negativity (EPN); e.g., Weber et al., 2009]. In depression, smaller N200 for happy than sad faces (e.g., Deldin et al., 2000) or no modulation of N200 amplitude in response to emotional stimuli (e.g., Kayser et al., 2000) has been found. In anxiety, larger N200 amplitude has been associated with processing emotionally arousing than neutral stimuli in the context of an emotion-word Stroop task (Sass et al., 2010). Taken together, P100 and N200 amplitude results indicate stronger evidence for an early bias in anxiety but mixed evidence for an early bias in depression, mirroring the behavioral literature. The posterior N200 component in the present study can be distinguished from a fronto-central N200 component that is thought to be associated with effortful processing (such as inhibition and conflict monitoring; e.g., Donkers and van Boxtel, 2004), and which typically peaks later in time (between 200 and 500 ms; e.g., Thomas et al., 2007; Enriquez-Geppert et al., 2010).

P300 amplitude is associated with context updating and event categorization processes (e.g., Donchin and Coles, 1988) as well as increased resource engagement (e.g., Yee and Miller, 1994). P300 amplitude is often modulated by emotional arousal, with larger amplitude for emotionally arousing than neutral stimuli interpreted as reflecting more attentional resources devoted to processing these stimuli (e.g., Schupp et al., 2004; Fischler and Bradley, 2006; Thomas et al., 2007; Li et al., 2007; Franken et al., 2009; Sass et al., 2010). In anxiety, P300 amplitude has been larger for unpleasant than neutral words (Li et al., 2007) and for emotionally arousing (pleasant and unpleasant) than neutral words (no difference between anxious and control participants, Sass et al., 2010) in the context of an emotion-word Stroop task. In comorbid anxiety and depression, no P300 effects were found in a visual oddball task including happy, sad, and neutral faces (Rossignol et al., 2008). Thus, inconsistent P300 amplitude evidence exists for a later attentional resource allocation bias for unpleasant or emotionally arousing stimuli in both depression and anxiety.

In order to address questions concerning the role of emotional valence, emotional arousal, co-occurring anxiety, and gender on the nature and timing of attentional biases in depression, the present study examined ERPs in three groups of participants: depression only (scored high on an 8-item Mood and Anxiety Symptom Questionnaire (MASQ) anhedonic depression measure and low on anxiety measures), combined (scored high on Penn State Worry Questionnaire (PSWQ) and MASQ measures of anxiety and high on an 8-item MASQ anhedonic depression measure), or control (scored low on anxiety and depression measures. The control group was included in order to investigate whether patterns of preferential attentional processing of unpleasant or emotionally arousing stimuli were specific to the depression only or combined groups. Pleasant and unpleasant stimuli were matched on emotional arousal level.

Critical differences in the nature and timing of attention to emotion were explored in the three groups. (1) It was unclear whether early effects would be present in the depression only or combined group, given inconsistency in the literature of early effects in depression and a general lack of consideration of co-occurring anxiety. If attentional bias is relatively automatic and specific to unpleasant stimuli in depression with and without anxiety, then P100 and/or posterior visual N200 amplitude should be larger for unpleasant than pleasant words. Alternatively, if initial bias is more broadly associated with emotional arousal, then P100 and/or posterior visual N200 amplitude should be larger for both unpleasant and pleasant than neutral words. (2) Later effects were predicted to occur in depression with and without co-occurring anxiety, given a literature documenting later effects in both depression and anxiety. If later, more strategic processing is specific to unpleasant stimuli in depression with and without co-occurring anxiety, then P300 amplitude should be larger for unpleasant than pleasant words. Alternatively, if later, more strategic processing is more broadly associated with emotional arousal, then P300 amplitude should be larger for both unpleasant and pleasant than neutral words. (3) If unpleasant or emotionally arousing words are attended followed by avoidance, than P100 and/or posterior visual N200 amplitude should be larger and P300 amplitude smaller for unpleasant or emotionally arousing stimuli in depression with and without co-occurring anxiety. (4) Gender may moderate early or later attentional processing and was included as an exploratory variable in present analyses. (5) P100, posterior visual N200, and P300 effects may be more pronounced over right posterior regions in the combined group and less pronounced in the depressed group, consistent with previous research regarding regional EEG, ERP, and fMRI patterns in depression with and without co-occurring anxiety (e.g., Heller and Nitschke, 1998; Keller et al., 2000; Sumich et al., 2006; Engels et al., 2010).

MATERIALS AND METHODS

Much of the methods section, including stimuli and experimental design, EEG recording procedure, and data reduction and analysis procedures overlap with Sass et al. (2010) and to some extent with Fisher et al. (2010) and Stewart et al. (2010). Method details are included here in slightly modified (not verbatim) form from Sass et al. (2010).

All participants provided informed consent, and all procedures were approved for ethical considerations by the University of Illinois Institutional Review Board. A total of 4,457 college undergraduates were screened for the study. Participants were 92 (49 female) paid volunteers (mean age = 19.0, SD = 1.9) recruited via group questionnaire screening sessions[1],[2]. Participants were 82% European American and were classified as high anhedonic depression ($n = 24$; 11 female), combined ($n = 27$; 19 female), or control ($n = 41$; 19 female) on the basis of responses on the PSWQ and MASQ. Compared to the total sample screened for the study, the anhedonic depression group scored at or above the 80th percentile on an eight-item depressed-mood subscale (Nitschke et al., 2001) of the MASQ Anhedonic Depression scale, shown to predict diagnostic category membership (Bredemeier et al., 2010). The anhedonic depression group also scored at or below the 50th percentile on the PSWQ and on the MASQ Anxious Arousal scale. The combined group scored at or above the 80th percentile on all three scales. The control group scored at or below the 50th percentile on all three scales. **Table 1** presents the means and standard deviations of the groups for the questionnaire measures.

The Structured Clinical Interview for Axis I Disorders, Non-Patient Edition (First et al., 1997), was administered to all participants to assess to what degree selecting participants based on the questionnaire measures yielded significant Axis I disorders. Participants were not selected based on DSM diagnosis, because DSM-defined anxiety and depression disorders include (to varying degrees) anxious apprehension, anxious arousal, and anhedonic depression. Selecting participants based on DSM category would likely result in missed sensitivity in uncovering brain mechanisms (e.g., see Engels et al., 2007, 2010; Herrington et al., 2010) distinctly associated with attentional processing as a function of the transdiagnostic dimensions of anhedonic depression and anhedonic depression co-occurring with anxious arousal and anxious apprehension.

Lifetime DSM-IV-TR (American Psychiatric Association, 2000) diagnoses were determined by a trained clinical psychology doctoral student interviewer and reviewed by a consensus team consisting of a second trained clinical psychology doctoral student interviewer and a clinical faculty supervisor (Gregory A. Miller). Although participants were not selected based on DSM-IV-TR depression or anxiety disorder diagnosis, approximately 25% of the individuals in the depression only and 59% of the combined group met criteria for a lifetime history of major depressive disorder (MDD) and/or an anxiety disorder. Specifically, of the 24 individuals in the depressed group, six met full criteria for a lifetime history of MDD. Of the 27 individuals in the combined group, 11 had a lifetime history of MDD (three were in a current major depressive episode) and 13 had a lifetime history of an anxiety disorder (anxiety disorder not otherwise specified, generalized anxiety disorder, obsessive compulsive disorder, posttraumatic stress disorder, social phobia). Control participants did not meet criteria for any lifetime DSM-IV-TR disorder. Therefore, the questionnaire measures used to screen individuals for the present combined and depressed groups provided a substantial number of participants meeting criteria for DSM-IV-TR diagnoses of MDD and/or an anxiety disorder.

The groups did not differ in age. All participants were determined to be right-handed by the Edinburgh Handedness Inventory (Oldfield, 1971), had self-reported normal color vision, and

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[1] Ninety-six participants qualified for the present study. Of these, two were disqualified due to missing RT data, and two were disqualified due to having one or more ERP component scores (P100, N200, or P300 amplitude) > 3 SD from the mean.

[2] Participants from the present study overlapped with three previous ERP investigations. Fifty-nine participants in the present sample were included in Stewart et al. (2010). Stewart et al. (2010) investigated attentional biases as a function of approach and withdrawal anger styles and did not focus on depression with and without co-occurring anxiety. That study also focused on different ERP components than the present study, including N100, P200, N200, and N400 amplitude at fronto-central and central sites, not investigated in the present study. The only overlapping ERP component investigated in Stewart et al. (2010) and the present study is P300 amplitude, with different sensors used. Forty-two participants were shared with Fisher et al. (2010), who investigated emotional information processing as a function of perceived emotional intelligence and focused on different ERP components including P100 and P300 amplitude (defined using different temporal scoring windows and different sensors than the present study), and P200 and slow wave (neither used in the present study). Finally, 32 controls from the present study were used in Sass et al. (2010), which focused on ERP evidence of the timing of attention to emotional information in pure anxious apprehension vs. pure anxious arousal.

were native speakers of English. Participants were informed of the procedures of the study and given a laboratory tour. Exclusion criteria included DSM-IV-TR alcohol or drug abuse or dependence within the past 3 months, experience with electroshock therapy, multiple sclerosis, epilepsy, current pregnancy, claustrophobia, moderate to severe head injury, loss of consciousness for 10 min or more, and contraindications for MRI participation (including metal present in the body).

STIMULI AND EXPERIMENTAL DESIGN

STIM software (James Long Company, Caroga Lake, NY, USA) controlled word presentation and button-press response recording. Neutral blocks were interleaved between blocks of pleasant and unpleasant emotion words. Two hundred fifty-six words were delivered to participants in 16 blocks (four pleasant, eight neutral, four unpleasant) of 16 trials. A word was presented in the center of the computer screen for 1500 ms at the beginning of each trial, followed by a fixation cross for 275 to 725 ms (onset-to-onset ITI 2000 +/− 225 ms). Each trial consisted of a single word presented in one of four colors (red, yellow, green, blue) on a black background. Each color appeared equally often with each word type (pleasant, neutral, unpleasant). Participants completed an emotion-word Stroop task in both EEG and fMRI sessions that were counterbalanced to precede each other equally often. The present report is based on the EEG data. Participants were randomly assigned one of eight possible orders. In half of the presentation orders, the first and third blocks were neutral words, and the second and fourth blocks were pleasant and unpleasant, with valence order counterbalanced across participants. In the remaining half of the presentation orders the first and third blocks were either pleasant or unpleasant emotion words and the second and fourth blocks were neutral words. These eight presentation orders were designed to avoid order effects by ensuring that the neutral and emotional words preceded each other equally often. A given word was presented only once per session to control stimulus familiarity. Each color appeared four times within a block and no more than two trials featuring the same color appeared in a row. A brief rest period occurred after every fourth block. In addition to 16 word blocks, four fixation blocks were presented: one at the beginning, one at the end, and two in the middle of the experiment. Specifically, a bright fixation cross was presented for 1500 ms instead of a word, followed by a dimmer fixation cross that always followed word stimuli.

Sixty-four pleasant, 64 unpleasant, and two sets of 64 neutral words were carefully selected from the Affective Norms for English Words set (ANEW; Bradley and Lang, 1999) on the basis of norms for valence, arousal, and frequency of usage in the English language (Bradley and Lang, 1999). Pleasant and unpleasant words were chosen to be high in arousal (arousal mean = 6.53 for pleasant, 6.56 for unpleasant, and 3.81 for neutral stimuli). Words ranged from three to eight letters in length and were presented in capital letters using Tahoma 72-point font. The visual display was 1.35 m from the participant's eyes for a vertical span of 1.5° and a horizontal span of 2.5–9.3°. The average luminance values of the words presented in red, yellow, green, or blue were 15, 72, 45, and 20 lux, respectively. Verbatim instructions were read by experimenters to ensure consistency. Each participant performed 32 practice trials before the actual task began. There were four buttons on the response box, with each button corresponding to a color. The left middle and index fingers were used to indicate red and green, respectively. The right middle and index fingers were used to indicate yellow and blue, respectively. All participants understood task instructions and the mapping between colors and buttons after the practice trials were completed.

EEG RECORDINGS

Participants were seated in a quiet room that was connected via intercom to an adjacent room where the experimenter controlled EEG data collection and stimulus presentation. A custom Falk Minow 64-channel cap with equidistantly spaced Ag/AgCl electrodes was used to record EEG. The left mastoid was the online reference for all EEG and electrooculogram (EOG) sites. Vertical and horizontal EOG was recorded with electrodes placed above and below each eye and near the outer canthus of each eye for off-line eye-movement artifact correction of EEG. Electrode impedances were below 20 Kohms. Data were digitized at 250 Hz, and half-power amplifier bandpass was 0.1–100 Hz. A Zebris ELPOS digitizer recorded electrode positions (Zebris Medizintechnik, Tübingen, Germany).

DATA REDUCTION

Muscle, movement, and other artifacts were removed manually. Eye blinks were corrected using Brain Electrical Source Analysis (BESA 5.1.8) software (Berg and Scherg, 1994). If a channel was off-scale for more than 10% of trials, all trials for that channel for a given subject were removed from analyses. All channels for epochs in which a single channel was off-scale were discarded. Artifact correction did not differentially affect the number of remaining pleasant, neutral, and unpleasant trials, Emotion $F(2,89) = 1.45$, $p = 0.237$[3], and did not differ by group or gender. Only correct trials were averaged for the pleasant, neutral, and unpleasant conditions. The electrode configuration was transformed using spherical spline interpolation to BESA's standard 81-channel montage (Perrin et al., 1989), reflecting the 10–10 system. An average reference (the mean voltage over the 81 standard virtual scalp electrodes) was computed for each time point, and data were exported from BESA. Each channel was baseline-adjusted in custom Matlab software by subtracting the average amplitude for the 200 ms before stimulus onset.

Three ERP components were scored: P100 (88–128 ms), N200 (160–240 ms), and P300 (448–580 ms). A 101-weight, 0.1–20 Hz digital filter was used for P100 and N200, and a 101-weight, 0.1–8 Hz digital filter was used for P300 (Cook and Miller, 1992; Nitschke et al., 1998; Edgar et al., 2005). A combination peak/area measure was used to avoid spurious peaks driving amplitude measures. Voltage 48 ms around the peak was averaged for the early (P100, N200) components, and voltage 96 ms around the peak was averaged for the late (P300) component. Latency associated with each peak was also recorded.

Sites for P100 and N200 were chosen based on examination of current source density (CSD) estimates across conditions and

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[3] Because the number of neutral trials was double the number of each emotion condition, this analysis involved dividing the number of neutral trials by two and then comparing the number in each condition after artifact removal.

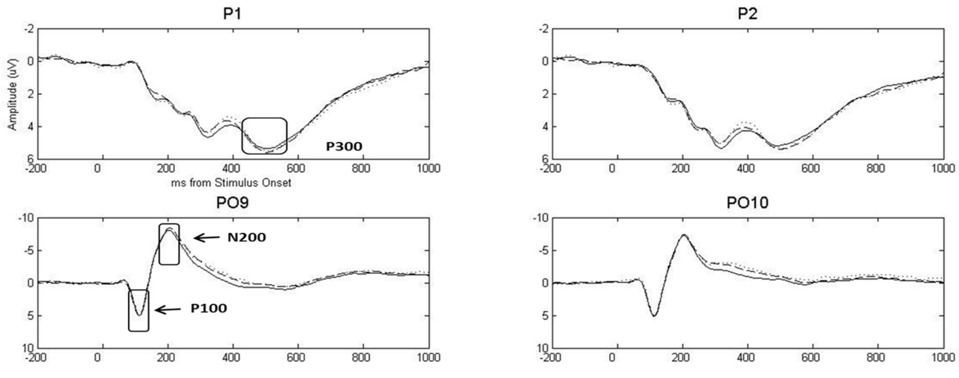

FIGURE 1 | Grand-average event-related potential waveforms for representative posterior sensors, highlighting P100, N200, and P300. Dotted, solid, and dashed lines represent pleasant, neutral, and unpleasant stimuli, respectively. Stimulus onset was at time = 0 ms.

groups. CSD estimates were used as an estimate of the contribution of the immediately underlying cortical surface to the recorded electrode signal (Nunez et al., 1999). Voltage associated with amplitude values at sites where CSD activity was maximal for P100 (P7, P8, PO7, PO8, O1, O2) and N200 (P7, P8, P9, P10, PO7, PO8, PO9, PO10) were averaged together by hemisphere for these bilaterally distributed components. Voltage associated with amplitude values at sites for P300 (P1, P2, P3, P4, CP1, CP2, CP3, CP4) were averaged by hemisphere. Sites for P300 were chosen based on previous emotion-word Stroop studies (e.g., van Hooff et al., 2008; Sass et al., 2010) and inspection of the grand-average waveforms where effects were maximal.

RESULTS

BEHAVIORAL PERFORMANCE

Pleasant, neutral, and unpleasant RT was analyzed for correct trial responses between 350 and 1400 ms ($M = 671$ ms, SD = 106 ms). 4.5% of RT data were lost due to the RT criterion of < 350 ms or > 1400 ms. Performance accuracy was high (mean number of errors = 4.0, SD = 4.1, of 256 trials). Participants were excluded from EEG analyses if they were excluded from RT analyses, and from RT analyses if they were excluded from EEG analyses. A Group (depression only, combined, control) x Gender (female, male) x Emotion (pleasant, neutral, unpleasant) multivariate analysis of variance (MANOVA) was conducted. Levels of the emotion factor were ordered pleasant, neutral, and unpleasant in order to take advantage of *a priori* orthogonal linear (valence: comparing pleasant with unpleasant) and quadratic (arousal: comparing pleasant and unpleasant with neutral) univariate trends on the emotion factor. All tests were 2-tailed using an alpha level of 0.05 and *p*-values reflect the Huynh–Feldt correction for sphericity where appropriate. No main effects or interactions were significant for RT.

EARLY EMOTION-WORD PROCESSING

A Group (depression only, combined, control) x Gender (female, male) x Emotion (pleasant, neutral, unpleasant) x Hemisphere (left, right) MANOVA including linear and quadratic trends (described above) was conducted separately for P100 and N200 (see **Figure 1** for grand-average waveforms for representative channels). Reported *p*-values reflect the Huynh–Feldt correction for sphericity where appropriate.

P100 amplitude

P100 was larger over right than left hemisphere, $F(1,86) = 19.67$, $p < 0.001$, and a Group main effect, $F(2,86) = 3.93$, $p = 0.023$, was qualified by a Group x Hemisphere interaction, $F(2,86) = 3.09$, $p = 0.050$. Separate Group ANOVAs were done for each hemisphere. The Group effect was significant over left, $F(2,89) = 3.34$, $p = 0.040$, and right, $F(2,89) = 4.29$, $p = 0.017$, hemispheres, dissected with orthogonal Group contrasts for each hemisphere. The first contrast compared the combined with the depressed group, and the second contrast pooled depressed groups and compared them with controls. P100 amplitude was smaller over left hemisphere in the depressed than combined group, $p = 0.015$, and the combined group did not differ from controls (see **Figure 2**). P100 amplitude was smaller over right hemisphere in both depressed

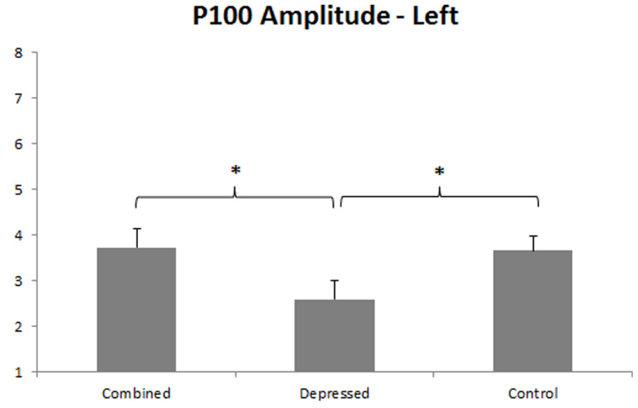

FIGURE 2 | P100 amplitude in left hemisphere. Error bars represent 1 SE. Asterisks indicate significant differences ($p < 0.05$), with reduced P100 amplitude in the depressed compared to the combined group, and in the depressed compared to the control group.

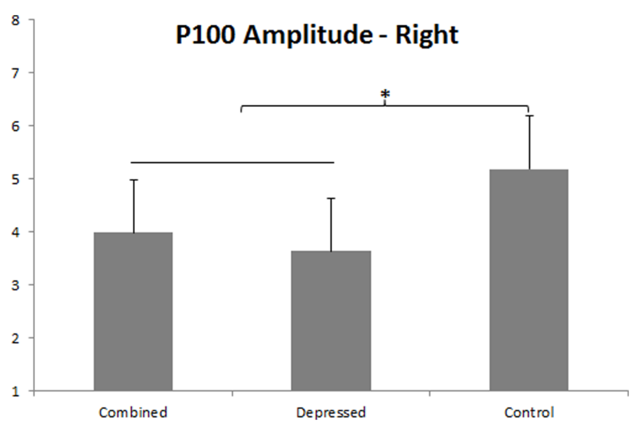

FIGURE 3 | P100 amplitude effect in right hemisphere. Error bars represent 1 SE. The asterisk indicates ($p < 0.05$), that both depressed groups showed reduced P100 amplitude compared to the control group.

groups compared to controls, $p = 0.005$, and the depressed and combined group did not differ from one another (see **Figure 3**).

N200 amplitude

N200 amplitude was larger over left than right hemisphere, $F(1,86) = 7.59$, $p = 0.007$. A main effect of Emotion, $F(2,86) = 3.52$, $p = 0.032$, was evaluated with linear and quadratic contrasts. N200 amplitude was larger for emotionally arousing than neutral words, $F(1,86) = 11.43$, $p = 0.010$. In addition, a Gender x Emotion effect, $F(2,86) = 3.40$, $p = 0.036$, was qualified by a Group x Gender x Emotion trend, $F(4,86) = 2.32$, $p = 0.059$. This latter interaction was dissected with Gender x Emotion ANOVA analyses for each group, following hypotheses 1, 3, and 4. A Gender x Emotion interaction was present in the combined, $F(2,25) = 3.83$, $p = 0.028$, and control, $F(2,39) = 4.51$, $p = 0.013$, but not depressed group. This interaction was dissected with separate Emotion ANOVAs for each gender within the combined and control groups using linear and quadratic contrasts. In the combined group, N200 amplitude was larger for unpleasant than pleasant words in women but not men, linear $F(1,18) = 5.00$, $p = 0.038$. In the control group, N200 amplitude was larger for pleasant than unpleasant words in men but not women, linear $F(1,21) = 9.65$, $p = 0.005$ (see **Figure 4**).

LATER EMOTION-WORD PROCESSING

P300 amplitude

A Group x Gender x Emotion x Hemisphere MANOVA including linear and quadratic trends (described above) was conducted for P300 amplitude. An Emotion effect, $F(2,86) = 3.95$, $p = 0.021$, was followed up with linear and quadratic contrasts. As expected, P300 amplitude was larger for pleasant and unpleasant than neutral words, quadratic Emotion $F(1,86) = 7.22$, $p = 0.009$. A Gender x Hemisphere interaction, $F(1,86) = 8.84$, $p = 0.004$, was investigated with separate Hemisphere ANOVAs for each gender. Only men had larger P300 amplitude over the right than left hemisphere, $F(1,42) = 4.72$, $p = 0.036$. A Group x Hemisphere effect, $F(1,86) = 3.51$, $p = 0.034$, was qualified by a Group x Emotion x Hemisphere interaction, $F(4,86) = 2.44$, $p = 0.049$, dissected with separate Emotion x Hemisphere ANOVAs for each group, following hypotheses 2, 3, and 5. A linear Emotion x Hemisphere interaction, $F(2,26) = 3.32$, $p = 0.044$, emerged in the combined group only. This interaction was dissected with separate Emotion ANOVAs for each hemisphere within the combined group using linear and quadratic contrasts on the emotion factor. P300 amplitude was larger for unpleasant than pleasant stimuli over the right

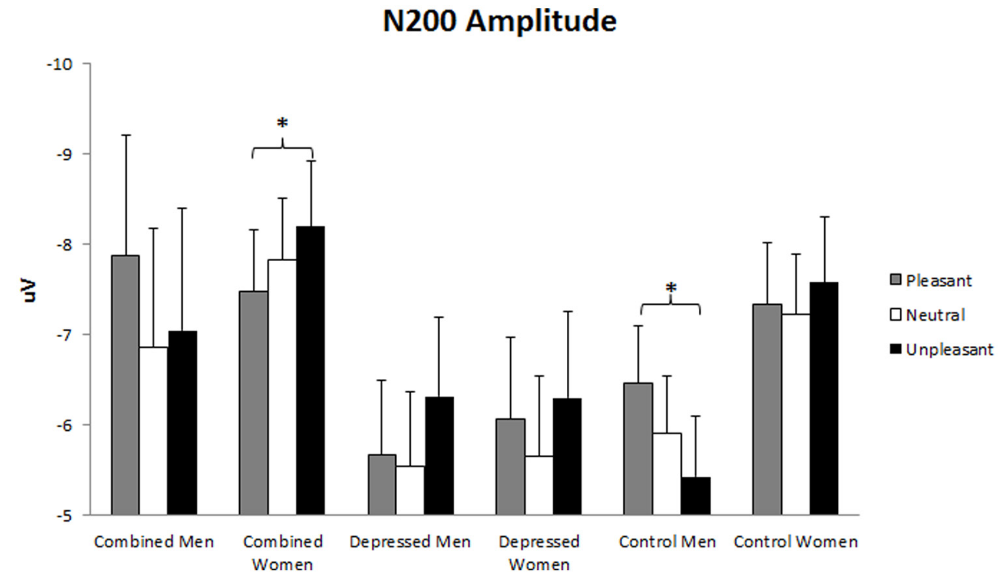

FIGURE 4 | N200 amplitude. Error bars represent 1 SE. N200 amplitude valence effect in combined women and control men. The asterisks represent significant differences with $p < 0.05$ in combined women (unpleasant > pleasant) and control men (pleasant > unpleasant).

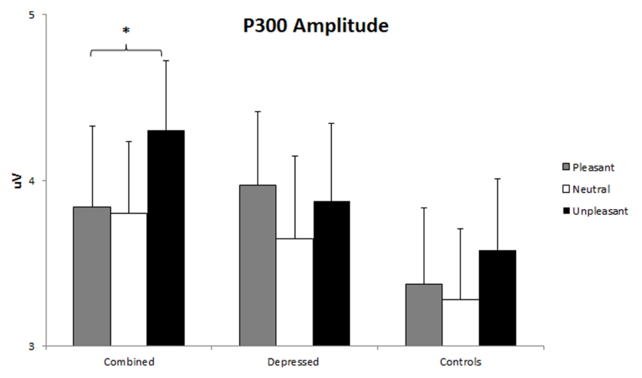

FIGURE 5 | P300 amplitude over right hemisphere. Error bars represent 1 SE. P300 amplitude valence effect (unpleasant > pleasant) over right hemisphere sensors (P1, P3, CP1, CP3) in the combined group. The asterisk represents $p < 0.05$ in the combined group (unpleasant > pleasant).

but not left hemisphere, linear $F(1,25) = 4.35$, $p = 0.047$ (see **Figure 5**).

DISCUSSION

Previous literature provides inconsistent evidence regarding the nature and timing of attentional biases in depression, and unexamined co-occurring anxiety may contribute to this inconsistency. The present study investigated the role of co-occurring anxiety and whether early, relatively automatic, or later, less automatic phenomena manifested in the ERP evidence would support attentional bias in favor of unpleasant or emotionally arousing stimuli in depression. Gender was included as an exploratory variable.

Evidence of early biased processing of unpleasant or emotionally arousing stimuli was absent in the depression-only group. The combined group, however, showed evidence of both an early and a later attentional bias in favor of unpleasant information. Posterior N200 amplitude was larger in women with both depression and anxiety symptoms for unpleasant than for pleasant stimuli, presumably reflecting relatively automatic processing of unpleasant words, consistent with other emotion-word Stroop studies finding modulation of an EPN for emotional compared to neutral stimuli (Franken et al., 2009; Sass et al., 2010). That an early effect modulated by emotion was not evident in the depression-only group suggests that high levels of anxiety are necessary to elicit evidence of attentional bias in depression, consistent with findings of Markela-Larenc et al. (2011).

The combined depression and anxiety group also showed evidence of biased processing later in the trial (larger P300 amplitude for unpleasant than pleasant over right hemisphere), reflecting biased processing of unpleasant information at a later, more elaborative stage. This finding is consistent with emotion-word Stroop studies showing P300 amplitude modulation by emotion (e.g., Li et al., 2007; Franken et al., 2009; Sass et al., 2010). This finding is also consistent with studies finding greater right-lateralized posterior brain activity in depression co-occurring with anxiety (e.g., Engels et al., 2010).

The later preferential attention to unpleasant information seen in the combined group was not observed in the depression-only group. The present depression sample was unusual in that participants were selected only if they scored high on a measure of anhedonic depression and low (in the control group range) on two measures of anxiety, allowing the relatively pure influence of anhedonic depression to be examined. Given present evidence of attentional bias effects in the combined but not depressed group, results indicate that attentional bias effects sometimes found in depressed samples may be due to co-occurring anxiety.

The depression-only group produced smaller P100 amplitude over left hemisphere than the combined group, consistent with EEG studies showing less left than right hemisphere activity in depressed compared to anxious individuals. These findings are also consistent with fMRI results revealing reduced left dorsolateral prefrontal cortical activity in depression when co-occurring anxiety is taken into account (Engels et al., 2010; Herrington et al., 2010).

In addition, both depressed groups showed reduced P100 amplitude over right hemisphere compared to controls that was not specific to emotional stimuli. This result suggests that depressed groups' detection of visual stimuli is generally dampened at this early time point (~100 ms), despite early (~200 ms), and later (~500 ms) ERP effects showing differential detection and processing of unpleasant stimuli in the combined group. This effect is in contrast to early P100 amplitude effects modulated by anxiety in previous emotion-word Stroop studies (Li et al., 2007; Sass et al., 2010), suggesting that high levels of anxiety and lower levels of depression are necessary to elicit early (~100 ms) emotion effects. P100 amplitude was not modulated by emotion in the present study, consistent with a number of previous studies failing to find early emotion effects in depression (e.g., Rossignol et al., 2008; Gotlib and Joormann, 2010).

There are several caveats and limitations associated with the present study. First, RT evidence of attentional bias was not obtained in the present study. RT is the end-stage of a number of different brain processes, and a lack of RT effects despite ERP effects in the present sample indicates biased processing at stages prior to response execution. This pattern of effects is consistent with and informs studies finding small or no RT effect sizes using the emotion-word Stroop task in samples with subclinical depression (for review, see Epp et al., 2012) and anxiety (Koven et al., 2003). Second, a larger sample and equal gender distribution would be better suited to examining gender differences in emotional information processing that may exist in the depression-only and combined groups. Third, because the Group x Gender x Emotion N200 amplitude effect was at trend level, this result should be interpreted tentatively pending replication. Fourth, in averaging across conditions for ERP analyses, pleasant and unpleasant trials were averaged separately before pooling them for comparison to neutral using quadratic contrasts. It is possible that the lower number of trials contributing to the pleasant and unpleasant ERP averages ($n = 64$ for each emotion condition) would contribute to higher amplitude scores than for the neutral averages ($n = 128$), due to the possibility of more noise in the averages with fewer trials. This issue is only relevant to comparisons of emotionally arousing with neutral stimuli and not to comparisons of pleasant with unpleasant stimuli (thus not affecting the main findings in the present paper, of greater N200 and P300 amplitude for unpleasant than pleasant stimuli in the combined group). Given that signal-to-noise reduction is a function of a ratio of the square root of the number of trials comprising an average, and 64 is a reasonable number of trials to begin with, the difference in the number of trials contributing to the emotionally arousing and neutral averages should not have much differential impact on noise reduction. Furthermore, present emotional arousal main effects for posterior visual N200 and P300 amplitude are consistent with previous research employing an equal number of pleasant, unpleasant, and neutral trials (e.g., Franken et al., 2009), suggesting that the emotional arousal effects found in the present study are not a function of differing trial numbers. Finally, the use of a block design in the present study is helpful in eliciting more sustained emotion effects as might occur in everyday emotional contexts. A block design may not be optimal in distinguishing early, more automatic processing from later, more strategic processing, as top-down expectancy effects may influence early processing (e.g., see van Hooff et al., 2008, for a similar discussion).

Present results can inform interventions for depression with and without co-occurring anxiety. Computerized attention-training programs have been successful in modifying attentional bias and reducing symptoms of depression and anxiety (for meta-analyses see Hakamata et al., 2010; Hallion and Ruscio, 2011). In a study targeting mild to moderate depression with mild levels of co-occurring anxiety, participants in an attention-training condition showed a greater reduction in depressive symptoms than did participants receiving a control intervention (Wells and Beevers, 2010). Another study found small improvements in symptom severity after computerized training among students showing mild depression symptoms, but symptoms worsened in those with moderate to severe depression (Baert et al., 2010). In this latter study, co-occurring anxiety was in the mild to moderate range, leaving open the question of whether individuals without co-occurring anxiety would show similar effects. Future research should build on these initial studies, targeting both earlier and later attentional biases and systematically examining the role of co-occurring anxiety.

The present study indicates that the nature and time course of attention prompted by emotional stimuli differentiates depression with and without combined anxiety, and both depressed groups from controls. In the absence of bias effects in the depression-only group, the combined group showed evidence of both an early and a later attentional bias in favor of unpleasant information. Co-occurring anxiety therefore appears to be an important factor in inconsistent results in previous studies regarding attentional biases in depression. Present findings support previous recommendations for careful experimental control of co-occurring anxiety and for including gender and hemisphere when investigating behavioral and brain correlates of attentional biases in depression. Systematic examination of these issues can yield insights into cognition-emotion phenomena in depression that may improve understanding of etiology and treatment, providing valuable directions for future research.

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Neural correlates of suspiciousness and interactions with anxiety during emotional and neutral word processing

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Suspiciousness is usually classified as a symptom of psychosis, but it also occurs in depression and anxiety disorders. Though how suspiciousness overlaps with depression is not obvious, suspiciousness does seem to overlap with anxious apprehension and anxious arousal (e.g., verbal iterative processes and vigilance about environmental threat). However, suspiciousness also has unique characteristics (e.g., concern about harm from others and vigilance about social threat). Given that both anxiety and suspiciousness have been associated with abnormalities in emotion processing, it is unclear whether it is the unique characteristics of suspiciousness or the overlap with anxiety that drive abnormalities in emotion processing. Event-related brain potentials were obtained during an emotion-word Stroop task. Results indicated that suspiciousness interacts with anxious apprehension to modulate initial stimulus perception processes. Suspiciousness is associated with attention to all stimuli regardless of emotion content. In contrast, anxious arousal is associated with a later response to emotion stimuli only. These results suggest that suspiciousness and anxious apprehension share overlapping processes, but suspiciousness alone is associated with a hyperactive early vigilance response. Depression did not interact with suspiciousness to predict response to emotion stimuli. These findings suggest that it may be informative to assess suspiciousness in conjunction with anxiety in order to better understand how these symptoms interact and contribute to dysfunctional emotion processing.

Keywords: suspiciousness, anxiety, emotional stroop, paranoia, event-related brain potentials

INTRODUCTION

Anxiety, depression, and psychotic disorders are often comorbid (e.g., Sands and Harrow, 1995; Braga et al., 2004; Baillie and Rapee, 2005). Researchers have increasingly emphasized the importance of determining which symptoms are unique to each disorder or shared with other disorders and how these symptoms interact (e.g., Braga et al., 2004). Suspiciousness (or paranoia in its extreme) is a symptom that involves the exaggerated tendency to believe that other people intend harm, especially to oneself. It is believed to exist on a continuum (Combs et al., 2002) and is associated with anxiety and depression in the general population (Martin and Penn, 2001; Messias and Kirkpatrick, 2001; Ellett et al., 2003; von Gemmingen et al., 2003; Combs and Penn, 2004) and in schizophrenia-spectrum disorders (Kirkpatrick et al., 1996; Freeman and Garety, 1999; Messias et al., 2001; Candido and Romney, 2002; Goodwin et al., 2002; Drake et al., 2004; Spitznagel and Suhr, 2004; Huppert and Smith, 2005). Although rarely investigated, a better understanding of the role of suspiciousness in the context of anxiety and depression could foster improved definition, classification, and treatment of psychological disorders.

Suspiciousness may be a manifestation or consequence of severe anxiety and depression. This possibility is consistent with Foulds and Bedford's (1975) hierarchical model of psychopathology in which individuals with disorders at higher levels (e.g., psychosis) have all the symptoms of the lower levels (e.g., mood disorders and anxiety). They proposed that comorbidity exists because severe symptoms at higher levels are episodic and therefore do not camouflage lower-level symptoms at all times. The model is also supported by the fact that individuals with one disorder are at increased risk for meeting criteria for another diagnosis, suggesting that the presence of certain symptoms makes more severe symptoms more likely. Since suspiciousness is more often associated with psychosis than with anxiety and depression, it may be a "higher-level" symptom that may develop as a consequence of depression and/or anxiety.

Other models also propose that emotional disorders have common trait characteristics, such as general distress or negative affect

(Clark and Watson, 1991; Zinbarg and Barlow, 1996; Brown et al., 1998). As these models are based on assessments of depression and anxiety, and suspiciousness is usually considered characteristic of psychosis, suspiciousness has not been studied in relation to these models. However, given the comorbidity outlined above and evidence that emotional disorders and psychosis are not as distinct as classification systems imply (Freeman and Garety, 2003), suspiciousness, like negative affect, may be another factor common to these disorders. In light of Foulds and Bedford's (1975) model and the other models cited above, it is possible that suspiciousness is a dimensional phenomenon common to both anxiety and depression that exacerbates the symptoms of these disorders. If so, one may be able to target suspiciousness in treatment in order to reduce the severity of depression, anxiety or psychosis.

An association between suspiciousness and anxiety and depression is not unexpected, considering their common impairment in processing emotion information (e.g., Bentall and Kaney, 1989; Gur et al., 1992; Green and Phillips, 2004). However, anxiety appears more related to suspiciousness than to depression, given similar misperceptions and attributional styles. For example, both paranoia and anxiety involve a tendency to misinterpret threat which can lead to emotional arousal. However, the emotional reaction to the panic symptoms may be vague or inaccurate, leading to inaccurate judgments and delusional or peculiar beliefs (e.g., suspiciousness) about the arousal (Maher, 1974, 1988; Clark, 1986; Boden and Berenbaum, 2007). This process is supported by the fact that suspiciousness is negatively associated with clarity of emotions (e.g., the ability to identify an experienced emotion; Berenbaum et al., 2006) and positively associated with boredom proneness, which in turn is associated with hyperfocus on one's feelings (von Gemmingen et al., 2003). In addition to a lack of clarity regarding emotions, paranoid individuals tend to blame external rather than internal sources for negative events (for a review, see Kinderman and Bentall, 1998), specifically the actions of other people (Fear et al., 1996; Kinderman and Bentall, 1997, 2000). Thus, suspiciousness may be due in part to an inability to accurately identify emotions associated with arousal in combination with an external attribution bias.

There also seems to be a cognitive component of suspiciousness that may overlap with anxiety disorders characterized by worry. For example, both suspiciousness and anxiety due to worry involve anticipation of danger (Freeman and Garety, 2003). Anxiety also contributes to the strength of suspiciousness or paranoia (i.e., conviction of persecutory delusions, Garety et al., 2005) and is a predictor of paranoia in a college student sample (Tone et al., 2011). In addition, cognitive-behavioral therapy for anxiety disorders can reduce paranoid beliefs (Key et al., 2003).

Depression and suspiciousness appear more distinct. Individuals with depression or suspiciousness do share a tendency toward hopelessness or powerlessness (Alloy and Clements, 1998) but may have different attributional styles. Paranoia is sometimes associated with attributing positive events to internal sources (e.g., Zigler and Glick, 1988; Chadwick and Trower, 1997) and attributing negative events to external sources (Fear et al., 1996; Kinderman and Bentall, 1997; Fornells-Ambrojo and Garety, 2009), whereas individuals who are depressed make negative internal attributions, in which they blame themselves for negative events (Wall and Hayes, 2000). Consequently, the association between suspiciousness and depression appears to be weaker, more indirect, or more complex than the association between suspiciousness and anxiety.

In summary, suspiciousness and anxiety share similar processes. As outlined in Grupe and Nitschke (2013), there are five processes common to anxiety disorders. It is likely that suspiciousness and anxiety associated with worry [e.g., generalized anxiety disorder (GAD)] are both associated with two of these processes: the tendency to inflate the cost and probability of threat and behavioral and cognitive avoidance. Suspiciousness and anxiety associated with arousal likely share hypervigilance under uncertainty of threat and heightened reactivity to threat. In combination, suspiciousness is likely a consequence of misinterpreting threat which leads to arousal. The inability to identify the source of these emotions in response to arousal in combination with an external attribution bias leads to apprehension and further misinterpretation of threat (anticipation of danger regarding other people). Thus, individuals who have tendency toward suspiciousness likely alternate between anxiety types (arousal vs. apprehension).

It is well-established that anxiety and depression are each associated with deficits in emotion processing (e.g., Gur et al., 1992; Heller et al., 1997; Surguladze et al., 2004; Engels et al., 2007, 2010), but suspiciousness has also been associated with deficits in emotion processing. Impaired fear processing (e.g., rating neutral antecedents of events as fear-provoking) was correlated with suspiciousness in patients with schizophrenia (Trémeau et al., 2009). This relationship is similar to the tendency of individuals with anxiety to anticipate danger in situations that others perceive as harmless. Another study indicated that individuals with persecutory delusions and a subclinical group with high paranoia scores had poorer emotion perception than did moderate and low subclinical paranoia groups. In addition, the high subclinical group showed more interference from words with paranoid content on an emotion-word Stroop task (Combs et al., 2006; see also Bentall and Kaney, 1989). This reaction to negative words has also been observed in numerous studies of anxiety (e.g., Mathews and MacLeod, 1985; Fox, 1993; Egloff and Hock, 2003). Given the clinical and conceptual overlap between suspiciousness and anxiety, it is important to determine the degree to which these two symptom dimensions reflect similar processes. In addition, given that dysfunctional emotional information processing contributes to psychopathology in general (e.g., Freeman and Garety, 2003), to the maintenance of anxiety and depression (e.g., Turk et al., 2005) and has been associated with paranoia (see above), understanding the effects of suspiciousness on emotion processing may clarify the mechanisms involved in anxiety and depression onset and maintenance as well as point to avenues for more effective intervention.

The emotion-word Stroop task is useful for investigating the role of suspiciousness in emotion processing in anxiety and depression. Behavioral interference from threat-related words has been demonstrated in this task (for review, see Williams et al.,

1996) in anxiety (e.g., Mathews and MacLeod, 1985; Fox, 1993; Egloff and Hock, 2003), schizophrenia-spectrum disorders (e.g., Bentall and Kaney, 1989; Combs and Penn, 2004; Mohanty et al., 2005), and depression (Williams et al., 1996; Lim and Kim, 2005). Indices of neural activity have provided valuable information about stages of processing during this task. For example, N200 and P200 components of the event-related brain potential (ERP) have been associated with early attention to emotional stimuli in this task (e.g., Pérez-Edgar and Fox, 2003; Thomas et al., 2007; Sass et al., 2010). In other tasks, P200 is sensitive to threat perception (Carretié et al., 2001a,b; Correll et al., 2006) and N200 to cognitive control or response inhibition (Correll et al., 2006). Later processing can be measured by P300, a component that can track task difficulty and is generally interpreted as an index of the cognitive resources allocated to a task (Donchin and Coles, 1988; Yee and Miller, 1994). Negative stimuli on the emotion-word Stroop task have been associated with larger P300 (Li et al., 2007; Thomas et al., 2007), interpreted as allocation of additional resources for categorization of stimuli. Metzger and Orr (1997) reported a trend for later P300 latency to trauma-related words in patients with post-traumatic stress disorder (PTSD), indicating delayed or prolonged evaluation of such words. In combination, ES ERP studies suggest that emotion words are associated with enhanced early perception and increased allocation of resources.

Given the association between suspiciousness and anxiety, neural activity associated with suspiciousness may co-occur with and possibly influence the time course and lateralization of neural activity associated with anxiety. As psychophysiological research has supported psychometric distinctions between two dimensions of anxiety (e.g., Nitschke et al., 2001), anxious apprehension (worry, a major component of GAD) and anxious arousal (fear or somatic anxiety, a component of panic disorder and phobias), lateralization and time course of activity could be affected by the relationship of suspiciousness to each of these dimensions. Anxious apprehension or worry is associated with more left than right prefrontal activity (Heller et al., 1997; Engels et al., 2007, 2010; Mathersul et al., 2008), and there is mixed evidence for an association between apprehension and enhanced early sensory processing of emotional stimuli (e.g., Drake et al., 1991; Turan et al., 2002; Li et al., 2007; Sass et al., 2010). In contrast, anxious arousal is associated with more right than left prefrontal activity (Nitschke et al., 1999; Mathersul et al., 2008), increased right-posterior activity (e.g., Heller and Nitschke, 1998; Engels et al., 2007, 2010), and enhanced early processing (larger amplitude and shorter latency of P200; Yee and Miller, 1988; Hanatani et al., 2005; Pauli et al., 2005).

Overlapping characteristics of anxiety and suspiciousness may be associated with similar patterns of regional activity. For instance, anxious arousal and suspiciousness are both associated with arousal and consequent vigilance to threat. Thus, suspiciousness and anxious arousal combined could exaggerate vigilance characteristics, leading to increased ERP activity recorded over right-posterior cortex. In contrast, anxious apprehension and suspiciousness share verbal iterative processes (such as rumination or worry that engage areas associated with verbal processing) which involve extended processing of stimuli. Suspiciousness could combine with characteristics of anxious apprehension to augment activity manifested at left-frontal sites. Alternatively, these shared aspects associated with vigilance and verbal iterative processes could suppress the effects of each other, leading to reduced amplitude of ERP components at right-posterior and left-frontal sites.

In addition to overlap with anxiety, suspiciousness has distinct characteristics. Suspiciousness and anxious apprehension share verbal iterative processes, but suspiciousness involves concerns specifically about harm intended by others (Kinderman and Bentall, 1998), rather than excessive worry across a number of life domains (American Psychiatric Association [APA], 2000). Similarly, suspiciousness and anxious arousal are both associated with sympathetic nervous system arousal, but suspiciousness is associated with such arousal due to vigilance to external threat (e.g., people are out to get me), rather than arousal due to specific stimuli which could be external or internal (e.g., spiders, interoceptive cues). These distinct characteristics may be reflected in distinct patterns of brain activity. There is some evidence that suspiciousness is associated with activity in right temporal brain regions that have also been associated with vigilance (Robertson and Garavan, 2004). Enhanced N200 was observed over right temporal-parietal cortex during an auditory oddball task (Sumich et al., 2014), suggesting increased early attentive processes, which is consistent with a tendency toward vigilance. Fractional anisotropy in the right uncinate fasiculus, a white-matter tract that connects the temporal and frontal lobes, was correlated with suspiciousness (Nakamura et al., 2005). As anxious arousal is also associated with activity in a similar right-posterior region, it is possible that the association of suspiciousness with this area is due to overlap with anxiety.

The present study sought to identify the shared and distinct effects of trait suspiciousness and anxiety on processing of emotional information by measuring both behavioral interference and ERPs. It was hypothesized that suspiciousness would be related to early attention to all stimuli, reflected in right-temporal activity, consistent with vigilance. Second, suspiciousness would interact with anxiety dimensions to affect vigilance and verbal iterative processes, reflected in activity over right posterior and left frontal regions. P200 and N200 would index early attentive processes, and P300 would index allocation of resources to process stimuli. Depression was assessed in order to demonstrate that predicted associations with anxiety were not due to general psychopathology and because some neuropsychological, fMRI, and ERP findings for anxiety have emerged only after partialling out depression (e.g., Keller et al., 2000; Herrington et al., 2010; Sass et al., 2010). Depression was not expected to interact with suspiciousness to predict ERP measures.

MATERIALS AND METHODS

Much of the methods section, including stimuli and experimental design, EEG recording procedure, and data reduction and analysis procedures overlap with Fisher et al. (2010) and to some extent with Sass et al. (2010, 2014) and Stewart et al. (2010).

PARTICIPANTS

Over 1000 participants in undergraduate psychology classes filled out the Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990; Molina and Borkovec, 1994) and the Anxious Arousal and Anhedonic Depression scales of the Mood and Anxiety Symptom Questionnaire (MASQ; Watson et al., 1995a,b). Five groups were recruited for a larger fMRI and EEG study based on combinations of scores on three scales: the PSWQ, the MASQ Anxious Arousal scale, and an eight-item subscale of the MASQ Anhedonic Depression scale that emphasizes depressed mood rather than low positive affect (Nitschke et al., 2001). Individuals who had scores at the 80th percentile or higher on one scale and at the 50th percentile or lower on the other two scales were recruited for three pure high-scoring groups: high anxious apprehension only ($n = 14$), high anxious arousal only ($n = 14$), or high depression only ($n = 15$). A fourth group had scores at the 80th percentile or higher on all three scales ($n = 18$), and controls had scores at the 50th percentile or lower on all three questionnaires ($n = 27$). Group criteria were for recruitment purposes only; present analyses were conducted across all participants to investigate dimensional relationships between suspiciousness, anxiety and depression[1]. All participants were right-handed, native speakers of English with self-reported normal color vision. Participants were given a laboratory tour, informed of the procedures of the study, and screened for claustrophobia or contraindications for MRI participation. The study was approved by the University of Illinois Urbana-Champaign IRB. All participants gave their informed consent prior to their inclusion in the study.

Present analyses are based on the 88 paid participants (55% female and 84% Caucasian) from which both EEG data and Suspiciousness scores from the Schizotypal Personality Questionnaire (SPQ; Raine, 1991) were obtained[2]. Participants were 18–34 years old (mean = 19.0, SD = 1.8), medically healthy by self-report, and right-handed as determined by the Edinburgh Handedness Inventory (Oldfield, 1971). Participants completed a recruitment session, a Structured Clinical Interview for DSM-IV Axis I Disorders (SCID; First et al., 1997), an fMRI session, and an EEG session. Participants completed the emotion- and color-word Stroop tasks during fMRI data and EEG. The order of presentation of the two tasks within a session was counterbalanced across subjects, as was the order of the fMRI and EEG sessions, with the SCID session in-between for most subjects. Only data from the emotion-word Stroop task during the EEG session were considered for the present report.

MEASURES

During the recruitment session, participants were administered the SPQ and re-administered the MASQ and PSWQ. Analyses are based on these scores, since they were obtained closer in time to the EEG measurements. The test–retest reliabilities were: PSWQ, $r(81) = 0.91$, $p < 0.001$; MASQ Anxious Arousal, $r(84) = 0.71$, $p < 0.001$; and MASQ Anhedonic Depression eight-item subscale, $r(84) = 0.64$, $p < 0.001$. Data from the PSWQ was missing for four participants, so analyses using the PSWQ are based on 84 participants.

Suspiciousness scores were obtained from the eight-item true–false SPQ subscale. Examples of these items are: “I am sure I am being talked about behind my back;” “Do you sometimes get concerned that friends or coworkers are not really loyal or trustworthy?;” and “Do you often pick up hidden threats or put-downs from what people say or do?”

TASK

Word presentation and response recording were controlled by STIM software (James Long Company, Caroga Lake, NY, USA). The present task was implemented as blocks of positive or negative emotion words alternating with blocks of neutral words, a design that has been effective (Compton et al., 2000; Bar-Haim et al., 2007). Participants received 256 trials in 16 blocks (four positive, eight neutral, four negative) of 16 trials. A trial began with the presentation of a word for 1500 ms, followed by a fixation cross for 275–725 ms (onset to onset ITI 2000 ± 225 ms). Each trial consisted of one word presented in one of four colors (red, yellow, green, blue) on a black background, with each color occurring equally often within word type (positive, neutral, negative). Each participant received one of eight orders designed to minimize stimulus order effects. In four of the eight presentation orders, the first and third blocks were neutral words, with positive and negative blocks second or fourth and valence order counterbalanced across participants. The remaining four presentation orders complemented these, with the first and third blocks being either positive or negative words and the second and fourth blocks being neutral words.

Emotional and neutral words preceded each other equally often, and no word was repeated within an experimental session. Within a block, each color appeared four times, and trials were pseudorandomized such that no more than two trials featuring the same color appeared in a row. After every fourth block, there was a brief rest period. In addition to the 16 word blocks, there were four fixation-only blocks – one at the beginning, one at the end, and two in the middle of the session. In the fixation condition, instead of a word, a brighter fixation cross was presented for 1500 ms.

The 256 word stimuli were selected from the Affective Norms for English Words (ANEW) set (Bradley and Lang, 1999). Sixty-four were positive (e.g., birthday, ecstasy, laughter), 64 were negative (e.g., suicide, war, victim), and two sets of 64 were neutral (e.g., hydrant, moment, carpet). The words were selected on the basis of established norms for valence, arousal, and frequency of usage in the English language (Toglia and Battig, 1978; Bradley and Lang, 1999) and ranged from three to eight letters in length. Words were presented in capital letters using Tahoma 72-point font at a

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[1] As discussed in Fisher et al. (2007), it can be problematic to use correlation or regression analysis with an extreme-groups design. However, the present study included subjects with scores that encompassed most of the distribution. Simulations with randomly generated data indicated that results were not substantially affected for the distribution used here versus the full simulated dataset (Fisher and Miller, 2005).

[2] The selection method did not provide a sample of suspiciousness representative of the general population but instead allowed controlled representation of specific types and amounts of anxiety and depression. Of the present sample, 38 of the 88 participants were included in Sass et al. (2010), which analyzed data from anxious apprehension, anxious arousal, and control groups and did not address suspiciousness. The present sample also included a combined anxiety and depression group. In addition, all of the present sample was included in Fisher et al. (2010), which investigated the role of perceived emotional intelligence, anxiety, and depression in processing emotion stimuli and did not address suspiciousness.

distance of 1.35 m from the participant's eyes, for a vertical span of 1.2° and a horizontal span of 3.2° to 9.1°. Instructions were read verbatim by experimenters to assure that participants understood task requirements. The participant performed 32 practice trials before the actual tasks began. No participants failed to understand the task instructions or the mapping between colors and buttons after completing practice trials. Participants responded with the middle and index fingers of each hand using a four-button response box.

ELECTROPHYSIOLOGICAL RECORDING

Subjects were seated in a comfortable chair in a quiet room connected to the adjacent equipment room by intercom. EEG was recorded with a custom-designed Falk Minow (Munich, Germany) 64-channel cap with Ag/AgCl EEG electrodes spaced equidistantly. The left mastoid served as the reference during recording (Miller et al., 1991; Keil et al., 2014). By placing electrodes above and below each eye and near the outer canthus of each eye, vertical and horizontal EOG were recorded. Electrode impedances were maintained below 20 kohms. This impedance threshold was appropriate because the amplifier (James Long Company, Caroga Lake, NY, USA) had a high input impedance (10 GΩ; Keil et al., 2014). Half-power amplifier bandpass was 0.1–100 Hz, with digitization at 250 Hz.

DATA REDUCTION

Artifacts were removed and eye movement artifact corrected with Brain Electrical Source Analysis (BESA v. 5.1.8) software (Berg and Scherg, 1994). Trials were rejected if reaction time (RT) was not between 200 and 1000 ms, as responses less than 200 ms would be made too soon after stimulus onset and thus would not be credible, and responses greater than 1000 ms would likely reflect trials in which the participant was not engaged in the task. Mean RT across all trials and participants was 633 ms, SD 97 ms. For each subject, all trials for each emotion word type were averaged, since the error rate was low (4.5%, SD 3.9%), and the phenomena of interest were not expected to vary according to error rates. The electrode configuration was transformed to BESA's standard 81-channel virtual montage placed according to the 10–10 system (Perrin et al., 1989) to facilitate comparison with literature that reports data from conventional electrode sites. An average reference was computed for each time point as the mean voltage over the 81 virtual electrodes. Data were exported from BESA and baseline-adjusted by subtracting the average amplitude for the 200 ms before stimulus onset. Waveform averages were smoothed using a 101-weight, 0.1–10 Hz (half-amplitude) FIR digital filter (Cook and Miller, 1992; Nitschke et al., 1998; Edgar et al., 2005). Amplitude and latency scores were obtained for ERP components at each of the 81 electrodes.

Scoring windows for ERP components were chosen by examining the data and consulting previous Stroop and other ERP studies. **Figure 1** illustrates temporal scoring windows, and **Figure 2** illustrates grouping of adjacent sites by region for analysis purposes. For each participant, peak amplitude was calculated within the following latency windows and regions: P200 (148–248 ms; frontal), N200 (148–248 ms; temporal), and P300 (348–768 ms; centroparietal). P200 and N200 were scored as the peak amplitude in the same 148–248 ms latency window. These two peaks are readily apparent in **Figures 1** and **2**. Whether they represent distinct phenomena, or opposite poles of the same dipole, is not as clear. Of the three traditional criteria for defining an ERP component, they share latency, and their topographies are sufficiently complementary to be compatible with a single dipole per hemisphere. On the third criterion, however, they diverge consistently, showing distinct relationships to experimental manipulation[3]. Thus, they were analyzed separately (see **Table 1**).

Groups of four adjacent electrodes were selected to create three composite region scores in each hemisphere to obtain a stable measure of activity. For each ERP component, a regional score was calculated for each hemisphere by averaging the scores of the individual electrodes over the region, thus creating six scores (frontal: left: AF3, F1, F3, F5; right: AF4, F2, F4, F6; Centroparietal: left: C1, C3, CP3, CP5; right: C2, C4, CP4, CP6; Temporal: left: FT9, FT7, T9, T7; right: FT8, FT10, T8, T10; see **Figure 2**). Frontal sites were chosen for P200, as maximal effects were observed there in this dataset and in prior literature (Luck, 2005; Pauli et al., 2005). Temporal sites were chosen for N200 as maximal effects associated with suspiciousness were observed there in prior literature (Sumich et al., 2014). Centroparietal sites were chosen for P300 (Luck, 2005). Scores from each hemisphere were obtained separately in order to establish whether the results were specific to sites over the predicted hemisphere[4]. Thus, there were three ERP measures (P200 over frontal; N200 over temporal; P300 over centroparietal) in each hemisphere.

DATA ANALYSIS

A number of ERP studies of emotion have observed P300 amplitude modulation by emotional stimuli, with positive and negative stimuli prompting large responses (e.g., Schupp et al., 1997, 2003; Herbert et al., 2006). Accordingly, P300 was examined to determine whether the task manipulation was effective. To determine whether the task manipulation was successful and to facilitate comparison with prior literature, a 2 × 3 (hemisphere × emotion) repeated-measures MANOVA including linear and quadratic trends was conducted for P300 scores.

Because the primary constructs and measures of interest were continuous, regressions were used to test whether anxiety, depression and suspiciousness scores predicted ERP component scores at hypothesized scalp regions. In order to minimize the number of regressions calculated and to simplify interpretation, multivariate linear regressions were conducted so that multiple dependent variables could be simultaneously entered in the models, instead of predicting each ERP in each condition over each hemisphere.

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[3] To ensure that the components observed during the same time window over different regions of the scalp were not manifestations of the same dipole, time-point-by-time-point correlations were done between an exemplar electrode in the frontal region (AF3) and one in the posterior region in the opposite hemisphere (PO4). Correlations were generally <0.1 for P200/N200 indicating that these components are independent.

[4] Laterality effects were calculated for an earlier set of analyses (Fisher, 2006). However, they were mostly redundant with the present findings and were not included in order to simplify presentation of results.

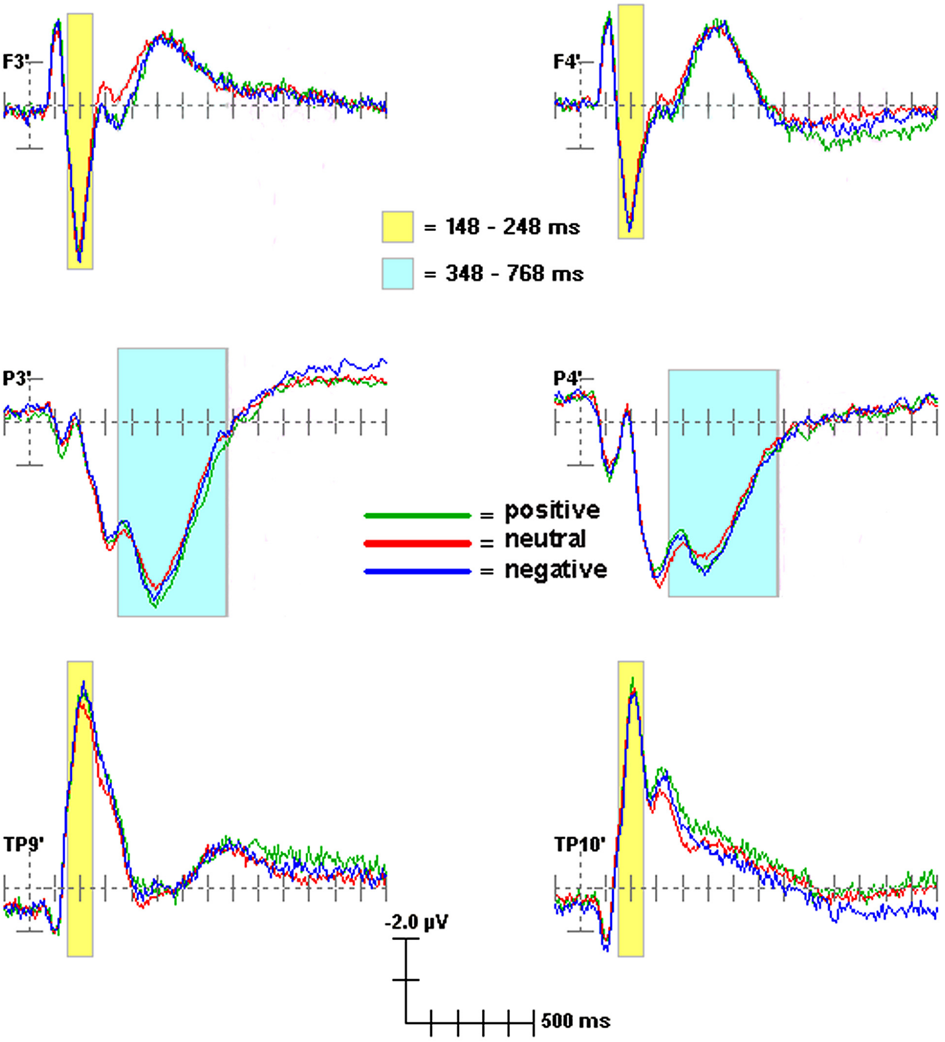

FIGURE 1 | ERP scoring windows illustrated for representative channels. All channels relative to an average reference. Waveforms from 100 ms prior to stimulus onset to 1400 ms after stimulus onset. Each tick mark on the x-axis represents 100 ms. The apostrophes after the channel names indicate that the channel locations were digitized.

This analysis strategy is more conservative and is less likely to uncover spurious effects than running separate analyses. Two sets of hierarchical regressions were conducted to investigate whether suspiciousness has either an additive or interactive relationship with anxiety (or depression), and how this relationship affects emotional information processing. The first set of regressions determined whether suspiciousness accounted for additional variance when added last to regression models in which anxious apprehension, anxious arousal, and anhedonic depression scores were already entered simultaneously as predictors of ERP amplitude scores for emotion (positive, neutral, and negative) stimuli over right and left hemispheres. Thus, there were four predictors and six dependent variables in each regression model (Model 1).

The second set of regressions investigated whether anxiety or depression interacted with suspiciousness or provided additive effects. Either an anxious apprehension (Model 2), anxious arousal (Model 3) or anhedonic depression (Model 4) score was entered first, suspiciousness was entered second, and an interaction term (product of two centered variables, per Cohen et al., 2003) was added third to predict the six ERP amplitude scores for each ERP component. All statistical analyses were conducted using SPSS v. 12 for Windows.

RESULTS

PSYCHOPATHOLOGY MEASURES AND BEHAVIORAL PERFORMANCE

Mean RT did not differ as a function of emotion (positive: 634 ms, SD 95 ms; neutral: 631 ms, SD 96 ms; negative: 633 ms, SD 101 ms)[5]. Although correlations with RT were

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[5] Both positive and negative stimuli produced very slightly and non-significantly slower responses than did neutral stimuli. Other studies that have used the ES task

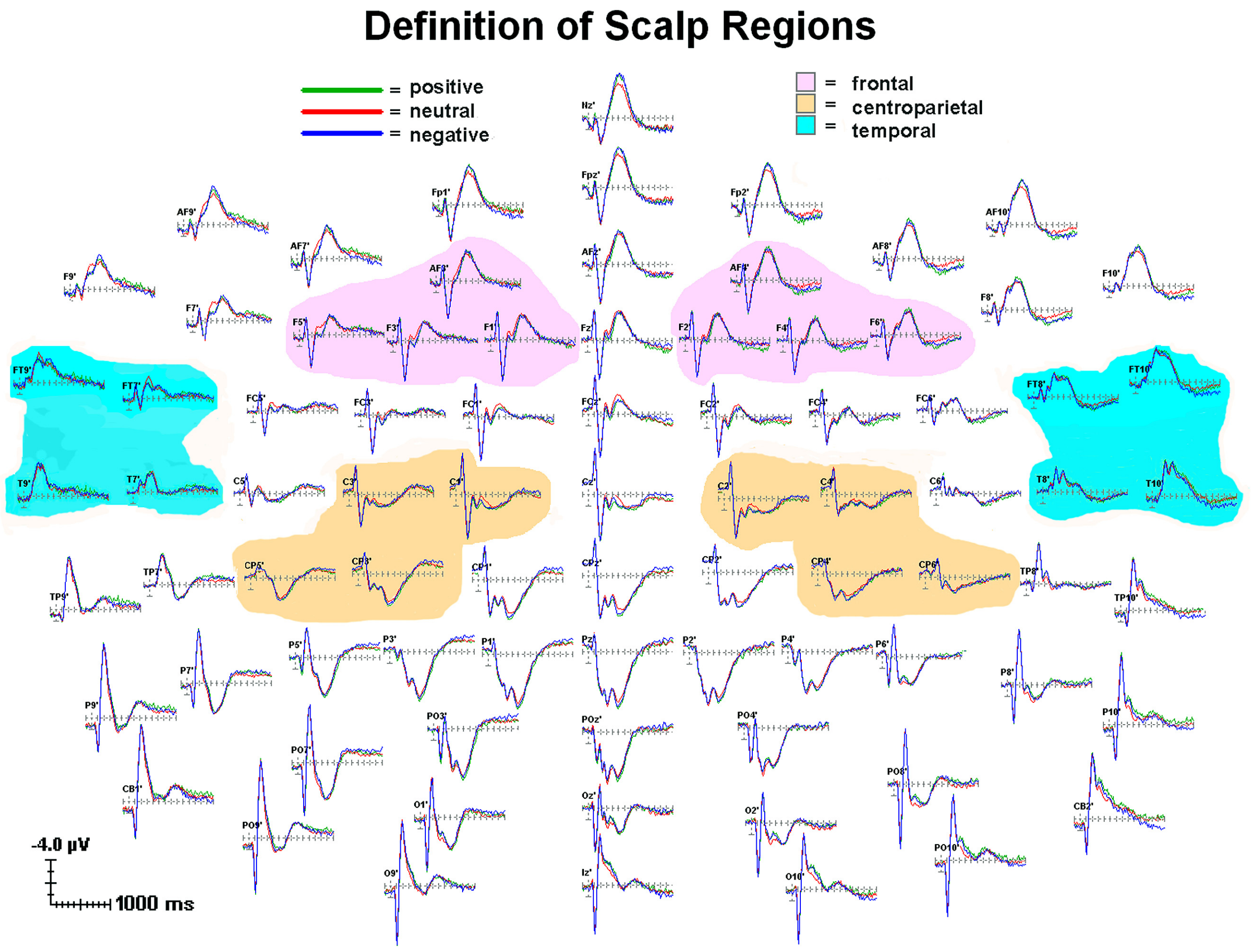

FIGURE 2 | Grand average ($n = 88$) for each emotion condition showing scalp topography for individual channels and regional groupings of channels. All channels relative to an average reference. Waveforms from 100 ms prior to stimulus onset to 1400 ms after stimulus onset. Each tick mark on the x-axis represents 100 ms. The apostrophes after the channel names indicates that the channel locations were digitized.

somewhat higher for suspiciousness than for anxiety and depression, only one was significant (see **Table 2**). Thus, self-reported symptoms were generally not related to overt performance, avoiding some potential interpretive confounds such as individuals scoring high in depression being less motivated for task engagement.

Table 2 shows that zero-order correlations among self-reported anxiety, depression and suspiciousness scores were positive and reliable. The relationship of suspiciousness to anxious apprehension, anxious arousal, and anhedonic depression was further examined in a hierarchical regression. The full three-predictor model accounted for 33% of the variance in suspiciousness, $F(3,79) = 13.05$, $p < 0.001$. Each predictor contributed unique variance when added last (anxious apprehension: $\Delta R^2 = 0.05$, $p = 0.02$; anxious arousal: $\Delta R^2 = 0.05$, $p = 0.02$; depression: $\Delta R^2 = 0.04$, $p = 0.03$), indicating that depression and the two dimensions of anxiety have distinct as well as overlapping relationships to suspiciousness.

ERP ANALYSES

Manipulation check

To determine whether the emotion-word Stroop task manipulation was successful and to facilitate comparison with prior literature, a 2 × 3 (hemisphere × emotion) repeated-measures MANOVA including linear and quadratic trends was conducted to predict P300. As expected, there was a main effect of Emotion, $F(2,86) = 6.04$, $p = 0.004$. A quadratic effect confirmed that P300 to positive and negative emotion stimuli was larger than P300 to neutral stimuli, $F(1,87) = 12.17$, $p = 0.001$.

P200

Model 1. In order to determine whether suspiciousness, in the context of anxiety and depression, had an additive effect on initial stimulus perception, it was entered in a multivariate model

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in non-clinical samples have not found reliable behavioral effects but have observed changes in neural activity (e.g., Whalen et al., 1998; Compton et al., 2003). The absence of behavior effects likely reflects small sample sizes, as a large sample is needed to reveal this effect in a non-clinical sample (Koven et al., 2003).

Table 1 | Mean (SD) ERP scores for negative stimuli in each region.

	P200 peak amplitude and latency	N200 peak amplitude and latency	P300 peak amplitude and latency
Left frontal	3.2 (2.3) μV 200 (19) ms		
Right frontal	2.9 (2.2) μV 200 (22) ms		
Left temporal		–2.5 (1.3) μV 217 (34) ms	
Right temporal		–2.4 (1.7) μV 216 (27) ms	
Left centroparietal			2.8 (1.9) μV 530 (91) ms
Right centroparietal			2.8 (1.6) μV 540 (105) ms

with the three other psychopathology measures. None of the four predictors was significant (p's = 0.16–0.93).

Model 2. Neither anxious apprehension ($p = 0.51$) nor suspiciousness ($p = 0.62$) predicted P200, but the interaction of suspiciousness and anxious apprehension did ($F(6,75) = 2.38$, $p = 0.04$). Univariate multiple linear regressions indicated that the interaction predicted P200 to positive stimuli over the right hemisphere ($B = –0.54$, $\Delta R^2 = 0.05$, $p = 0.05$), and predicted P200 to each condition (including neutral) over the left hemisphere, though the positive condition was only marginally significant (positive: $B = –0.50$, $\Delta R^2 = 0.04$, $p = 0.06$; neutral: $B = –0.56$, $\Delta R^2 = 0.05$, $p = 0.03$; negative: $B = –0.74$, $\Delta R^2 = 0.07$, $p = 0.01$). As illustrated in **Figure 3**, individuals with high scores on both suspiciousness and anxious apprehension had a reduced amplitude compared to those with a combination of low suspiciousness and high anxious apprehension. The same was true for high suspiciousness/low anxious apprehension compared to individuals with low scores on both measures.

Neither Model 3 nor Model 4 accounted for variance in P200.

N200

Model 1. With all four predictors (anxious apprehension, anxious arousal, anhedonic depression, and suspiciousness) in the multivariate linear regression model, suspiciousness was the only significant predictor ($F(6,73) = 2.27$, $p = 0.05$). This effect was examined further with univariate multiple regressions. When added last to the model, suspiciousness predicted N200 to positive ($B = –0.19$, $\Delta R^2 = 0.06$, $p = 0.03$) and to negative ($B = –0.27$, $\Delta R^2 = 0.10$, $p = 0.002$) stimuli over the right hemisphere. It accounted for variance at a trend level for P200 to neutral stimuli over the right hemisphere ($B = –0.15$, $\Delta R^2 = 0.03$, $p = 0.10$). Though not significant in the multivariate model, anxious arousal ($B = 0.05$, $\Delta R^2 = 0.05$, $p = 0.02$) and suspiciousness were both predictors of N200 in response to negative stimuli over the right hemisphere, though in opposite directions.

Model 2. Model 2 did not account for significant variance in N200.

Model 3. Suspiciousness was a significant predictor ($F(6,78) = 4.41$, $p = 0.001$) in the model with anxious arousal and the interaction between anxious arousal and suspiciousness. Consistent with the Model 1 regression, univariate regressions for each of the emotion conditions indicated that suspiciousness predicted N200 over the right hemisphere to positive ($B = –0.18$, $\Delta R^2 = 0.05$, $p = 0.02$), neutral ($B = –0.19$, $\Delta R^2 = 0.07$, $p = 0.01$) and negative ($B = –0.34$, $\Delta R^2 = 0.19$, $p < 0.001$) stimuli.

Model 4. Suspiciousness predicted N200 at a trend level ($F(6,78) = 2.03$, $p = 0.07$). Anhedonic depression and the interaction between anhedonic depression and suspiciousness were not significant predictors of N200 (anhedonic depression $p = 0.53$; interaction $p = 0.86$).

P300

Model 1. Anxious arousal predicted P300 at a trend level ($F(6,73) = 2.16$, $p = 0.06$). None of the other predictors was significant.

Model 3. When anxious arousal, suspiciousness, and their interaction were predictors, only anxious arousal was significant at a trend level ($F(6,78) = 2.00$, $p = 0.08$). Univariate regressions with the same predictors indicated that anxious arousal predicted P300

Table 2 | Correlations among suspiciousness, anxiety, and depression measures and behavioral performance on the emotion-word Stroop task.

	Anxious apprehension	Anxious arousal	Anhedonic depression	Positive-word RT	Neutral-word RT	Negative-word RT
Suspiciousness	0.46**	0.41**	0.42**	–0.22*	–0.12	–0.18
Anxious apprehension		0.35**	0.43**	–0.07	–0.11	–0.08
Anxious arousal			0.42**	0.01	0.06	0.01
Anhedonic depression				–0.02	0.05	0.02
Positive-word RT					0.93**	0.91**
Neutral-word RT						0.92**

*Note. For n = 88, two-tailed Pearson correlation. *p ≤ 0.05, **p ≤ 0.01.*

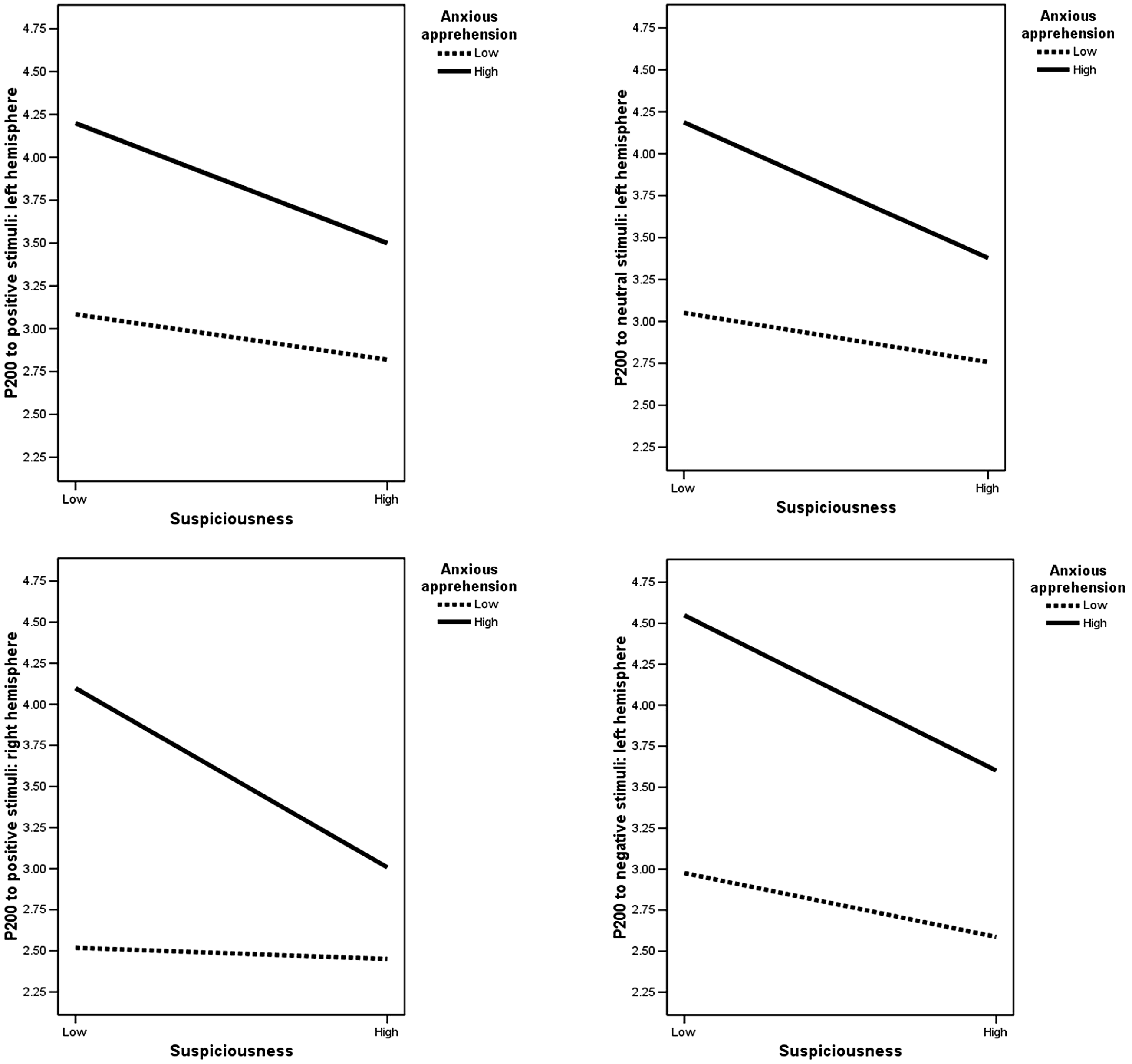

FIGURE 3 | Anxious apprehension × suspiciousness interactions. *Y* values are P200 amplitude scores to stimuli over the specified hemisphere. Low and high labels represent suspiciousness and anxious apprehension scores divided according to a median split.

to positive ($B = 0.05$, $\Delta R^2 = 0.04$, $p = 0.04$) and negative stimuli ($B = 0.06$, $\Delta R^2 = 0.08$, $p = 0.01$) over the right hemisphere. However, when the order of predictors was reversed and suspiciousness was entered first, it was a significant predictor, but only for P300 to negative stimuli over the right hemisphere ($B = 0.15$, $\Delta R^2 = 0.04$, $p = 0.05$). Once anxious arousal was entered in the model, suspiciousness was no longer significant. These regressions indicate that shared characteristics of suspiciousness and anxious arousal (e.g., vigilance) predict P300 to negative stimuli over the right hemisphere.

Models 2 and 4 were not significant.

DISCUSSION

The present study investigated whether suspiciousness affects processing of emotional information in a unique manner, or whether it overlaps with anxiety to affect processing. Teasing apart the relationship between anxiety and suspiciousness would foster improved definition, classification, and treatment of psychological disorders. Behavioral and ERP indices were used. The larger P300 for positive and negative than neutral stimuli indicated a successful emotional arousal manipulation. Study hypotheses specified that suspiciousness alone would be related to early attention to emotion information, reflected by right-temporal activity, and that suspiciousness would interact with anxiety to affect vigilance and verbal iterative processes in response to emotion information, reflected in activity over right posterior and left frontal regions.

Supporting the first hypothesis, high suspiciousness was associated with enhanced right temporal N200 to all stimuli. The other psychopathology measures did not show such a relationship. This finding for suspiciousness is consistent with the limited research available that links activity of the temporal region to suspiciousness (Li et al., 2011), especially the right temporal region (Nakamura et al., 2005; Sumich et al., 2014). Associations with the right temporal lobe may be due to involvement of a right frontoparietal vigilance system that modulates arousal (Nitschke et al., 2000; Robertson and Garavan, 2004). The right ventral frontoparietal network has been implicated in attention to behaviorally relevant stimuli and has been activated during "theory of mind" cognition (involving judgments of another person's mental state), thus requiring a combination of perceptual processes and judgment of other people's actions (Corbetta et al., 2008). The temporal N200 in the present study can be distinguished from a fronto-central N200 that is thought to be associated with effortful processing (e.g., Donkers and van Boxtel, 2004), and typically peaks later in time (between 200 and 500 ms; e.g., Thomas et al., 2007; Enriquez-Geppert et al., 2010). Present results suggest that suspiciousness is associated with an overactive early attentive response (manifested in N200) to any type of stimulus, regardless of its emotion content. Thus, individuals with high suspiciousness scores likely judged all stimuli to be behaviorally relevant. Though this study investigated suspiciousness in a non-clinical sample, this hyperactive response to all stimuli is consistent with reports of misattribution of salience to neutral stimuli in patients with schizophrenia (Holt et al., 2006; Heerey and Gold, 2007). Similarly, neutral and negative stimuli led to increased mesotemporal and ventral striatal activity and reduced prefrontal activity in patients with hallucinations and delusions, whereas controls showed this response to negative stimuli only (Epstein et al., 1999). Thus, it appears that suspiciousness, even at non-clinical levels in the general population, influences the perception of stimuli in the same manner as that observed in clinical populations.

The second hypothesis was that suspiciousness would combine with anxiety to affect emotion processing, specifically that the interaction of suspiciousness and anxious apprehension would affect left-frontal activity and that suspiciousness and anxious arousal would affect right-posterior activity. The interaction of suspiciousness and anxious apprehension did predict P200 (index of stimulus perception) to neutral and negative stimuli over the left hemisphere, consistent with fMRI evidence that left-hemisphere activity is associated with anxious apprehension (e.g., Engels et al., 2007, 2010). To interpret this P200 interaction, characteristics of anxious apprehension and anxious arousal that are shared with suspiciousness were used as a guide (see **Figure 3**). Given that suspiciousness and anxious apprehension presumably share verbal-iterative processing, an additive effect might have been expected. Instead, individuals with high scores on both suspiciousness and anxious apprehension had a reduced amplitude compared to those with a combination of low suspiciousness and high anxious apprehension. The same was true for high suspiciousness/low anxious apprehension compared to individuals with low scores on both measures. Thus, the presence of high levels of suspiciousness in the context of anxiety reduced P200 amplitude. It is possible that the aspect of suspiciousness associated with arousal and vigilance (involving the right hemisphere) may have reduced the degree to which verbal-iterative processing (left hemisphere processes) associated with anxious apprehension was reflected. Unexpectedly, the suspiciousness × anxious apprehension interaction also predicted P200 to positive stimuli over the *right* hemisphere in the same manner. This finding could be explained by the fact that suspiciousness is associated with responses to all types of stimuli. Therefore, instead of finding an expected association between anxious apprehension and a response to negative stimuli only, the presence of suspiciousness led to a more generalized response to all stimuli.

Contrary to hypotheses, suspiciousness did not interact with anxious arousal to predict ERP measures over the right-posterior region. Instead, anxious arousal and suspiciousness each were independent, but overlapping predictors of N200 and P300 to negative stimuli over the right hemisphere. When both were entered in a regression model, only one accounted for variance (suspiciousness for N200 over right temporal region and anxious arousal for P300 over right centroparietal region), indicating that suspiciousness and anxious arousal share overlapping characteristics that predict response to negative stimuli over the right hemisphere. The association of anxious arousal with P300 over the right centroparietal region is consistent with evidence that right-central (e.g., inferior temporal gyrus, Engels et al., 2007) and right-temporoparietal (Heller et al., 1997; Keller et al., 2000; Compton et al., 2000, 2003) areas are associated with anxious arousal and a network involved in vigilance to behaviorally relevant stimuli (Tucker and Williamson, 1984; Heller, 1990; Heller et al., 1998; Nitschke et al., 1999, 2000; Herrington et al., 2005; Corbetta et al., 2008).

Present results indicate that anxiety and suspiciousness each affects emotion processing alone, but also in combination. Suspiciousness interacts with anxious apprehension to modulate initial stimulus perception processes, manifested in P200 recorded over frontal cortex. In addition, suspiciousness and anxious arousal share overlapping characteristics that predict response to negative stimuli over the right hemisphere. Finally, suspiciousness is uniquely associated with a hyperactive early response (enhanced N200 to all stimuli). Together, these results suggest that suspiciousness is associated with hypervigilance to all incoming stimuli and interacts with anxiety to modulate early attention to emotion stimuli. As predicted, suspiciousness did not interact with anhedonic depression to predict ERP measures.

Given that suspiciousness is present in individuals with anxiety symptoms and that it is uniquely associated with early processing of incoming information (with emotion content or otherwise), suspiciousness should be assessed more frequently in individuals who present with anxiety symptoms in order to determine whether these individuals perceive threat in positive or neutral situations. Potential treatments could target reducing vigilance to perceived threat (common in anxious arousal) and minimizing verbal-iterative processes about anxiety-provoking events or thoughts (common in anxious apprehension). Depression, which often co-occurs with anxiety and suspiciousness, should be treated separately, as it appears to be a distinct construct. In summary, these results identify how characteristics of suspiciousness, both unique and those that overlap with two anxiety dimensions, affect processing of emotion information. The characterization of these symptom dimensions provides additional support for the recent emphasis (e.g., NIMH Research Domain Criteria (RDoC) project) on using dimensions to classify psychopathology. In addition, these results extend prior findings to a non-clinical population and suggest ways to refine treatments for individuals with clinically significant levels of suspiciousness and anxiety.

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Sex differences in event-related potentials and attentional biases to emotional facial stimuli

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Attentional processes play an important role in the processing of emotional information. Previous research reported attentional biases during stimulus processing in anxiety and depression. However, sex differences in the processing of emotional stimuli and higher prevalence rates of anxiety disorders among women, compared to men, suggest that attentional biases may also differ between the two sexes. The present study used a modified version of the dot probe task with happy, angry, and neutral facial stimuli to investigate the time course of attentional biases in healthy volunteers. Moreover, associations of attentional biases with alexithymia were examined on the behavioral and physiological level. Event-related potentials were measured while 21 participants (11 women) performed the task, utilizing also for the first time a difference wave approach in the analysis to highlight emotion-specific aspects. Women showed overall enhanced probe P1 amplitudes compared to men, in particular after rewarding facial stimuli. Using the difference wave approach, probe P1 amplitudes appeared specifically enhanced with regard to congruently presented happy facial stimuli among women, compared to men. Both methods yielded enhanced probe P1 amplitudes after presentation of the emotional stimulus in the left compared to the right visual hemifield. Probe P1 amplitudes correlated negatively with self-reported alexithymia, most of these correlations were only observable in women. Our results suggest that women orient their attention to a greater extent to facial stimuli than men and corroborate that alexithymia is a correlate of reduced emotional reactivity on a neuronal level. We recommend using a difference wave approach when addressing attentional processes of orientation and disengagement also in future studies.

Keywords: sex differences, dot probe paradigm, attentional bias, difference wave approach, probe P1 amplitudes, alexithymia

INTRODUCTION

Attentional processes play an important role in the processing of emotional information and with regard to the development and maintenance of symptoms of anxiety and depression: anxious and depressive subjects allocate more attention to threatening stimuli and less attention to pleasant stimuli and cues of reward (e.g., Bar-Haim et al., 2007; Frewen et al., 2008; Staugaard, 2010; Yiend, 2010). Women are known to have higher prevalence rates of anxiety disorders than men (e.g., Kessler et al., 2005) and it is also an established finding that there are sex differences in the processing of emotional stimuli (Cahill, 2006). Killgore and Yurgelun-Todd (2001) observed enhanced activation in the right amygdala when presenting happy faces only in their male participants. In contrast, the presentation of fearful faces evoked enhanced left amygdala activation in both sexes. Derntl et al. (2009) also reported subtle sex differences in amygdala activation. Enhanced bilateral amygdala activation was positively related to better fear recognition only in their male participants. Importantly, the amygdala is considered to be actively involved in driving emotional enhancement, i.e., exerting modulatory influence on visual processing of emotional stimuli (Vuilleumier et al., 2004).

Based on the enhanced prevalence rates for anxiety disorders in women and reported sex differences during the processing of emotional stimuli, it can be hypothesized that also attentional biases differ between the two sexes. There is indeed a growing number of attentional bias studies that reported sex differences on the behavioral level (Tan et al., 2011; Donges et al., 2012; Tran et al., 2013), but also on the neuronal level (Sass et al., 2010). Women have a greater ability than men to perceive and respond to positive stimuli at an automatic processing level (Donges et al., 2012), and show enhanced neural activity during early visual processing stages compared to men, regardless of the emotional content of the stimuli (Sass et al., 2010). Moreover, in anxiety, attentional biases toward threat may be a phenomenon that is limited to women as suggested by recent studies (Tan et al., 2011; Tran et al., 2013).

A widely used paradigm to investigate attentional biases is the dot probe task (see Bar-Haim et al., 2007). In the original version (MacLeod et al., 1986), participants were shortly presented with two words on the left and right side of a computer screen. One of these words was emotionally valenced, the other one was neutral. Immediately after display offset, a dot (i.e., the "probe") appeared in the location of one of the words; either in the location of the emotional stimulus (consequently a "congruent trial") or in the position of the neutral one (consequently an "incongruent trial"). Participants' task was to indicate visual detection of the probe by pressing a corresponding button as fast as possible. Following theoretical considerations (MacLeod et al., 1986), response times should be shorter for congruent trials in case attention is captured by the emotional stimulus. If attention is directed away from the emotional stimulus (i.e., an avoidance reaction), response times should be shorter for incongruent trials. Subtracting mean response times in congruent and incongruent trials results in a commonly used bias index (BI). This BI is positive when attention is drawn to emotional stimuli and negative when emotional stimuli are avoided. However, this BI might not be able to distinguish different attentional processes from each other. Positive scores may either be due to fast reactions in congruent trials (suggesting increased attention toward target stimuli) and/or due to slow reactions in incongruent trials (suggesting delayed disengagement from target stimuli; Salemink et al., 2007; Cisler and Koster, 2010). To avoid this ambiguous constellation, adding trials with two neutral stimuli may allow to differentiate fast orienting more clearly from a difficulty to disengage as reaction times in the neutral–neutral trials may serve as a baseline measure. Studies applying these modified task version suggested that anxiety-related attentional biases seem to reflect specifically effects of delayed disengagement, but not of increased attention (Cisler and Koster, 2010).

Reaction times are one dependent measure to assess attentional biases, but may be prone to measurement error (e.g., Waechter et al., 2014). To further elucidate attentional processes, previous studies have also investigated physiological measures during the dot probe task. Event-related potentials (ERPs) evoked by visual task displays of the dot probe task are a useful tool to further disentangle underlying attentional processes since they provide millisecond precision. The current study particularly focused on an ERP evoked by the probe presentation—the P1 component (termed "probe P1" in the following)—as did several other attentional bias studies (see below).

The P1 is a positive-going ERP with peak latencies between 100 and 130 ms after visual presentation at parieto-occipital and occipital electrode positions (Luck, 2005). It indexes an early stage of visual processing, as with regard to luminance or contrast (i.e., low-level visual features; Luck, 2005). However, apart from low-level visual processing, the P1 amplitude is also modulated by top-town attentional processes. P1 amplitudes were reported to be enhanced for attended, compared to unattended, stimuli in spatial attention paradigms (Hillyard and Anllo-Vento, 1998; Luck and Ford, 1998) and have also been linked to emotional face categorization processes (Linkenkaer-Hansen et al., 1998; Pizzagalli et al., 2002). Specifically, modulation of P1 amplitudes is larger for negative emotional faces than for positive emotional faces (Ito et al., 1998; Smith et al., 2003).

Recent studies in healthy participants reported enhanced probe P1 amplitudes after angry compared to happy faces (Santesso et al., 2008) and after fearful compared to happy faces (Pourtois et al., 2004) in a dot probe paradigm, and enhanced P1 amplitudes to emotionally congruently primed targets (i.e., fearful faces) in contrast to incongruently primed ones in a spatial cueing paradigm (Brosch et al., 2011). Santesso et al. (2008) interpreted their results as indicative of increased sensory gating for emotionally cued stimuli in the visual cortex and in line with theories on hyper vigilance toward threat. However, in their tasks, Santesso et al. (2008) and Pourtois et al. (2004) were not able to distinguish increased vigilance from disengagement difficulties. Brown et al. (2010) specifically reported that both evolutionary relevant (e.g., pictures of snakes or spiders) and irrelevant threatening stimuli (e.g., pictures of knives and syringes) evoke enhanced probe P1 amplitudes in congruently primed trials, compared to incongruently primed ones. This finding speaks for the universality of the so-called threat-superiority effect—meaning that any threatening stimuli accompanied by fear or danger easily capture attention compared to non-threatening ones (Öhman et al., 2001; Smith et al., 2003; Blanchette, 2006). In contrast, Eldar et al. (2010) found no probe P1 amplitude variation in response to angry or happy faces in anxious and non-anxious participants, using a block design to present the different emotions.

In summary, the results of extant studies on probe P1 amplitudes are rather inconsistent. Notably, participant sex was not controlled for in these studies and most of them did not include neutral stimuli in their paradigms (but see Eldar et al., 2010, and O'Toole and Dennis, 2012) or did not analyze them. The internal validity and generalizability of previous studies thus appears, both on the behavioral and the physiological level, limited. For the ERPs, neutral trials allow the calculation of differences waves to further extract relevant ERP amplitude variation. Neutral–neutral stimulus pairs could thus serve as individual baselines when calculating participant- and emotion-wise difference waves. The difference wave approach might be better suited to disentangle vigilance and disengagement effects (Luck, 2005).

In order to elucidate and to expand on previous inconsistent findings, the present study focused on the examination of sex differences in probe P1 amplitudes, utilizing the dot probe paradigm with emotional facial stimuli. As a novel and unique procedure in neuroscientific attentional bias research, we implemented a difference wave approach for the study of attentional biases. This was possible because we included neutral–neutral trials in our electroencephalogram (EEG) paradigm, as suggested by Salemink et al. (2007) for behavioral data. Given previous results on sex differences with regard to attentional processes and attentional biases (see above), we expected overall enhanced ERP amplitudes among women compared to men. As we expected ERP amplitudes to be specifically enhanced during early stimulus processing stages for attended stimuli (Luck, 2005), the P1 component time interval lay in the focus of the present study. Data of a community sample were used, as we were interested in sex differences in the general population. Psychological symptoms were assessed in the course of data acquisition. Potential sex effects were explored both in behavioral and neuronal correlates of the dot probe task, using alternative bias indices as proposed by Salemink et al. (2007) to differentiate fast orienting from a difficulty to disengage on the behavioral level (see Materials and Methods) and a difference wave approach for ERPs. Additionally, we assessed associations of attentional biases on the behavioral and physiological level with alexithymia, which has been repeatedly reported to be related to emotion processing (e.g., Franz et al., 2004; Eichmann et al., 2008; Reker et al., 2010). Alexithymia can be described as the inability to identify, describe, regulate, and express emotions (Sifneos, 1976) and is considered to be a continuous personality trait (Jessimer and Markham, 1997). Previous research has linked disturbed emotion regulation in alexithymia with deficits in the processing stream of emotional stimuli (Lane et al., 2000; Berthoz et al., 2002; Mantani et al., 2005). This relation might be also seen in attentional biases. Moreover, several studies reported P1 amplitude variation in relation to alexithymia when participants were presented with emotional stimuli (Schaefer et al., 2007; Pollatos and Gramann, 2011). Therefore, we assessed alexithymic traits in the participants of the current dot probe experiment to assess a possible link between attentional biases and alexithymia.

MATERIALS AND METHODS

PARTICIPANTS

Twenty-one volunteers (11 women; all sampled from the community) participated in the present study. Mean age of all participants was 27.3 ± 3.58 years, ranging from 23 to 34. All participants were right-handed (Edinburgh Handedness Inventory; Oldfield, 1971), had normal or corrected-to-normal vision, and reported no past or present neurological or psychiatric disorder. Most participants ($n = 20$; 95%) reported non-clinical levels of current psychological symptoms [T scores <63 in the relevant scales of the SCL-90-R (Symptom Checklist-90-Revised); see below]. One male participant (5%) reported elevated levels in anxiety, depression, and global psychological distress. This was not unexpected as using a cutoff of 63, roughly 10% of the general population are expected to show elevated scores.

This study was conducted in accordance with the Declaration of Helsinki (1983) and local guidelines of the University of Vienna and the Faculty of Psychology. All participants gave written informed consent prior to the experiment.

QUESTIONNAIRES

Prior to the EEG data collection, participants completed several psychological questionnaires.

Psychological symptoms

Current psychological distress, depression, and anxiety were assessed with the 90-item Symptom Checklist (SCL-90-R; German version: Franke, 2002). The SCL-90-R assesses the prevalence and distress caused by a variety of symptoms during the last 7 days. Depression and anxiety were assessed with 13 and 10 items, respectively. Psychological distress (Global Severity Index; GSI) is operationalized as the mean of all 90 items. Items were scored from 0 (*not at all*) to 4 (*extremely*). In the current sample, Cronbach alpha for scales of depression, anxiety, and psychological distress was 0.81, 0.85, and 0.95, respectively. T scores ≥63 may be considered clinically relevant, according to the published norm data of the instrument.

Self-reported alexithymia

Ratings of alexithymia were obtained with the 26-item Toronto Alexithymia Scale (TAS-26; German version: Kupfer et al., 2001). The TAS-26 assesses three components of alexithymia: difficulties in the identification of feelings (DIF; seven items), difficulties describing feelings (DDF; five items), and externally oriented thinking (EOT; six items). Items were scored from 1 (*strongly disagree*) to 5 (*strongly agree*). Cronbach alpha in the current sample was 0.68 (DIF), 0.61 (DDF), and 0.33 (EOT), which is in accordance with published validity data, except for EOT, where Cronbach alpha appeared unacceptably low in the current sample. The TAS-26 also allows the computation of a total score (Cronbach alpha = 0.64 in the current sample) that was, however, not used in the present study.

TASK AND PROCEDURE

The synchronization of the stimulus presentation with the EEG recording was implemented by E-Prime 2.0 software (Psychology Software Tools, Inc., Sharpsburg, PA, USA) running on a Pentium IV, 3.00 GHz machine. During EEG data collection, participants were seated comfortably in a sound-attenuated room in front of a 21 inch cathode ray tube monitor (Sony GDM-F520; 75 Hz refresh rate) with approximately 70 cm distance to the screen. A modified version of the dot probe paradigm by MacLeod et al. (1986) was applied. Participants' task was to indicate the location of a probe stimulus on the screen via corresponding button press. Each trial started with the central presentation of a black fixation cross against a white background for 750 ms. Subsequently, two pictures depicting faces were presented to the left and to the right of the fixation cross (i.e., left or right visual hemifield; picture size: 4 cm × 5 cm; distance from fixation cross to picture center: 4 cm). These pictures were taken from the FACES database (Ebner et al., 2010), utilizing emotional and neutral facial expressions of four female posers and four male posers, and presented for 500 ms. Afterward, the faces disappeared and a black dot (the "probe") was blended in for at most 3000 ms, either on the position of the left or the right face picture. Participants had to indicate dot location, i.e., right or left half of the screen, by pressing a corresponding button on a standard keyboard with their right ("j") or left ("f") index finger. Immediately after the button press, the dot disappeared and the fixation cross was presented again with a variable duration of 750–1000 ms. Each trial consisted either of the combination of an emotional and a neutral face picture by the same poser or of the combination of two neutral face picture by the same poser. Emotional facial expressions depicted anger, disgust, fear, happiness, and sadness (Ekman, 1992). Each emotion-neutral pair was presented twelve times per poser, the location of the emotional face picture and the location of the subsequent dot were counterbalanced across trials. Each neutral–neutral face pair was presented six times per poser. Overall, the experiment consisted of 528 trials. Emotional and neutral pairs were presented randomly. Prior to the experiment, participants completed 16 training trials with neutral–neutral pairs with eight different posers (four female, four male posers) to get familiar with the experimental paradigm. Concerning emotional-neutral pairs, congruent trials were defined as trials where the dot replaced an emotional face, whereas trials where the dot replaced the neutral facial expression were considered as incongruent trials. For the neutral–neutral face pairs, no congruency effect was assessable. Thus, each dot replacement was considered as neutral. After blocks of 44 trials, participants were given short breaks if needed. Overall, EEG data collection took around 45 minutes.

DATA ACQUISITION

Electroencephalogram was recorded from 59 Ag/AgCl ring electrodes which were embedded in a fabric electrode cap in an equidistant fashion (EASYCAP GmbH, Herrsching, Germany; model M10). A further four electrodes were placed at both outer canthi and 1 cm above and below the left eye to record horizontal and vertical electrooculogram (EOG). These bipolar EOG recordings were used off-line for eye-movement correction. Electrodes on the seventh vertebra and on the right sternoclavicular joint served as reference site (Stephenson and Gibbs, 1951). Subsequently, a skin-scratching procedure was applied to each electrode site to keep electrode impedances below 2 kΩ (Picton and Hillyard, 1972). EEG signals were amplified using an AC amplifier set-up with a time constant of 10 s (Ing. Kurt Zickler GmbH, Pfaffstätten, Austria). EEG was recorded within a frequency range of 0.016–125 Hz and sampled at 250 Hz for digital storage.

DATA ANALYSIS

As prior evidence (Tran et al., 2013) revealed strongest effects for happy and angry faces among both men and women, only happy, angry, and neutral face pairs were considered for analysis in the present study.

Behavioral data analysis

Response times were defined as the interval from dot onset to button press. Trials with response times faster than 200 ms and slower than 1000 ms were discarded. Only correct trials were considered for analysis (less than 1.5% of all trials were incorrect). Mean reaction times were assessed and used to calculate emotion-wise bias indices. First, the commonly used BI was calculated via subtracting mean response times of congruent trials from mean response times of incongruent trials. Secondly, the orienting index (OI) was calculated via subtracting the mean response times of congruent trials from mean response times of neutral trials. Thirdly, the disengaging index (DI) was calculated via subtracting mean response times of neutral trials from mean response times of incongruent trials. OI and DI, as proposed by Salemink et al. (2007), are considered to disentangle processes of increased and facilitated orientation toward target stimuli (i.e., OI) from processes of delayed and decreased disengagement from target stimuli (i.e., DI). Previous research indicated that OI and DI scores may be more valid indicators of attentional biases than the commonly applied BI scores (Tran et al., 2013). Note that the index scores OI and DI add up to the BI, but depend on neutral–neutral trials for computation. The presence of attentional biases was assessed using one-sample t-tests versus 0 in the overall sample, as well as among women and men separately. Moreover, we tested whether or not sex differences were also observable in reaction times and questionnaire data.

EEG data analysis

Prior to data analysis, participant- and channel-specific coefficients were calculated for weighting vertical and horizontal eye movements which were assessed during two calibration trials administered prior to the experiment. Subsequently, these weighted EOG signals were subtracted from experimental EEG data (Bauer and Lauber, 1979). Off-line data analysis was carried out using EEGLAB 6.0.3b (Delorme and Makeig, 2004) with Matlab 7.9.0 (The MathWorks, Inc., Natick, MA, USA). A low-pass filter with a cut-off frequency of 30 Hz (roll-off 6 dB/octave) was applied to the data. Data were epoched starting 100 ms prior to dot presentation with each epoch lasting 500 ms. The mean of the first 100 ms served as baseline interval. Data were epoched according to emotion (anger, happy, neutral), congruency condition (congruent, incongruent) and according to the hemifield in which the emotional face was presented prior to dot onset (right, left hemifield). The factors congruency and hemifield was only available for angry and happy faces. Nine experimental conditions were derived after averaging participant- and condition-wise: *anger-congruent-R*, *anger-congruent-L anger-incongruent-R*, *anger-incongruent-L*, *happy-congruent-R*, *happy-congruent-L*, *happy-incongruent-R*, *happy-incongruent-L*, and *neutral*.

A semi-automatic artifact removal procedure was applied to these epochs. Artifact-afflicted trials with voltage values exceeding ±70 μV or with voltage drifts of more than 50 μV were automatically marked by EEGLAB. During subsequent visual inspection, the automatic markings were controlled and artifact-afflicted trials were discarded from further analysis. Mean amplitudes were assessed for probe P1 amplitudes (interval: 80–120 ms) at midline electrode location Oz for all conditions.

Probe P1 mean amplitudes were investigated with a linear mixed model, examining the factors sex, emotion (anger vs. happy vs. neutral), congruency (incongruent vs. congruent), and hemifield (right vs. left); congruency and hemifield were both nested within emotion (effects of congruency applied only to the emotions anger and happy, but not neutral; the same was also true with regard to hemifield). Such a doubly nested design may not be directly investigated with classical ANOVA, but demands utilization of specific analysis tools, like the linear mixed model. Parameters in the linear mixed model were estimated with maximum likelihood, using an unstructured covariance matrix. In addition to the results of the effect tests, we report here Cohen's d of significant effects, derived from the effect estimates of the fitted model, as no direct estimates of explained variance are provided in linear mixed models.

Additionally, difference waves were calculated with the artifact-corrected EEG data, yielding measures on a physiological level that were comparable to Salemink et al.'s (2007) bias indices on the behavioral level. However, in order to account for the nature of the ERP data, neutral trials always served as subtrahend in our calculations to allow direct comparison of ERP amplitude variation. Eight experimental conditions were derived: *anger-congruent-R-diff*, *anger-congruent-L-diff*, *anger-incongruent-R-diff*, *anger-incongruent-L-diff*, *happy-congruent-R-diff*, *happy-congruent-L-diff*, *happy-incongruent-R-diff*, and *happy-incongruent-L-diff*. Mean amplitudes for probe P1 amplitudes were also extracted at Oz, 80–120 ms after probe onset. These probe P1 mean difference wave amplitudes were subjected to a four-way mixed-model ANOVA with the between-subject factor sex, and the within-subject factors emotion (anger vs. happiness), congruency (incongruent vs. congruent), and hemifield (right vs. left). Classical ANOVA could be utilized here, as all factors were fully crossed with one another (balanced design). Significant interaction effects in the ANOVA were explored with *t*-tests.

Furthermore, Spearman correlations (r_s) were calculated to explore the associations between probe P1 and probe P1 difference wave amplitude variations, behavioral measures, and questionnaire data. Significance was set at $p < 0.05$ (two-sided) for all tests; $p < 0.10$ was interpreted as borderline significant. Partial eta-squared (η_p^2) and Cohen's *d* are reported as effect sizes, values of $\eta_p^2 = 0.01/d = 0.20$, $\eta_p^2 = 0.06/d = 0.50$, and $\eta_p^2 = 0.14/d = 0.80$ representing small, medium, and large effects. Fisher's *z*-test was applied to assess significant differences in correlation coefficients. Statistical analysis was performed using PASW 18 (SPSS Inc., IBM Corporation, NY, USA).

RESULTS

BEHAVIORAL DATA

Table 1 provides descriptive statistics on all bias indices and psychological measures. The overall sample showed BI scores for happy faces that were significantly lower than 0 [$t(20) = -4.49$, $p < 0.001$, $d = -0.98$], indicating avoidance of happy faces following the classical BI interpretation (MacLeod et al., 1986). Taking the neutral trials into account, this result was reflected in the borderline significance of OI happy scores [$t(20) = 1.74$, $p = 0.097$, $d = 0.38$] which rather speaks for increased orienting toward happy faces (Salemink et al., 2007; Cisler and Koster, 2010). No other BI reached the significance level (all *p*-values ≥0.280). When splitting the sample in male and female subgroups, BI scores for happy faces were still significantly lower than 0 in the male group [$t(9) = -4.92$, $p = 0.001$, $d = -1.56$], and borderline significant in the female group [$t(10) = -2.14$, $p = 0.058$, $d = -0.65$]. No other BI reached significance in the subgroups (all *p*-values ≥0.139). Using a more stringent significance level of $p < 0.01$ to control for multiple testing, only BI scores for happy faces in the overall sample and among men reached significance. *Per se*, bias indices did not differ between women and men (all *p*-values ≥0.128).

Reaction times did not differ between woman and men in the current study (all *p*-values ≥0.137). Concerning questionnaire data and sex differences, significant differences between women and men were only found in the TAS-26 subscale DDF [$t(19 = -3.21$, $p = 0.005$, $d = 1.40$]. Male participants reported more difficulties describing feelings than female participants. The other comparisons did not reach significance level (all *p*-values ≥0.383).

Table 1 | Means and standard deviations of bias index scores, reaction times, and psychological measures.

	Total sample	SD	Men (*n* = 10)	SD	Women (*n* = 11)	SD	Statistics sex differences
Bias indices							
BI anger	2.17	9.10	4.39	9.14	0.16	9.00	$t(19) = -1.07$, $p = 0.300$
BI happy	-5.35^{***}	7.80	-8.08^{***}	7.92	-2.86^{+}	7.13	$t(19) = 1.59$, $p = 0.128$
OI anger	2.44	11.63	4.82	14.76	0.27	7.97	$t(19) = -0.89$, $p = 0.348$
OI happy	-3.31^{+}	13.00	−3.39	16.37	−3.23	9.83	$t(19) = 0.03$, $p = 0.978$
DI anger	−0.26	8.80	−0.44	8.37	−0.11	9.58	$t(19) = 0.08$, $p = 0.935$
DI happy	−2.04	10.97	−4.69	11.70	0.37	10.19	$t(19) = 1.06$, $p = 0.303$
Reaction times							
Anger congruent	359.00	34.81	369.01	44.17	349.90	21.85	$t(19) = -1.28$, $p = 0.218$
Anger incongruent	361.17	37.88	373.39	45.70	350.07	26.56	$t(19) = -1.45$, $p = 0.164$
Happy congruent	364.75	36.29	377.22	44.23	353.41	23.99	$t(19) = -1.55$, $p = 0.137$
Happy incongruent	359.40	36.23	369.14	45.01	350.55	24.91	$t(19) = -1.19$, $p = 0.250$
Neutral	361.44	37.28	373.83	47.29	350.17	21.77	$t(12.4) = -1.45$, $p = 0.172$
SCL-90							
Depression	4.71	5.25	4.00	6.83	5.36	3.50	$t(19) = 0.58$, $p = 0.566$
Anxiety	3.05	3.81	2.90	4.15	3.18	3.68	$t(19) = 0.17$, $p = 0.871$
GSI	0.27	0.25	0.24	0.32	0.29	0.18	$t(19) = 0.44$, $p = 0.663$
TAS-26							
DIF	11.00	2.77	11.00	2.31	11.00	3.26	$t(19) < 0.01$, $p > 0.999$
DDF	10.76	2.70	12.40	2.07	9.27	2.37	$t(19) = -3.21$, $p = 0.005$
EOT	12.10	2.30	11.60	3.06	12.55	1.29	$t(11.9) = 0.91$, $p = 0.383$

$^{+}p < 0.10$, $^{***}p < 0.001$ in t-tests against 0 (see text).

EEG DATA

Probe P1 mean amplitudes differed between sexes [$F(1,21) = 8.31$, $p = 0.009$] and hemifield [$F(1,21) = 5.94$, $p = 0.009$], but not between emotions [$F(1,20.92) = 0.93$, $p = 0.412$] or congruency conditions [$F(1,21) = 0.97$, $p = 0.397$]. Moreover, there was an interaction of sex by emotion [$F(1,21) = 3.96$, $p = 0.035$]. In marginal means, women ($M = 0.51\ \mu$V, SE = 0.50) and men ($M = -1.81\ \mu$V, SE = 0.52) differed overall by a large effect size, $d = 1.42$[1] (see **Figure 1**); this effect was more pronounced for happy faces ($d = 1.76$, $p < 0.001$ in simple effects analysis) than for angry ($d = 1.14$, $p = 0.016$) or neutral faces ($d = 0.53$, $p = 0.239$). The hemifields differed overall by a medium effect size, $d = 0.65$ (left: $M = -0.31\ \mu$V, SE = 0.39; right: $M = -0.90\ \mu$V, SE = 0.39).

Probe P1 mean difference wave amplitudes differed between right and left hemifield [$F(1,19) = 8.08$, $p = 0.010$, $\eta_p^2 = 0.30$], but not between sexes [$F(1,19) = 1.93$, $p = 0.181$, $\eta_p^2 = 0.09$], emotions [$F(1,19) = 0.32$, $p = 0.597$, $\eta_p^2 = 0.02$], or between congruency conditions [$F(1,19) = 0.81$, $p = 0.381$, $\eta_p^2 = 0.04$]. Probe P1 mean difference wave amplitudes were more positive when the emotional face was presented in the left compared to the right hemifield. All first-order interactions were not significant [sex by emotion: $F(1,19) = 0.72$, $p = 0.407$, $\eta_p^2 = 0.04$; sex by congruency: $F(1,19) = 0.78$, $p = 0.389$, $\eta_p^2 = 0.04$; sex by hemifield [$F(1,19) = 0.25$, $p = 623$, $\eta_p^2 = 0.01$; emotion by congruency: $F(1,19) = 0.42$, $p = 0.523$, $\eta_p^2 = 0.02$; emotion by hemifield: $F(1,19) = 0.43$, $p = 0.522$, $\eta_p^2 = 0.02$; congruency by hemifield: $F(1,19) = 1.15$, $p = 0.298$, $\eta_p^2 = 0.06$]. However, the triple interaction sex by emotion by congruency yielded a significant result [$F(1,19) = 4.66$, $p = 0.044$, $\eta_p^2 = 0.20$]. This could be traced to a significant difference between men and women in the *happy-congruent-diff* conditions [merged for both hemifields; $t(19) = 2.42$, $p = 0.026$, $d = 1.06$; see **Figure 2**]; probe P1 mean amplitudes in this condition were enhanced among women, but diminished among men relative to the *neutral* condition (see also **Figure 1**). This resulted in relatively higher amplitudes (i.e., more positive amplitudes) of the difference wave

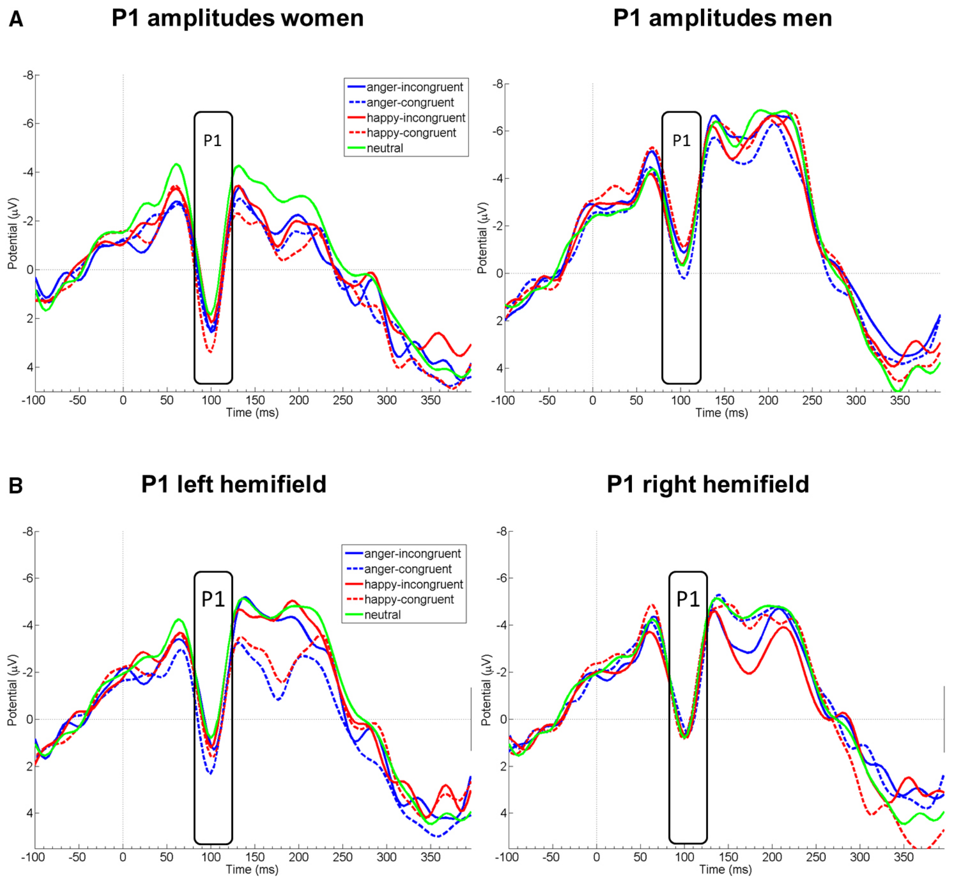

FIGURE 1 | EEG amplitude courses elicited by probe presentation at electrode Oz. Panel **(A)** displays P1 amplitudes separately for women (left, n = 11) and men (right, n = 10). Panel **(B)** displays P1 amplitudes for all participants, separately after emotional face presentation in the left and right visual hemifield. Note that the condition neutral is included in both figures of panel **(B)** for demonstrational purposes. Negative is drawn upward per convention. The rectangles denote probe P1 analysis time window.

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[1] Excluding the one male participant who reported elevated levels in anxiety, depression, and global psychological distress (see Participants), did not substantially change results [$F(1,20) = 9.01$, $p = 0.007$; marginal means: women: $M = 0.49\ \mu$V, SE = 0.50; men: $M = -1.91\ \mu$V, SE = 0.55; $d = 1.49$].

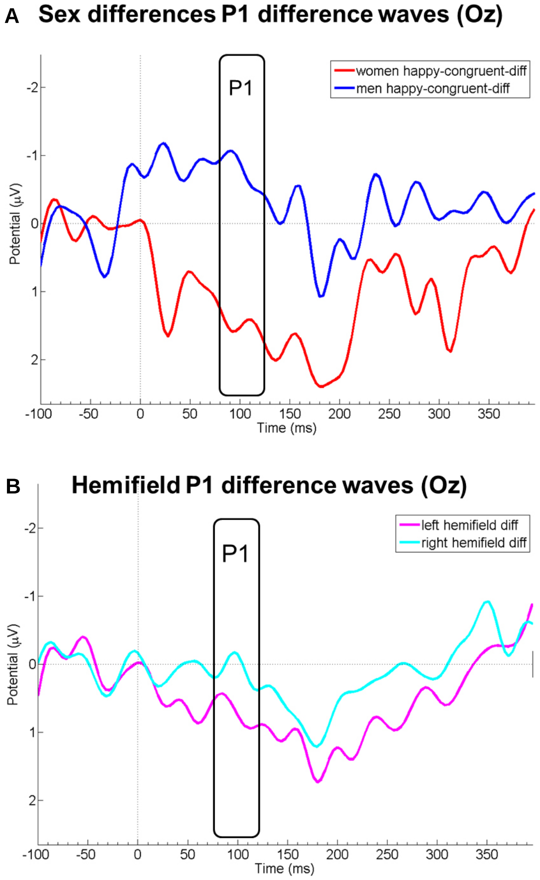

FIGURE 2 | Panel **(A)** displays EEG courses at electrode Oz of the difference wave amplitudes of the condition happy-congruent minus neutral for women (red) and men (blue). Panel **(B)** displays P1 difference wave amplitudes at Oz merged for all conditions in which the emotional face was presented in the left (pink) and right (light blue) visual hemifield. Negative is drawn upward per convention. The rectangles denote probe P1 analysis time window.

among women than men in the P1 time range. Men and women did not differ significantly in any of the other merged conditions (*anger-incongruent-diff*: $t(19) = 1.32$, $p = 0.204$, $d = 0.58$; *anger-congruent-diff*: $t(19) = 0.47$, $p = 0.645$, $d = 0.21$; *happy-incongruent-diff*: $t(19) = 0.21$, $p = 0.834$, $d = 0.09$). The remaining triple and the four-way interaction were not significant (all *p*-values ≥ 0.217).

We observed no significant correlations between bias indices and ERP amplitudes following angry (all *p*-values ≥ 0.074) or happy faces (all *p*-values ≥ 0.127) in the total sample. Separately for women and men, no significant correlations were found in women (all *p*-values ≥ 0.096). However, in men significant correlations were observed for DI happy scores and probe P1 mean amplitudes in the conditions *anger-congruent-R* ($r_s = -0.70$, $p = 0.025$) and *happy-incongruent-R* ($r_s = -0.72$, $p = 0.019$). The correlation between DI happy scores and probe P1 mean amplitudes of *anger-congruent-R* revealed the only significant difference between both sexes in these correlational analyses ($z = 1.99$, $p = 0.048$). Men showed a negative correlation whereas woman a non-significant positive one ($r_s = 0.16$, $p = 0.631$).

ALEXITHYMIA ANALYSES

The TAS-26 subscales did not significantly inter-correlate (all *p*-values ≥ 0.236). Anger BI scores correlated borderline significantly with TAS-26-DDF scores ($r_s = 0.42$, $p = 0.056$; all other *p*-values ≥ 0.116). Probe P1 mean amplitudes correlated negatively with TAS-26-DIF scores in the following conditions: *anger-congruent-L* ($r_s = -0.49$, $p = 0.025$), *happy-incongruent-R* ($r_s = -0.45$, $p = 0.041$), and *happy-congruent-L* ($r_s = -0.53$, $p = 0.013$). Negative correlations between probe P1 mean amplitudes and TAS-26-DDF scores were observed in the *happy-congruent-L* ($r_s = -0.44$, $p = 0.046$) and *happy-congruent-R* conditions ($r_s = -0.56$, $p = 0.008$). Neither of the associations with TAS-26-DIF scores were fully qualified by participant sex (controlling for sex, the partial Spearman correlation coefficients were $r_s = -0.57$, $p = 0.008$; $r_s = -0.49$, $p = 0.026$; $r_s = -0.64$, $p < 0.001$), nor was the negative association of TAS-26-DDF scores with amplitudes in the *happy-congruent-R* condition (partial $r_s = -0.61$, $p = 0.002$); however, the association of TAS-26-DDF scores with amplitudes in the *happy-congruent-L* condition lost its significance (partial $r_s = -0.31$, $p = 0.180$), even though still pointing in the same direction. Probe P1 difference wave amplitudes correlated negatively with TAS-26-DDF in *happy-congruent-R-diff* ($r_s = -0.44$, $p = 0.045$). However, controlling for sex, this correlation was substantially reduced in magnitude and rendered insignificant, partial $r_s = -0.28$, $p = 0.233$.

Calculating the correlations separately in women and men, correlations with TAS-26-DIF scores remained mostly significant. In women, significant correlations were observed for probe P1 mean amplitudes for the conditions *anger-congruent-L* ($r_s = -0.66$, $p = 0.027$), *happy-incongruent-R* ($r_s = -0.62$, $p = 0.043$), and *happy-congruent-L* ($r_s = -0.82$, $p = 0.002$); in men only for *happy-incongruent-L* ($r_s = -0.67$, $p = 0.033$). The correlations between TAS-26-DIF scores and probe P1 difference wave scores yielded only a significant correlation in women for the condition *happy-congruent-L-diff* ($r_s = -0.71$, $p = 0.015$). No significant correlations were found for TAS-26-DDF scores and any ERPs (all other *p*-values ≥ 0.068). Spearman correlations between TAS-26-DIF scores and probe P1 mean amplitudes and difference wave amplitudes did not significantly differ between women and men (all *p*-values ≥ 0.095), neither did the correlations between TAS-26-DDF scores and the ERPs (all *p*-values ≥ 0.131).

DISCUSSION

This study examined sex differences in probe P1 amplitudes, using the dot probe paradigm, and explored the usefulness of a difference wave approach for investigating attentional biases. We found that probe P1 amplitudes were overall enhanced among women, compared to men, in particular after rewarding facial stimuli. This adds to prior evidence, suggesting that neural activity during early visual processing stages is enhanced in women compared to men (Sass et al., 2010). It further underlines that neuroscientific studies may need to control and adjust for participant sex both with regard to study design and analysis (see Cahill, 2006). Moreover, we found that probe P1 amplitudes were enhanced when the emotional face was presented beforehand in the left compared to the right visual hemifield which might be due to component overlap with offset potentials of the preceding emotional stimuli. Only a few previous studies considered the factor hemifield in their analyses. Some studies observed no significant influence of hemifield (Pourtois et al., 2004; Eldar et al., 2010). However, in line with the current results, Brosch et al. (2011) observed larger probe P1 amplitudes when the probes were presented in the left hemifield. Our results can further be related to early research on hemispheric control of spatial attention. Kinsbourne (1974) postulated neuronal control networks in both hemispheres which interact in a mutually inhibitory way. Subsequent research showed that activation in one hemisphere led to orienting attention to the side of the other hemisphere (Reuter-Lorenz et al., 1990). Moreover, the bias of the right hemisphere executed on left-hemispheric activations was observed to be stronger than vice versa (Reuter-Lorenz et al., 1990). More recent theoretical assumptions emphasize competitive interactions between the hemispheres controlling spatial attention though (Szczepanski and Kastner, 2013). In any case, the observed effects of hemifield on probe P1 amplitudes further indicate hemispheric lateralization during stimulus processing in the dot probe task. Future studies should by default include hemifield in their analyses to allow stronger testing of hypotheses concerning lateralized emotional stimulus processing and spatial attention effects.

Using neutral–neutral stimulus pairs in the dot probe task, which served as a baseline in a difference wave approach, we further obtained additional preliminary evidence that, relative to this baseline, women showed specifically enhanced probe P1 amplitudes with regard to rewarding (i.e., happy) facial stimuli after congruent stimulus presentation. Previous behavioral research has suggested that healthy women show delayed disengagement specifically from happy faces (Tran et al., 2013). This could not be confirmed with the behavioral data in the present study. However, our results corroborate previous findings on the neuronal level. It may be speculated that this effect is more readily observable on a neuronal level, but demands larger sample sizes to also be observed on the behavioral level (Tran et al., 2013, investigated the data of 173 women and 174 men). Differences between men and women in the allocation of attention toward rewarding and threatening stimuli need to be investigated in much more detail in the future. Our results suggest that a difference wave approach might be optimally suited for such an endeavor and should therefore be followed up.

Using the classical mean amplitudes approach, no threat-related probe P1 amplitude variation in response to target processing was observed. This is in line with a study by Eldar et al. (2010), but is contradictory to others (Santesso et al., 2008; Brosch et al., 2011). As suggested by Eldar et al. (2010), however, different results may have been partially caused by differences in the experimental paradigms and set-ups. Task demands such as giving a motor response or withholding a motor response might have top-down influence on early stimulus processing ERPs as a function of attentional load (Fu et al., 2010). Even the temporal distance (i.e., stimulus onset asynchrony) between face pair onset and probe onset may be important, both on the behavioral (e.g., Brosch et al., 2011) and the neuronal level (Wykowska and Schubö, 2010).

The difference wave approach utilized in the present study revealed additional information which could not be captured with the classical analysis approach. The significant differences between men and women during the probe P1 time range following congruently primed happy faces suggests that women allocated more attentional resources to the happy face stimuli than men, and that their attention was still captured by these stimuli during probe presentation. In Salemink et al.'s (2007) notion, this might be interpreted as delayed disengagement from these stimuli. Emotional facial displays serve as social cues containing important information during social exchange situations (Rolls, 2000). Happy faces can be seen as approach signals initiating affiliative tendencies, whereas angry faces signal rejection and non-affiliation (Frijda et al., 1989; Bourgeois and Hess, 2008). It might be an evolutionary residue that women allocate more attentional resources to happy faces than men, thereby increasing their chances of affiliation. However, there is also ample evidence showing that gender role promotes specifically prosocial and supportive behavior in close relationships among women (Eagly, 2009), and that sociocultural influences and gender role socialization also contribute to sex differences in anxiety (McLean and Anderson, 2009).

Lastly, probe P1 and probe P1 difference wave amplitudes showed associations with self-reported difficulties in identifying and describing feelings. Participants with problems in the identification of feelings showed less activation after angry and happy faces, whereas participants with problems in describing feelings showed less activation only after happy faces in the congruent conditions. In particular women showed a negative association between difficulties in describing feelings and neuronal correlates after congruently presented happy facial displays—again in the one condition were sex differences were most evident. Previous research has suggested that alexithymia is not associated with a lack of emotional awareness *per se*, but may entail more effort in the processing of emotional information (Franz et al., 2004). Our results do not directly lend to this interpretation, but were instead suggestive of early processing deficits in alexithymia (e.g., Eichmann et al., 2008; Reker et al., 2010; Pollatos and Gramann, 2011). Applying a passive viewing paradigm, Pollatos and Gramann (2011) also observed reduced P1 amplitudes in response to emotional stimuli in participants with high levels of alexithymia, most pronounced for pleasant and neutral stimuli. Further in line with our results, these authors reported that in particular difficulties in describing feelings explained the variance in P1 amplitudes in their sample. In conclusion, our findings corroborate that alexithymia is a correlate of reduced emotional reactivity on a neuronal level. These findings need to be followed up in future studies.

Limitations pertain to the relatively small sample size and the mostly non-clinical nature of our sample which was not assessed via a structured clinical interview but via self-report. The previously reported delayed disengagement from happy faces among healthy women on the behavioral level could not be confirmed in the present study, which could, however, also be sample-related.

Generally, our present findings need to be replicated in larger samples and should be, therefore, regarded only as preliminary. Moreover, sex differences in (probe) P1 amplitudes need to be investigated also in clinical samples to determine the generalizability of our results also with regard to clinical populations. The internal consistency of the externally oriented thinking factor in the TAS-26 was unacceptably low. It may not be ruled out that probe P1 amplitudes correlate also with this factor in more heterogeneous or more alexithymic samples, where a higher internal consistency of this measure may be expected.

In conclusion, the current results demonstrate that even healthy men and women differ in their neural activation while allocating attention to emotional stimuli, in particular to rewarding ones. This needs to be considered in studies of attentional biases, both in samples from the general population, but also in clinical samples. The current study was the first to use a difference wave approach to investigate attentional processes of orientation and disengagement which allowed us to detect more subtle differences between the two sexes. We recommend that future attentional bias studies include neutral–neutral trials to be able to use the difference wave approach for their research questions. Furthermore, alexithymia may need to be considered more closely in studies on attentional biases with facial stimuli.

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Behavioral and ERP measures of attentional bias to threat in the dot-probe task: poor reliability and lack of correlation with anxiety

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The dot-probe task is often considered a gold standard in the field for investigating attentional bias to threat. However, serious issues with the task have been raised. Specifically, a number of studies have demonstrated that the traditional reaction time (RT) measure of attentional bias to threat in the dot-probe task has poor internal reliability and poor test-retest reliability. In addition, although threatening stimuli capture attention in other paradigms, attentional bias to threat has not usually been found in typical research participants in the dot-probe task. However, when attention is measured in the dot-probe task with the N2pc component of the event-related potential waveform, substantial attentional orienting to threat is observed, and the internal reliability is moderate. To provide a rigorous comparison of the reliability of this N2pc measure and the conventional behavioral measure, as well as to examine the relationship of these measures to anxiety, the present study examined the N2pc in conjunction with RT in the dot-probe task in a large sample of participants ($N = 96$). As in previous studies, RT showed no bias to threatening images across the sample and exhibited poor internal reliability. Moreover, this measure did not relate to trait anxiety. By contrast, the N2pc revealed a significant initial shift of attention to threat, and this measure was internally reliable. However, the N2pc was not correlated with trait anxiety, indicating that it does not provide a meaningful index of individual differences in anxiety in the dot-probe task. Together, these results indicate a serious need to develop new tasks and methods to more reliably investigate attentional bias to threat and its relationship to anxiety in both clinical and non-clinical populations.

Keywords: anxiety, attentional bias, dot probe, ERPs, IAPS, N2pc, reliability, threat

INTRODUCTION

Threatening stimuli convey important information about the surrounding environment and are thought to automatically capture attention (e.g., LeDoux, 1996; Eastwood et al., 2001; Öhman et al., 2001a). The preferential allocation of attention to threatening stimuli over emotionally neutral stimuli, typically termed an *attentional bias* to threat, has become an important topic of investigation in the fields of affective and clinical science. In particular, abnormal allocation of attention to threat is thought to play a key role in anxiety disorders, providing a possible mechanism for distinguishing between normal and abnormal responses to threatening information (Beck, 1976; Williams et al., 1988; Mathews, 1990; Eysenck, 1992; Mathews and MacLeod, 2002; Bar-Haim et al., 2007; Cisler and Koster, 2010).

The dot-probe task, developed by MacLeod et al. (1986), is considered a gold standard in the field for investigating attentional bias to threatening stimuli. In this task, a threatening stimulus and a neutral stimulus are presented simultaneously in different spatial locations (e.g., one to the left visual field and one to the right visual field), followed by the presentation of a target item at one of the cued locations. Reaction times (RTs) to targets that appear at the prior location of the threatening stimulus (i.e., threat-congruent trials) are compared with RTs to targets that appear at the prior location of the neutral stimulus (i.e., threat-incongruent trials); faster responses on threat-congruent trials are interpreted as evidence of an attentional bias to the location of the threatening stimulus.

The dot-probe task has been used in hundreds of studies over the past three decades to investigate attention to threat-related stimuli in typical individuals and in clinically and non-clinically anxious individuals (see Bar-Haim et al., 2007 for a review). However, serious concerns with the dot-probe task have been raised. For example, although typical research participants appear to allocate attention to threatening information in the context of a variety of *other* tasks and measures (see MacNamara et al., 2013 for a review), there is no evidence of an attentional bias to threat in typical individuals using RT measures derived from the dot-probe task (see Bar-Haim et al., 2007 for a review). The dot-probe task almost uniquely suggests that normative individuals do *not* attend to threat. By contrast, an attentional bias to threat has been found in the dot-probe task among clinically and non-clinically anxious individuals (see Bar-Haim et al., 2007 for a review). However, even these results vary, with some studies failing to find a bias to threat in anxious populations (see, for example, Broadbent and Broadbent, 1988; Bradley et al., 1997, 2000; Mogg et al., 1997, 2000a,b; Mogg and Bradley, 1999; Yiend and Mathews, 2001; Pineles and Mineka, 2005). Indeed, a recent study found that anxious individuals exhibit a range of threat biases in the dot-probe task, including a bias to threat, a bias away from threat, or a combination of bias to and away from threat that depends on the specific type of threatening images examined (Zvielli et al., 2014).

One possible reason for such discrepant findings in the literature as well as the failure to find a bias to threat among typical individuals may be the poor psychometric properties of the RT measure derived from the dot-probe task. Specifically, a number of studies have collectively demonstrated that the traditional RT measure of attentional bias to threat used in the dot-probe task has both poor test-retest reliability (Schmukle, 2005; Staugaard, 2009) and poor internal reliability (Schmukle, 2005; Staugaard, 2009; Waechter et al., 2013; Kappenman et al., 2014; however, see Bar-Haim et al., 2010 for one contradictory finding). Indeed, the first study to demonstrate poor reliability of the RT-based measure of attentional bias to threat in the dot-probe task was published nearly a decade ago (Schmukle, 2005); however, the majority of dot-probe studies published since then do not provide a quantification of the internal consistency of the RT bias measure.

Internal consistency can be derived easily by computing split-half reliability (for example, by computing the correlation between the RT-based measure of threat bias derived from odd- versus even-numbered trials). This produces a measure of internal reliability—the degree to which RT bias to threat is consistent across the task *within* an individual. A number of studies have found poor internal reliability for the RT-based measure of attentional bias to threat using different versions of the dot-probe task, including the original version (Schmukle, 2005; Staugaard, 2009) and more recent modifications of the task (Schmukle, 2005; Staugaard, 2009; Waechter et al., 2013; Kappenman et al., 2014), and across different types of threat stimuli, including words (Schmukle, 2005), faces (Staugaard, 2009; Waechter et al., 2013), and complex images (Schmukle, 2005; Kappenman et al., 2014).

The fact that the RT measure of attentional bias to threat in the dot-probe task has poor internal reliability limits its validity: a measure cannot be valid unless it is reliable. Moreover, the internal reliability of a measure places an upper bound on its ability to correlate with another measure. Therefore, poor internal reliability of the RT-based measure of threat bias in the dot-probe task limits its ability to correlate with another measure, such as anxiety.

Recently, event-related potentials (ERPs) have been used in conjunction with RT measures to examine the time course of attention to threat in the dot-probe task with millisecond resolution (Kappenman et al., 2014). In contrast to behavioral measures, which reflect the combined effects of a sequence of many distinct neural processes, ERPs provide a continuous measure of processing and can therefore show how the allocation of attention unfolds over the course of a trial. In contrast to RT findings, ERPs have revealed an initial shift of visual attention to the threatening stimulus in typical individuals in the dot-probe task, as measured with the N2pc component (Kappenman et al., 2014), described in greater detail below. In other words, this ERP measure showed that typical individuals do indeed allocate attention to threat in the dot-probe task, despite the fact that RT measures (in this and many other tasks) showed no bias for threat in these individuals. That is, even within the same task and individuals, ERPs *but not behavioral measures* suggested that attention was biased to threatening stimuli in typical individuals. Moreover, this ERP measure of attention to threat was internally reliable, whereas the RT measure of threat bias exhibited poor internal reliability (Kappenman et al., 2014).

One reason the N2pc was able to capture an attentional bias to threat that was not evident in behavior is likely related to the timing of the measures relative to the events in the task. That is, the N2pc component was present from approximately 150–250 ms after the onset of the images, whereas the behavioral response occurred several hundred milliseconds later (after the presentation of the target item). Given that covert attention can shift rapidly between locations (in as little as 50–100 ms; Müller and Rabbit, 1989), ample time was provided for attention to shift away from the location of the threatening stimulus prior to the onset of the target. This was further supported in our previous study by the absence of sustained engagement with the threatening stimulus subsequent to the initial shift of attention (Kappenman et al., 2014). Specifically, our previous study found an N2pc to the threatening stimulus but no late positive potential (LPP)—an ERP index of sustained engagement with emotional images (see Hajcak et al., 2012 for a review of the LPP). Thus, the shift of attention to the threatening image had already terminated when the target appeared, which explains why the behavioral response to the target did not show evidence of an attentional bias to the threatening image location.

The present study extended this work by examining the relationship between ERP and behavioral measures of attentional bias to threat with individual differences in anxiety across a large sample ($N = 96$) of participants. The primary goal of this study was to determine whether the more internally reliable N2pc component might provide a better index of individual differences in anxiety—specifically, in contrast to the internally *un*reliable RT-based measure that has been the primary focus of the attentional bias literature.

We focused on the N2pc component, which is a negative-going potential at posterior electrode sites contralateral to the location of an attended item. This component has been well validated and has been used to index covert visual attention in cognitive psychology for over 25 years (see Luck, 2012 for a review), and more recently, to examine the allocation of attention to emotional stimuli (Eimer and Kiss, 2007; Fox et al., 2008; Buodo et al., 2010; Brosch et al., 2011; Shaw et al., 2011; Ikeda et al., 2013; Weymar et al., 2013; Grimshaw et al., 2014; Kappenman et al., 2014). We examined the N2pc in conjunction with the traditional RT measure of threat bias in the dot-probe task, investigating both the internal reliability of these measures and how they correlate with individual differences in trait-level anxiety. To ensure that we could distinguish anxiety from depression—which are often comorbid but may show distinct patterns of results in the dot-probe task (see Bar-Haim et al., 2007)—we used the Mood and Anxiety Symptom Questionnaire (MASQ; Watson and Clark, 1991; Watson and McKee, 1996) to separately measure facets of anxiety and depression in our sample. In addition, to maximize the potential for the task-irrelevant threatening stimuli to capture attention, we used complex threatening images from the International Affective Picture System (IAPS; Lang et al., 2008), which may be stronger elicitors of emotion than the emotional faces often used in dot-probe studies (Britton et al., 2006).

In line with previous studies, we predicted that we would find no evidence of an attentional bias to threat on average across the sample of participants in the present study using the RT-based measure of threat bias, and further, that this measure would have poor internal reliability. Poor internal reliability for the RT-based measure of threat bias would severely restrict the ability of this measure to correlate with any of our other measures, and therefore we predicted that the RT-based measure of threat bias would not meaningfully correlate with self-reported anxiety or depression. By contrast, we predicted that the N2pc would provide evidence of an initial shift of attention to threatening images across the sample, and that this measure would show moderate reliability, replicating our previous findings (Kappenman et al., 2014). Finally, we tentatively predicted that the N2pc would correlate with self-reported anxiety.

MATERIALS AND METHODS

PARTICIPANTS

One hundred and eleven undergraduate students between the ages of 18 and 30 were tested. In our research with typical young adults, participants are always excluded if they exhibit EEG artifacts on more than 25% of trials. Fifteen participants were excluded for this reason, leaving 96 participants (50 female, 46 male; Mean age = 20.54, SD = 2.34, Range 18–29); all analyses reflect this final sample. The study was approved by the University of California, Davis Institutional Review Board (IRB), and participants received monetary compensation.

QUESTIONNAIRES

Prior to the start of the task, participants completed the MASQ, Short Form (Watson and Clark, 1991; Watson and McKee, 1996). The MASQ is a 62-item self-report measure consisting of four subscales, two that index anxiety symptoms, including "Anxious Arousal" (17 items) and "General Distress–Anxiety Symptoms" (11 items), and two that index depressive symptoms, including "Anhedonic Depression" (22 items) and "General Distress–Depressive Symptoms" (12 items). Participants are asked to indicate how much each item describes how they have felt "during the past week, including today" using a 5-point scale ranging from 1 ("Not at All") to 5 ("Extremely").

STIMULI AND TASK

The stimuli were 50 neutral and 50 threatening images selected from the IAPS images[1]. Neutral images included pictures of buildings, household objects, and people with neutral facial expressions. Threatening images included pictures of animals attacking the viewer, assault and abduction scenes, and pictures of guns.

Participants performed a dot-probe task. Example trial sequences are presented in **Figure 1**. Stimuli were presented on a gray background with a continuously visible fixation cross using a CRT monitor viewed at a distance of 70 cm. On each trial, a pair of IAPS images was presented for 500 ms, one image to the left and one image to the right of a continuously visible central black fixation cross. Each image in a pair subtended 10 × 7.3° of visual angle and was centered 6.2° to the left or right of the fixation cross. Immediately following the offset of the images, a target composed of either two horizontally or vertically arranged white dots outlined in black (each dot subtending 0.75° × 0.75° and separated by 0.15°) was presented for 100 ms, centered in the location of one of the previously presented images. Participants made a button press using the index or middle finger of the dominant hand to indicate whether the target item was a pair of vertically or horizontally arranged dots. A jittered intertrial interval of 1400–1600 ms (rectangular distribution) with a blank screen occurred following the offset of the target. Participants were told that the images were irrelevant to the task and were instructed to respond to the targets as quickly and accurately as possible. To ensure that eye movement artifacts would not contaminate the EEG recordings and influence measurement of the N2pc, participants were instructed to maintain eye fixation in the center of the screen throughout the trial (see Luck, 2014).

The threat image appeared with equal probability on the left and right sides, as did the target, but the threat and target locations were independently randomized. The target orientation was equally likely to be horizontal or vertical, and this was randomized independently of the other variables. The combinations of threat location, target location, and target orientation were

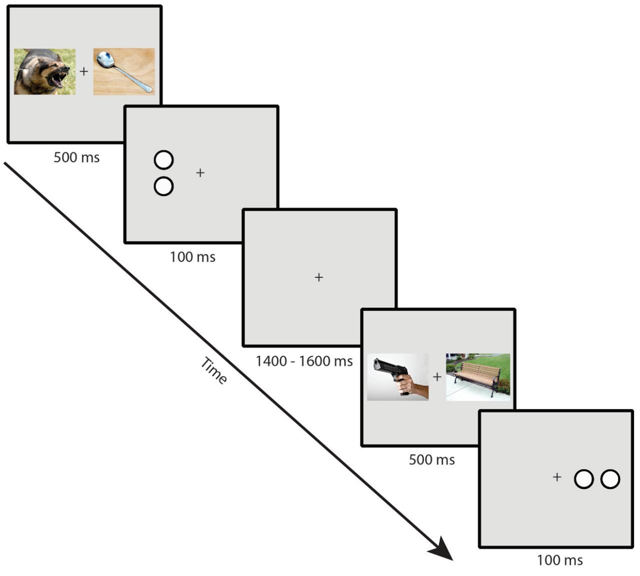

FIGURE 1 | Example trial sequence in the dot-probe task (note that stimuli are not to scale; see text for actual sizes used in the task).

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[1] Threatening IAPS images were: 1026, 1033, 1050, 1052, 1080, 1090, 1113, 1114, 1120, 1200, 1201, 1202, 1205, 1220, 1230, 1240, 1300, 1301, 1302, 1304, 1321, 1525, 1726, 1820, 1930, 1931, 1932, 2120, 2691, 2811, 3500, 6190, 6200, 6211, 6213, 6220, 6230, 6240, 6242, 6243, 6250.1, 6260, 6263, 6370, 6510, 6940, 9425, 9426, 9440, 6270.1. Neutral IAPS images were: 2102, 2191, 2383, 2384, 2411, 2745.1, 5390, 5395, 5500, 5530, 5731, 6150, 7000, 7001, 7002, 7004, 7006, 7009, 7010, 7012, 7018, 7019, 7020, 7021, 7025, 7026, 7031, 7032, 7033, 7035, 7036, 7037, 7040, 7041, 7042, 7056, 7057, 7059, 7060, 7061, 7080, 7081, 7090, 7150, 7175, 7211, 7233, 7512, 7546, 7547.

presented in an unpredictable order. Participants completed a total of 360 trials. Short self-paced breaks were provided every 40 trials.

ELECTROENCEPHALOGRAPHIC RECORDING AND DATA PROCESSING

The continuous EEG was recorded using a Biosemi ActiveTwo recording system (Biosemi B.V., Amsterdam, the Netherlands). The electrodes were mounted in an elastic cap using a subset of the International 10/20 System sites (FP1, FP2, F3, F4, F7, F8, C3, C4, T7, T8, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, PO3, PO4, PO7, PO8, O1, O2, Fz, Cz, Pz, POz, Oz, and Iz). A common mode sense (CMS) electrode was located at site FC1, with a driven right leg (DRL) electrode located at site FC2. The horizontal electrooculogram (EOG) was recorded from electrodes placed lateral to the external canthi and was used to detect horizontal eye movements; the vertical EOG was recorded from electrodes placed above and below the right eye and was used to detect eyeblinks and vertical eye movements. The EEG and EOG were low-pass filtered using a fifth order sinc filter with a half-power cutoff at 204.8 Hz and digitized at 1024 Hz with 24 bits of resolution. The single-ended EEG signals were converted to differential signals offline, referenced to the average of P9 and P10 (located adjacent to the mastoids).

Signal processing and analysis was performed in Matlab using EEGLAB toolbox (Delorme and Makeig, 2004) and ERPLAB toolbox (Lopez-Calderon and Luck, 2014). The EEG was high-pass filtered with a cut-off of 0.1 Hz (non-causal Butterworth impulse response function, half-amplitude cut-off, 12 dB/oct roll-off). Portions of EEG containing large muscle artifacts or extreme voltage offsets (identified by visual inspection) were removed. Independent component analysis (ICA) was then performed for each subject to identify and remove components that were clearly associated with eyeblink activity, as assessed by visual inspection of the waveforms and the scalp distributions of the components (Jung et al., 2000). The ICA-corrected EEG data were segmented for each trial beginning 200 ms prior to the onset of the images and continuing for 500 ms. Baseline correction was performed using the 200 ms prior to the onset of the images. Segments of data containing artifacts were removed by means of semi-automated ERPLAB algorithms, including eye movements larger than 0.1° of visual angle that were detected using the step function described by Luck (2014). Trials with incorrect behavioral responses or RTs of <200 or >1000 ms (relative to probe onset) were excluded from all analyses.

Reaction time was defined as the time of the button press relative to the onset of the target item on correct trials only; RTs were averaged separately for each condition. Accuracy was calculated as the percentage of correct trials per condition.

To determine whether attention was preferentially allocated to the threatening image, we isolated the N2pc time-locked to the onset of the image pairs at posterior electrode sites (P7 and P8, where the N2pc is typically maximal; Luck, 2012), relative to the location of the threatening image. Specifically, we first created separate waveforms for the hemisphere that was contralateral to the threatening image (i.e., left hemisphere electrode sites for right-side threatening images, and right hemisphere electrode sites for left-side threatening images) and the hemisphere that was ipsilateral to the threatening image (i.e., right hemisphere electrode sites for right-side threatening images, and left hemisphere electrode sites for left-side threatening images). We then created a contralateral-minus-ipsilateral difference waveform, and the N2pc was measured from the resulting difference wave in each subject. The mean amplitude of the N2pc was measured using an a priori time window of 175–225 ms following the onset of the image pairs, as in our previous study (see Kappenman et al., 2014).

Pearson's correlations were used to examine the relationship among measures[2]. Split-half reliability analyses were conducted by computing correlations of the averages of odd-numbered trials and even-numbered trials. All split-half reliability analyses were corrected for length using the Spearman–Brown formula (Anastasia and Urbina, 1997); all reported values reflect this correction.

RESULTS

BEHAVIOR

Mean RTs and mean accuracy (percent correct) are shown in **Table 1**. Participants were just as accurate for targets that replaced threatening images compared to targets that replaced neutral images [$t(95) = 0.128$, $p > 0.898$]. Consistent with previous studies, no significant RT difference was found between targets that replaced threatening images (threat-congruent trials) and targets that replaced neutral images (threat-incongruent trials) across the sample of participants [$t(95) = 1.01$, $p > 0.314$]. In other words, the sample as a whole demonstrated no evidence of bias toward or away from threat with RT, replicating many previous findings in the literature. We also examined the internal reliability of the RT bias measure (the difference between RT on threat-incongruent and threat-congruent trials); mean values for odd- and even- numbered trials are shown in **Table 2**. The threat bias measure derived from RT was uncorrelated between odd- and even-numbered trials ($r = 0.030$, $p > 0.772$), indicating poor internal reliability for this measure.

The logic of traditional null hypothesis statistical testing does not make it possible to conclude from the lack of statistically significant differences between threat-congruent and threat-incongruent trials that these conditions yielded equivalent RT or equivalent accuracy. However, it is possible to convert the t values from these analyses into *Bayes factor* values, which indicate the relative likelihood of the null and alternative hypotheses (Rouder et al., 2009). When we computed the Bayes factor for RT in the present study (using the calculator at http://pcl.missouri.edu/bayesfactor), we found that the null hypothesis was 7.5 times more likely to be true than the alternative hypothesis of a difference in RT between threat-congruent and threat-incongruent trials. Similarly, the null hypothesis was 12.3 times more likely to be true than the alternative hypothesis for accuracy. To provide a sense of the magnitude of these Bayes factor values, we also computed the Bayes factor that we would have obtained with this sample size if we had found a just-barely significant difference [i.e., $t(95) = 2.0$, $p = 0.049$] between threat-congruent and threat-incongruent trials. If we had obtained this t

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[2] We also examined non-parametric (i.e., Spearman's rank) correlations, which showed the same pattern of results.

Table 1 | Behavioral measures (SD in parentheses).

Trial type	Accuracy (% Correct)	Mean RT (ms)
All trials	95.57 (3.7)	527.93 (73.7)
Threat-congruent	95.58 (3.8)	529.15 (75.6)
Threat-incongruent	95.55 (3.9)	526.89 (74.2)

Table 2 | Split-half reliability measures (SD in parentheses).

Trial type	Mean RT-bias (ms)	N2pc mean amplitude (μV)
Odd-numbered	2.26 (16.35)	−0.66 (0.95)
Even-numbered	−0.08 (13.89)	−0.66 (1.03)

value, the corresponding Bayes factor value would have been 1.8, meaning that the alternative hypothesis would have been only 1.8 times more likely to be true than the null hypothesis. By comparison, the Bayes factor values of 7.8 and 12.3 that we actually found in favor of the null hypothesis are quite substantial.

N2pc

Grand average ERP waveforms time-locked to the onset of the IAPS images and collapsed across the P7 and P8 electrode sites are presented in **Figure 2**. The top panel overlays the waveforms contralateral to the location of the threatening image (dark blue line) and ipsilateral to the location of the threatening image (light red line). The bottom panel shows the contralateral-minus-ipsilateral difference waveform (dotted black line). Analyses revealed a significant N2pc ($M = -0.66$ μV, SD $= 0.82$) in the contralateral-minus-ipsilateral difference waveform [$t(95) = 7.96$, $p < 0.001$], reflecting a shift of covert visual attention in the direction of the threatening image following the onset of the image pair. The corresponding Bayes factor value indicated that the hypothesis of a real difference between the contralateral and ipsilateral voltages was over 1000 times more likely to be true than the null hypothesis.

Mean amplitude values for the N2pc on odd- and even-numbered trials are shown in **Table 2**. The amplitude of the N2pc on odd- versus even-numbered trials was moderately correlated ($r = 0.515$, $p < 0.001$), indicating that the N2pc was somewhat internally reliable (and much more reliable than the behavioral measures). These findings replicate our previous study conducted with participants from a different university (see Kappenman et al., 2014).

CORRELATIONS

Mood and Anxiety Symptom Questionnaire subscale measures are summarized in **Table 3**. To examine the relationship between threat bias and anxiety, we correlated each of the MASQ subscale scores separately with each of the measures of threat bias. The RT measure of attentional bias as a function of each of the MASQ subscale scores is shown in **Figure 3**. No significant correlation was found between the RT measure of threat bias and any of the MASQ subscales, including the anxiety and depression subscales (all *ps* > 0.10). Note that the non-significant correlations between the RT-based measure of threat bias and anxiety subscales were *negative* correlations, indicating that higher levels of anxiety were (non-significantly) associated with a *smaller* attentional bias to threat. This is the opposite of what would be predicted. The mean amplitude of the N2pc as a function of each of the MASQ subscale scores is shown in **Figure 4**. Despite the significant internal reliability of the N2pc, no significant correlation was obtained between the N2pc and the MASQ subscales (all *ps* > 0.29).

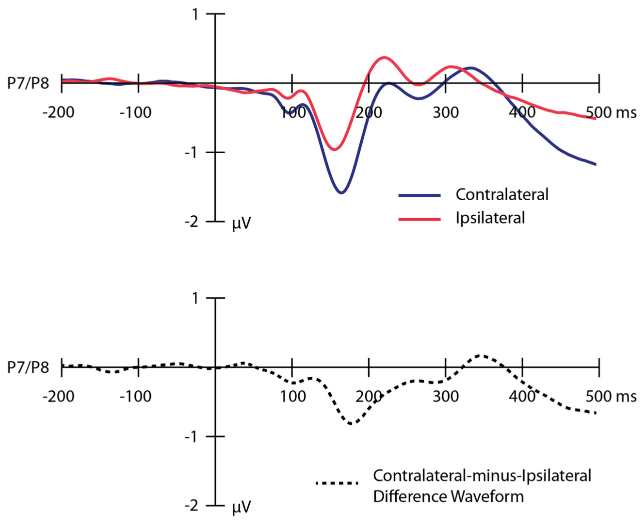

FIGURE 2 | Grand average event-related potential waveforms time-locked to the onset of the images collapsed across the P7 and P8 electrode sites. The top panel shows the waveforms contralateral to the location of the threatening image (dark blue line), ipsilateral to the location of the threatening image (light red line). The bottom panel shows the contralateral-minus-ipsilateral difference waveform (dotted black line). A digital low-pass filter was applied offline before plotting the waveforms shown here (Butterworth impulse response function, half-amplitude cutoff = 15.0 Hz, 12dB/oct roll-off).

Table 3 | MASQ measures (SD in parentheses).

Subscale	Mean score
General Distress: Anxiety symptoms	17.02 (4.0)
Anxious arousal	21.42 (4.4)
General Distress: Depressive symptoms	21.52 (7.0)
Anhedonic depression	54.49 (12.6)

We also examined whether attention to threat indexed by the N2pc was related to behavior by correlating the difference in RT on threat-incongruent and threat-congruent trials with the amplitude of the N2pc. No relationship between the N2pc and the RT measure of threat bias was found ($r = -0.079$, $p > 0.445$).

DISCUSSION

The present study examined the relationship between behavioral and ERP measures of attentional bias to threat in a dot-probe task and measures of trait anxiety in a large sample of participants. In line with previous dot probe studies, we found no

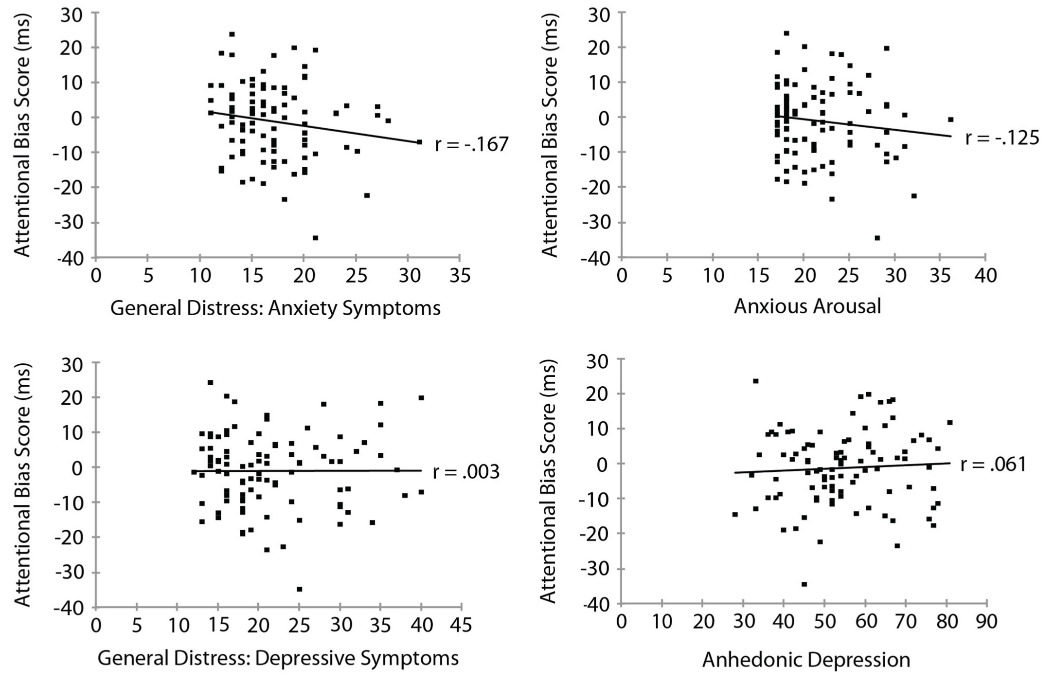

FIGURE 3 | Reaction time measure of attentional bias (threat-incongruent minus threat-congruent) as a function of MASQ subscale scores.

evidence of an attentional bias to threat across the sample using the traditional RT measure of threat bias in this task (i.e., the difference in RT on threat-incongruent and threat-congruent trials). In addition, the RT-based measure of threat bias was not internally reliable—RT-based measure of bias derived from odd and even trials were uncorrelated—a finding consistent with many previous studies (Schmukle, 2005; Staugaard, 2009; Waechter et al., 2013; Kappenman et al., 2014). Insofar as internal reliability limits both the validity of a measure and the ability of a measure to correlate with another trait-like measure, the RT-based measure of threat bias in this task was not an appropriate measure of individual differences in anxiety. Indeed, even if a significant relationship between RT-based threat bias and anxiety had emerged in the present study, the lack of internal reliability for the RT measure would have made the correlation uninterpretable.

Although we found no evidence of an attentional bias to threat using RT, ERPs revealed that there was an attentional bias to threat in our sample, as reflected by an N2pc to the location of the threatening stimulus. In addition, the N2pc showed highly significant (but not impressive) internal reliability, replicating the results of our previous study (Kappenman et al., 2014). These results showed that individuals are somewhat consistent in the degree to which they exhibit an electrocortically measured attentional bias to threatening stimuli in the dot-probe task. This is in direct contrast to the results obtained with behavior, which showed (1) no evidence of an attentional bias to threat and (2) no consistency within an individual.

One likely reason the N2pc was able to capture an attentional bias to threat that was not evident in behavior is that the N2pc is a direct measure of attention time-locked to the onset of the threatening stimulus. By contrast, the behavioral response is made to a separate target stimulus presented hundreds of milliseconds after the initial onset of the threatening stimulus. Given that covert attention can shift rapidly between locations, it is likely that although attention was initially allocated to the threatening image (as reflected by the N2pc), attention shifted away from the threatening stimulus location prior to the onset of the target. This was supported by our previous study, which found an initial shift of attention to threatening images but no evidence of sustained engagement with threat subsequent to the initial allocation of attention (Kappenman et al., 2014). A number of studies have found similar dissociations between the N2pc and RT-based measures (Fenker et al., 2009; Ikeda et al., 2013; Kiss et al., 2013). It is possible that a modification of the timing of the events in the dot-probe task—for example, by presenting the target during the time of the initial shift of attention reflected by the N2pc—might provide a way of capturing the initial shift of attention to threat with behavioral measures.

Despite the modest internal reliability of the N2pc measure of threat bias and the large sample size used in the present study, we found no evidence of a relationship between anxiety and the amplitude of the N2pc. A similar result was obtained in a recent study, which found an N2pc to angry faces but no relationship between the N2pc and self-reported levels of anxiety in a non-clinical sample (Grimshaw et al., 2014). Thus, the N2pc may provide a more reliable marker of attentional bias to threat than RT in the dot-probe task, but it appears to be unrelated to individual differences in anxiety in non-clinical samples.

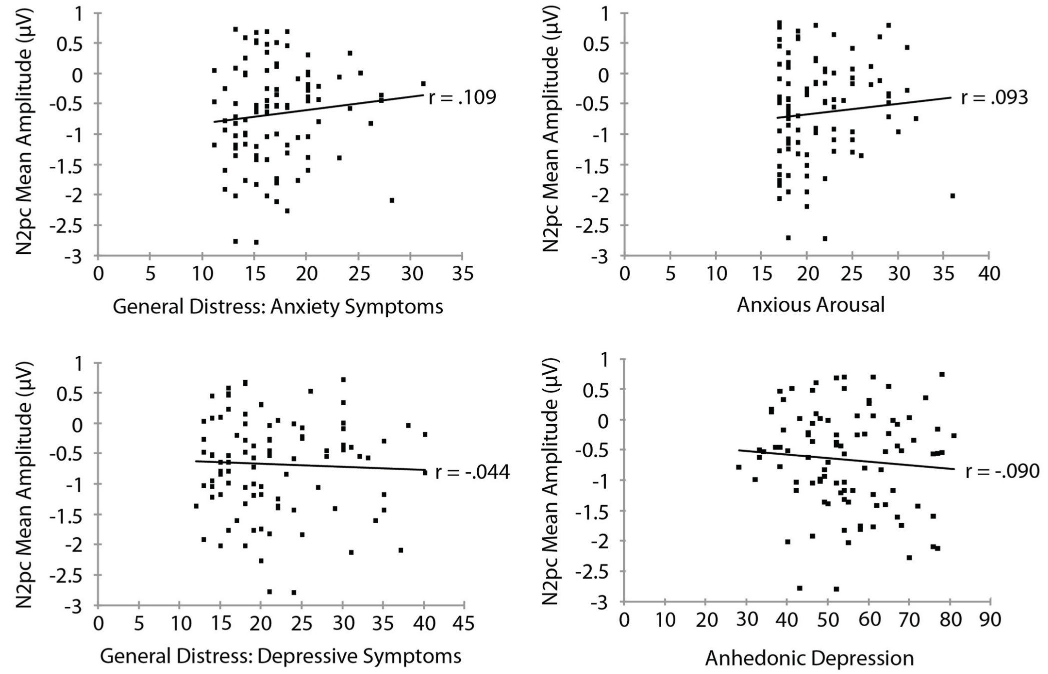

FIGURE 4 | Mean amplitude of the N2pc as a function of Mood and Anxiety Symptom Questionnaire subscale scores.

It is important to note that thus far dot-probe studies examining the N2pc have all used inherently threatening stimuli, which differ on low-level physical stimulus properties (e.g., luminance, spatial frequency, etc.). These low-level physical stimulus differences may influence the amplitude of early sensory-related ERP components, including the N2pc (see Luck and Kappenman, 2012; Luck, 2012, 2014). Therefore, it is impossible to determine on the basis of existing studies whether the initial shift of attention to threat in the dot-probe task indexed by the N2pc is specifically related to the *emotional* content of the stimuli, differences in low-level physical stimulus properties that are naturally conflated with emotional content, or a combination of both of these factors. Indeed, it may not be fully possible to separate out emotional content from differences in low-level physical stimulus properties (for example, see Larson et al., 2009). An important direction for future research could be to examine attentional bias to *conditioned* threat stimuli, which would provide a way of fully controlling for physical stimulus properties across participants. If the N2pc is partially determined by low-level physical stimulus differences and not by the emotional content of the images *per se*, this might help explain why this early signature of attentional bias to threat does not correlate with anxiety.

Together, the findings of the present study call into question the appropriateness of the dot-probe task as the primary method for examining attentional bias to threat across populations. Specifically, multiple studies have demonstrated that the RT-based measure of threat bias in the dot-probe task is unreliable, both in terms of internal reliability (Schmukle, 2005; Staugaard, 2009; Waechter et al., 2013; Kappenman et al., 2014) and test-retest reliability (Schmukle, 2005; Staugaard, 2009). However, this RT-based measure of attentional bias to threat in the dot-probe task has remained the primary measure used in the field, and the majority of dot probe studies still do not quantify the internal consistency of threat bias measures. This makes it difficult to interpret the results of studies, especially studies that include correlations between RT-based threat bias and other trait-based measures, such as anxiety.

The fact that typical individuals fail to show an attentional bias to threat using behavioral measures in the dot-probe task is also concerning in light of clear evidence that these individuals do exhibit biased attention to threat both in the dot-probe task (revealed by ERPs in the present study; also see Kappenman et al., 2014), and in other tasks (with RT and ERP measures; see MacNamara et al., 2013 for a review). In other words, the RT-based measure of threat bias in the dot-probe task is not capturing an attentional bias to threat among individuals who clearly show such a bias using alternative measures. This could directly impact the ability of this measure to elucidate differences in normal and abnormal reactions to threatening stimuli.

In contrast to the RT-based threat measure, the N2pc did suggest an attentional bias to threat in the dot-probe task; however, the internal reliability of the N2pc was somewhat unimpressive—suggesting that this measure too may not be an ideal individual difference measure of the initial allocation of attention to threatening stimuli in this task. This means that after decades of research we still lack a measure of attentional bias to threat in the dot-probe task that can reliably index individual differences in anxiety. Note, however, that the reliability of the N2pc depends on the number of trials being averaged together, so the reliability could be increased by using a much larger number of trials per subject—a possible direction for future research.

Collectively, these data suggest that it is time to develop new tasks and measures to index attentional bias to threatening information and assess the role of attentional bias to threat in anxiety. An alternative approach to continued reliance on the dot-probe task might involve adapting other tasks designed to measure attentional processes in the cognitive psychology literature for use in emotion and anxiety. For example, visual search appears to provide a promising alternative, including a long history of use in the basic science literature and easy integration with ERP and eye-tracking measures. Furthermore, visual search has been adopted already with modest success in the emotion, anxiety, and depression literatures (see, for example, Öhman et al., 2001b; Wisco et al., 2012; Lundqvist et al., 2014). However, note that one study found relatively low reliability for behavioral bias measures derived from visual search in children 8–10 years of age (Brown et al., 2014). Combining neural measures with the development of alternative tasks may provide the most promising avenue for future research to obtain conclusive evidence about the role of attentional bias to threat in anxiety. As a field, it is time to move beyond the dot-probe task as our primary paradigm.

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