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The neural correlates of attentional bias in blood phobia as revealed by the N2pc

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In the literature, a lack of attentional bias in blood phobia has been reported, using both behavioral and ERP measures. However, in the tasks employed so far, attentional resources to single stimuli, rather than attentional selection, were evaluated. The present study investigated whether in blood phobics disorder-relevant pictures can capture visuo–spatial attention when paired with neutral or non-specific unpleasant pictures (attack), and participants have to focus on a visual detection task. The N2pc component of the ERPs was measured as an index of spatial attentional selection. Results showed that in blood phobics, but not in controls, injuries elicited a larger early N2pc than attack pictures when paired with neutral material. Moreover, only in blood phobics a reliable N2pc to injury-attack pairs was found. The late N2pc reversal to injury pictures suggests that early orienting to phobic cues was followed by cognitive avoidance.

Keywords: attentional bias; blood phobia; N2pc

INTRODUCTION

A large body of experimental investigations has consistently demonstrated that anxiety and pathological fears are associated with an attentional bias, i.e. a systematic tendency to direct attention toward stimuli that are perceived as dangerous in the external (or internal) environment (e.g. Watts *et al.*, 1986; McNally *et al.*, 1990; Mogg *et al.*, 1992). In most of the relevant literature, the attentional bias is revealed by enhanced interference effect in the Emotional Stroop task, or facilitation in the dot-probe detection task, that are attributed to the selective capture of attention by disorder-relevant (although task-irrelevant) information (e.g. Hayes and Hirsch, 2007). However, it is unclear whether the attentional bias primarily reflects an early facilitation of the automatic encoding of threatening information (Öhman, 1997), or a difficulty in attentional disengagement from it, which might occur at later stages of information processing (Fox *et al.*, 2001). A further proposal is that automatic attentional allocation to disorder-related stimuli is immediately followed by cognitive avoidance as a strategic attempt to reduce anxiety, that could interfere with subsequent detailed processing of threat (Mogg *et al.*, 1997; Mogg and Bradley, 1998).

Complementary to behavioral studies based on reaction times, the results of investigations where the event-related potentials (ERPs) were recorded in response to disorder-relevant stimuli support the existence of an anxiety-related attentional bias. Larger P300 and late positive potential (LPP) in response to disorder-related words or pictures were observed in individuals with high trait anxiety or anxiety disorders, including specific phobias, as compared with healthy controls (e.g. Pauli *et al.*, 1997; Stanford *et al.*, 2001; Miltner *et al.*, 2005; Kolassa *et al.*, 2005; Schienle *et al.*, 2008). Since the P300 and LPP evoked by emotional pictures are thought to reflect the neural activity underlying the allocation of processing resources to motivationally relevant input (Palomba *et al.*, 1997; Cuthbert *et al.*, 2000), these findings have been interpreted as direct evidence that stimuli whose meaning specifically reflects the individual's critical concerns call for a greater amount of attentional resources.

Surprisingly, little attention has been devoted to the systematic investigation of information-processing biases in blood-injection-injury phobia, while it is widely acknowledged that this disorder has distinct clinical features and psychophysiological response characteristics that clearly differentiate it from other specific phobias (Page, 1994). Sawchuk *et al.* (1999) failed to demonstrate delayed color-naming latencies to phobia-related words in blood phobics as compared with phobia-unrelated words and with healthy controls on a Stroop task, indicating a lack of attentional bias. Similarly, Sawchuk *et al.* (2002) found no differences between blood phobics and controls in recognition memory for phobia-related pictures, further suggesting that these contents were not preferentially processed during the initial encoding phase. These findings are in striking contrast with evidence of attentional biases in other specific phobias (e.g. Watts *et al.*, 1986; Mogg and Bradley, 2006) and suggest that information processing might be qualitatively different in blood phobia as compared with other anxiety disorders.

The results of studies employing the recording of electro- and magnetoencephalographic responses are in line with the limited behavioral data in the literature demonstrating the

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lack of an attentional bias in blood phobics. Blood phobics and controls were found to respond to phobia-related and -unrelated pictures with comparable ERP P300 and LPP amplitudes, suggesting that blood phobics do not assign more attentional resources to phobic stimuli than non-fearful controls. In both blood phobics and controls, pictures of injuries and mutilated bodies elicited larger P300 than other unpleasant and arousing stimuli (Buodo *et al*., 2006). Recording magnetoencephalographic activity in response to phobia-related and -unrelated pictures, Buodo *et al*. (2007) observed that blood-fearful subjects displayed more intense occipito–parietal activation than healthy controls 190–250 ms after picture onset, regardless of picture content. Such result was interpreted as non-specifically enhanced sensory encoding of visual stimuli. Indeed, blood-related pictures did not elicit different activity patterns in blood-fearful subjects and controls, again supporting the hypothesis that the processing of phobogenic contents is not specifically enhanced in blood-fearful individuals.

In both studies by Buodo *et al*. (2006, 2007), a passive-viewing task was employed. That is, participants were required to simply look at pictures, presented one at a time, for their entire duration. In such experimental condition, neither selective processing nor attentional competition among concurrent stimuli is involved. That is, the individual's attentional resources are completely available for recruitment by the visual affective content presented singly. Although some ERP studies do highlight an attentional bias in phobic individuals by presenting phobogenic pictures one at a time (Kolassa *et al*., 2005; Miltner *et al*., 2005; Schienle *et al*., 2008), attentional biases in anxiety disorders have been mainly observed when two or more stimuli (stimulus attributes, or meanings) are processed under competitive conditions (Mathews and Mackintosh, 1998). When disorder-related information is simultaneously presented with disorder-unrelated information, the competition for attention would result in a biased advantage of disorder-related stimuli to the expense of disorder-unrelated cues (Mathews and Mackintosh, 1998). Following these considerations, it could be assumed that the passive-viewing task, while effective for other specific phobics, might not be sensitive enough to highlight an attentional bias in blood phobics.

A further issue to consider is that in non-fearful subjects, pictures of injuries and mutilated bodies presented singly engage larger amounts of processing resources than equally unpleasant and arousing material (human attack scenes), as indicated by greater ERPs positivity (Schupp *et al*., 2004) and higher cortical activation (Sarlo *et al*., 2005), larger HR deceleration and skin conductance changes (Palomba *et al*., 2000), reduced startle blink amplitude potentiation (Kaviani *et al*., 1999), reduced spontaneous blinking rate (Palomba *et al*., 2000), and longer reaction times (Buodo *et al*., 2002). Based on these findings, a 'ceiling effect' might explain the lack of an attentional bias in blood phobics when blood stimuli are presented singly. That is, blood stimuli seemingly saturate available attentional resources already in healthy individuals, so that fear of blood does not (or perhaps cannot) further increase attentional allocation. Blood phobia might be associated with an attentional bias only when phobic and non-phobic stimuli compete for attention. Therefore, an experimental paradigm that requires the cognitive system to distribute spatial attention among multiple discrete stimuli in the visual field might be effective in unmasking the possible presence of such attentional bias.

Aim of the present study was to assess the presence of a bias in orienting spatial attention in blood phobics, using an experimental paradigm involving the simultaneous presentation of disorder-related and -unrelated stimuli. To address this issue, the N2pc component of the ERPs was measured. When subjects focus attention onto an item within a bilateral stimulus array, the N2pc is observed as a negative-going voltage deflection at posterior electrodes, contra-lateral to the location of the item, between 200 and 300 ms post-stimulus. The N2pc is a well-validated correlate of spatial attentional selection and/or the suppression of irrelevant or conflicting information (Eimer, 1996; Woodman and Luck, 1999). Such selection can be stimulus-driven, with pre-defined target features attracting attention to its location, or guided by top–down processes that result in a shift of attention to stimuli possessing relevant features (Luck and Hillyard, 1994; Eimer, 1996). Given its lateralized nature, the N2pc appears particularly suitable for an online tracking of the allocation of attention to the visual field and for the assessment of any spatial bias created by phobic stimuli.

It has been recently demonstrated that emotionally salient stimuli are able to elicit an N2pc when attention is focused elsewhere and they can be completely ignored, providing direct electrophysiological evidence that emotional stimuli can bias the distribution of spatial attention (Eimer and Kiss, 2007; Holmes *et al*., 2009). Furthermore, Fox *et al*. (2008) showed that trait anxiety strongly modulates this early bias, in that angry facial expressions were found to elicit a larger N2pc than neutrals in individuals with high than low trait anxiety.

In the present study, the possible presence of an attentional bias in blood phobics was investigated by recording the ERPs in response to pictures depicting injuries, presented near fixation on the left or right side, each paired with a disorder-unrelated unpleasant or neutral picture. Participants had to perform a visual detection task on the fixation cross at the center of the screen (Eimer and Kiss, 2007). A bias in visuospatial attention would be revealed by an enhanced N2pc to phobic pictures not only when presented together with neutral pictures, but also with phobia-unrelated unpleasant and arousing pictures. This would indicate that in blood phobics the cognitive system assigns priority to phobic stimuli also when they compete for attention with other motivationally salient stimuli.

METHODS

Participants

Since blood phobia is known to have a higher prevalence among females (Bienvenu and Eaton, 1998), only women were recruited for the present study.

The Italian version of the Mutilation Questionnaire (MQ; Klorman *et al.*, 1974) was administered to 250 female undergraduates. The range of possible scores in this questionnaire is 0–30. Subjects scoring above the 90th percentile of the obtained scoring distribution ($\geq$19) were preliminarily included in the phobic group ($N = 22$). They were then invited to the laboratory and screened with a semi-structured interview (Anxiety Disorders Interview Schedule (Anxiety Disorders Interview Schedule, ADIS IV; Brown *et al.*, 1994) by a clinical psychologist, to assess whether they fulfilled DSM-IV criteria for specific phobia, blood-injection-injury type (American Psychiatric Association, 2000). If DSM criteria were met, the subject was asked to participate in the study, and an appointment was arranged for those who gave preliminary informal consent.

The final phobic sample included 12 women (mean age = 22.5 years, s.d. = 3.39, range = 19–32 years). Mean MQ score for the phobic group was 21.58 (range 19–29; s.d. = 3.45). Based on its characteristics, this sample can be considered as a clinical analogue.

Healthy control subjects (mean age = 23.23 years, s.d. = 2.45, age range = 20–29 years) were randomly selected from the initial pool of subjects. They were included in the sample if they scored below the 50th percentile of the obtained scoring distribution ($\leq$10) on the MQ and had no specific fears, as assessed before the experimental session by a 17-item reduced form of the Fear Survey Schedule (FSS-III; Wolpe and Lang, 1964). Given that none of the control subjects had specific fears, the ADIS IV interview was not administered. Their mean score on the MQ was 8 (range 2–10; s.d. = 2.88).

All participants were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). They had no history of psychiatric or neurological disorders (other than specific phobia in the phobic group), and were not taking medication.

The study was approved by the local Ethical Committee. Written informed consent was obtained from all volunteers prior to participation, according to the Declaration of Helsinki. Phobic subjects were informed that, if interested, psychological treatment was available.

Stimulus material and experimental task

The stimuli consisted of pairs of emotional pictures of 36 different exemplars divided into three categories according to their content (Injury: small injuries and minor surgical procedures; Attack: attacking humans and aimed weapons; Neutral: household objects, neutral people and landscapes). Neutral and Attack pictures were selected from the International Affective Picture System (IAPS; Lang *et al.*, 2008), whereas Injury pictures were downloaded from medical websites and standardized for valence (pleasant–unpleasant) and arousal (activated–calm) ratings on the Self-Assessment Manikin (SAM; Lang *et al.*, 2008) on a sample of 70 undergraduates (42 females) at the University of Padova. The two unpleasant picture categories were balanced for mean valence and arousal standardized ratings (Table 1).

Table 1 Standardized ratings of valence and arousal (means and standard deviations) for each emotional category

Emotional Category	Valence		Arousal	
	Mean	s.d.	Mean	s.d.
Injury	2.34	0.29	6.29	0.39
Attack	2.13	0.23	6.70	0.53
Neutral	4.91	0.25	3.39	0.84

Note: The range of possible scores for valence and arousal ratings is 1–9.

Each picture was modified to fit a square frame subtending to $3.6^\circ \times 3.6^\circ$ of the visual angle. Stimulus pairs were presented bilaterally, with the outer edge of each picture 9° from a $0.5^\circ \times 0.5^\circ$ central fixation cross that remained on the screen throughout the task. All pictures were equated for mean luminance using Photoshop (Adobe, San Jose, CA) and no significant differences were found among the three emotional categories in either mean luminance values (Injury: $M = 45.86$, s.d. = 17.37; Attack: $M = 30.39$, s.d. = 29.87; Neutral: $M = 25.82$, s.d. = 22.86; $F[2,33] = 2.3$, $P = 0.11$) or Michelson contrast calculations (Michelson, 1927) (Injury: $M = 0.90$, s.d. = 0.13; Attack: $M = 0.97$, s.d. = 0.03; Neutral: $M = 0.91$, s.d. = 0.18; $F[2,33] = 0.88$, $P = 0.42$). All stimuli appeared on a light grey background (28 cd/m^2). Stimulus pairs contained all the possible combinations of the 3 emotional categories taking the location into account (Injury–Attack, Attack–Injury, Injury–Neutral, Neutral–Injury, Attack–Neutral, Neutral–Attack). Each pair was presented with equal number of times during the task.

Twelve experimental blocks of 90 trials each were run, separated by breaks. On each trial, the stimulus pair was presented for 200 ms (Eimer and Kiss, 2007). The intertrial interval was 2000 ms. Stimulus pairs were presented in a new random order for each participant. Each stimulus pair had the same probability of occurrence within each block and throughout the experiment. Participants were requested to maintain fixation in order to detect an infrequent luminance change of the fixation cross from dark grey (21 cd/m^2) to light grey (23 cd/m^2). They had to press the keyboard spacebar upon occurrence of luminance changes (alternating the response hand for each successive block). Luminance changes occurred concurrently with the onset of the stimulus pair on 20% of all trials (18 trials per block), and lasted 200 ms until stimulus pair offset. The task was presented

on a 19-inch computer screen through a Pentium IV computer running E-prime presentation software (Psychology Software Tools, Pittsburgh, PA, USA), at a viewing distance of 1 m.

Procedure

Upon arrival at the laboratory, participants read and signed an informed consent form. They were then seated in front of the computer screen on a comfortable chair with the head positioned on an adjustable head-and-chin rest, in a dimly lit, sound-attenuated room. After electrode attachment, they were instructed that a reaction-time visual detection task would be performed. Participants were informed that pictures would be presented on the sides of a central fixation cross, but were instructed to keep their gaze focused on the cross throughout the task and to press the spacebar in response to its luminance changes as fast and accurately as possible. A practice block of 15 neutral stimulus-pair trials was completed. At the end of the experimental session, participants were thanked and debriefed.

Electrophysiological Recordings and Data Analyses

The electroencephalogram (EEG) was recorded with tin electrodes mounted in an elastic cap from 11 scalp sites (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, T5, T6) referenced to linked-mastoids, according to the International 10–20 System (Jasper, 1958). For artifact scoring, vertical and horizontal electro-oculograms (EOGs) were recorded. Electrode pairs (bipolar) were placed at the supra- and suborbit of the right eye and at the external canthi of the eyes. All electrode impedances were kept below 10 kΩ. The EEG and EOG signals were amplified with a V-Amp amplifier (Brain Products GmbH, Gilching, Germany), bandpass filtered (0.5–30 Hz), digitized at 250 Hz (16 bit AD converter, accuracy 0.08 uV/LSB) and stored on to a Pentium IV computer.

Continuous EEG data were corrected for eyeblinks and eye movements using a regression-based correction algorithm (Gratton *et al.*, 1983; Brain Vision Analyzer 1.05 software). EEG was then segmented off-line into 500 msec epochs from 100 ms before to 400 ms after stimulus onset. The EEG epochs were baseline-corrected against the mean voltage during the 100 ms prestimulus period. All EEG epochs were visually scored for eye movement and other artifacts, and each portion of data containing artifacts greater than ±100 uV in any channel was rejected for all the recorded channels prior to further analysis. Artifact-free trials with correct behavioral responses were separately averaged for each subject, for luminance change and no-change trials, and for all combinations of emotional category (Injury–Attack *vs* Injury–Neutral *vs* Attack–Neutral), emotional stimulus location (left *vs* right), and contralaterality (electrode ipsilateral *vs* contralateral to the location of the most salient emotional stimulus). Based on the studies demonstrating greater attentional engagement to stimuli depicting blood, injuries and mutilations compared with other unpleasant and arousing visual stimuli (Palomba *et al.*, 2000; Buodo *et al.*, 2002; Schupp *et al.*, 2004; Sarlo *et al.*, 2005), we decided to consider contralaterality relative to the spatial location of Injury pictures for the Injury–Neutral and Injury–Attack stimulus pairs. Similarly, we considered contralaterality relative to the spatial location of Attack pictures for the Attack–Neutral pair. Analyses focused on the luminance no-change trials only, and on the occipito–parietal electrodes T5 and T6, where the N2pc component is maximal. The ipsilateral waveform was computed as the average of the T5 electrode to the left-sided emotional stimulus and the T6 electrode to the right-sided emotional stimulus, and the contralateral waveform was computed as the average of the T5 electrode to the right-sided emotional stimulus and the T6 electrode to the left-sided emotional stimulus.

On the basis of inspection of grand average ERP waveforms and in line with previous studies employing similar paradigms (Eimer and Kiss, 2007; Holmes *et al.*, 2009), the N2pc was quantified on the basis of ERP mean amplitudes measured at T5 and T6 within two successive time windows (early N2pc: 180–240 ms post-stimulus; late N2pc: 240–310 ms post-stimulus). In addition, N2pc contralaterality scores were obtained by subtracting the mean amplitude recorded from the ipsilateral electrode (with respect to the visual field of the emotional stimulus) from that of the contralateral electrode. In addition, the P1 was specified as the most positive peak between 80 and 140 ms from stimulus onset and the N1 as the most negative peak between 145 and 195 ms from stimulus onset.

Initial analysis was specifically aimed at verifying the existence of an N2pc to different stimulus-pairs as a function of group. Mean ERP amplitudes were submitted to $2 \times 3 \times 2$ mixed analysis of variance (ANOVA) with Group (Phobics and Controls) as between-subjects factor, and Stimulus-Pair (Injury-Attack, Injury-Neutral, and Attack-Neutral) and Contralaterality (Contralateral *vs* Ipsilateral hemisphere relative to the visual hemifield where the most salient emotional stimulus was presented) as within-subjects factors. The same experimental design was used for mean P1 and N1 ERP amplitudes.

To simplify the comparison among stimulus-pairs between groups, 2×3 mixed ANOVA with Group as between-subjects factor and Stimulus-Pair as within-subjects factor was conducted on mean N2pc contralaterality scores. The same experimental design was employed for mean target reaction times and mean percentages of correct responses (accuracy) at the luminance changes detection task.

To minimize the risk for type I error in repeated measures ANOVAs, the Greenhouse-Geisser (G-G) correction was applied when appropriate. In the text, uncorrected degrees of freedom are reported together with adjusted probability values. Post-hoc means comparisons (Newman-Keuls) were

employed to further examine significant effects (using a $P < 0.05$ criterion for significance).

RESULTS

Behavioral data

No significant main effects or interactions were found for reaction times to changes in the luminance of the fixation cross (Phobics: mean = 483 ms, s.d. = 22.63; Controls: mean = 491 ms, s.d. = 21.74). However, a significant main effect of Stimulus-Pair was found for accuracy ($F[2,46] = 5.22$; $P < 0.01$; $\varepsilon = 0.93$). Accuracy rates were lower when stimulus-pairs included Injury. Post-hoc tests showed that accuracy rates for Injury-Neutral and Injury-Attack did not differ from each other ($P = 0.38$), whereas accuracy for Attack-Neutral differed from accuracy for both Injury-Neutral ($P = 0.009$) and Injury-Attack ($P = 0.03$). Mean accuracy rates as a function of group and stimulus-pairs are reported in Table 2.

Table 2 Mean accuracy rates (%) and standard deviations as a function of stimulus-pair and group

Stimulus-Pair	Phobics		Controls	
	Mean accuracy	s.d.	Mean accuracy	s.d.
Injury–Attack	96.91	3.41	96.23	5.57
Injury–Neutral	95.41	5.08	96.23	5.04
Attack–Neutral	98.87	1.33	97.96	3.01

Event-related potentials

Figure 1 shows grand-averaged ERPs obtained at electrodes T5 and T6 contralateral (solid lines) and ipsilateral (dashed lines) to the location of the most salient emotional stimulus (Injury pictures for the Injury–Neutral and Injury–Attack pairs and Attack pictures for the Attack–Neutral pair) when no luminance change occurred, for the different stimulus-pairs in phobics and controls. As can be seen from visual inspection, Phobics developed larger positivity than Controls starting at about 100 ms after stimulus onset. This effect is possibly related to generally enhanced processing in Phobics, as they knew in advance that they would have been presented with pictures of their phobic object (cf. Buodo *et al.*, 2007). Moreover, an enhanced negativity appeared contralateral to the location of the most salient

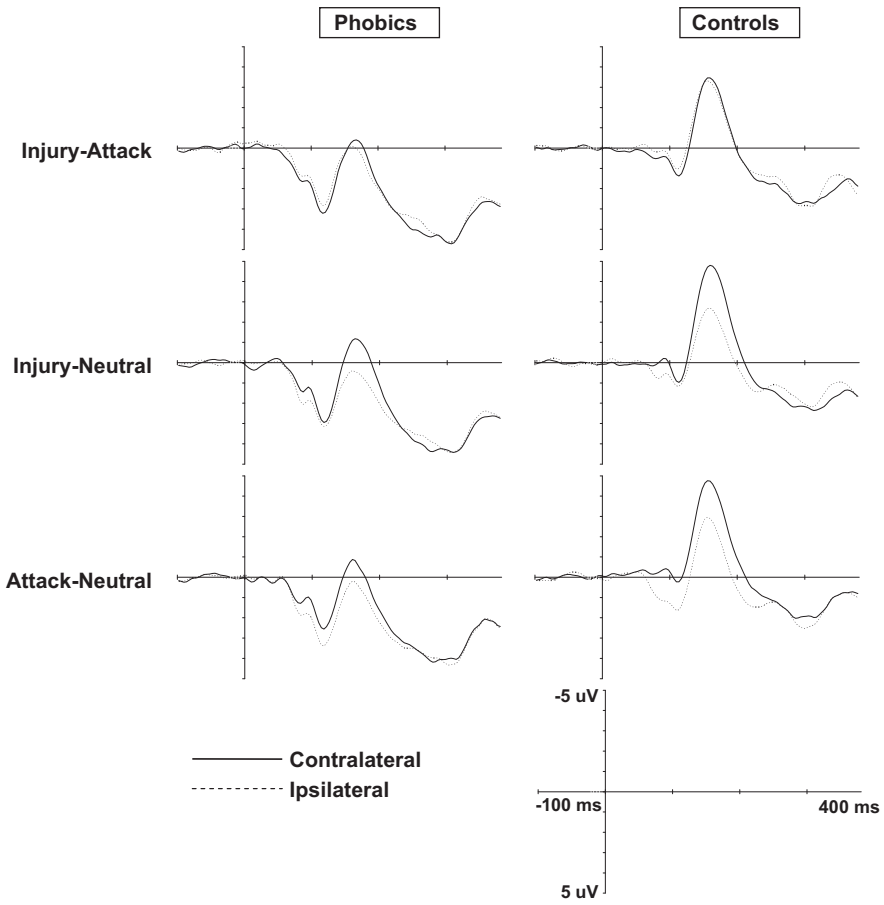

Fig. 1 Grand-averaged event-related potentials to the different stimulus pairs recorded at T5/T6 electrodes in Phobics and Controls contralaterally (solid lines) and ipsilaterally (dashed lines) to the location of the most salient emotional stimulus when no luminance change occurred.

emotional stimulus within the early phase of the N2pc (180–240 ms), overlapping with the N1 component.

P1. There were no group-based differences for this component. A main effect of Contralaterality was found ($F(1,23) = 13.92$; $P < 0.001$), showing that P1 amplitude was larger ipsilaterally than contralaterally. Moreover, as revealed by the significant Contralaterality × Stimulus-Pair interaction ($F(2,46) = 34.23$; $P < 0.0001$; $\varepsilon = 0.76$), this effect was observed only for stimulus-pairs including neutral pictures (Injury–Neutral: $P = 0.008$; Attack-Neutral: $P = 0.0001$), whereas for the Injury–Attack pair the opposite effect was found ($P = 0.001$).

N1. A significant main effect of Group was obtained for this component ($F(1,23) = 11.28$; $P < 0.003$), which showed a larger amplitude for Controls than Phobics. The significant Contralaterality effect ($F(1,23) = 38.36$; $P < 0.0001$) showed that N1 amplitude was larger contralaterally than ipsilaterally. Moreover, as revealed by the significant Contralaterality × Stimulus-Pair interaction ($F(2,46) = 23.54$; $P < 0.0001$; $\varepsilon = 0.91$), this effect was obtained only for stimulus-pairs including neutral pictures (Injury–Neutral: $P = 0.0001$; Attack-Neutral: $P = 0.0001$), whereas for the Injury–Attack pair no difference between amplitudes at contralateral and ipsilateral sites was found ($P = 0.11$).

Early N2pc (180–240 ms). In order to assess the reliable presence of an N2pc for each stimulus-pair within each group, we performed the post-hoc tests on the significant Group × Contralaterality × Stimulus-Pair interaction ($F(2,46) = 7.34$; $P < 0.003$; $\varepsilon = 0.89$) obtained in the preliminary ANOVA. N2pc amplitudes differed in Phobics and Controls specifically during the viewing of the Injury–Attack pair. Whereas in Phobics the N2pc amplitude was larger contralaterally than ipsilaterally for the Injury–Attack ($P = 0.04$), Injury–Neutral ($P = 0.0001$) and Attack–Neutral ($P = 0.0007$) stimulus-pairs, thus demonstrating that an N2pc was reliably elicited in all conditions, in Controls no difference between amplitudes at contralateral and ipsilateral sites was found for the Injury–Attack pair ($P = 0.59$), indicating that no N2pc was triggered in response to this stimulus pair. On the other hand, a reliable N2pc was found in Controls for both the Injury–Neutral ($P = 0.0001$) and Attack–Neutral ($P = 0.0001$) stimulus-pairs.

From the main analyses performed on N2pc contralaterality scores, a significant Stimulus-Pair effect emerged ($F(2,46) = 28.36$; $P < 0.0001$; $\varepsilon = 0.89$), showing larger amplitudes to the Injury–Neutral and Attack–Neutral pairs than to the Injury–Attack pair (P's < 0.0001). The significant Group × Stimulus-Pair interaction ($F(2,46) = 7.34$; $P < 0.003$; $\varepsilon = 0.89$) indicated that N2pc amplitudes differed between groups only in the Attack–Neutral pair ($P = 0.0006$), where Controls showed larger amplitude than Phobics. However, different response patterns were found for the two groups: Phobics showed larger amplitude to the Injury–Neutral pair than to the other stimulus conditions (P's < 0.03), whereas Controls showed larger amplitudes to the Injury–Neutral and the Attack–Neutral pairs than to the Injury–Attack pair (P's < 0.0001) (Figure 2).

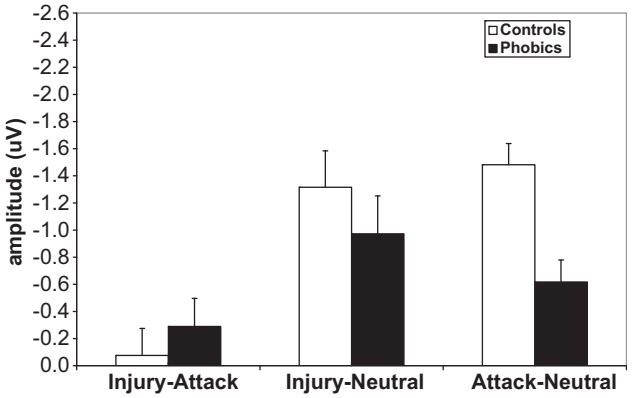

Fig. 2 Mean early N2pc contralaterality scores to the different stimulus pairs recorded at T5/T6 electrodes in Phobics and Controls. Negative values indicate larger contralateral amplitude. Standard errors are marked.

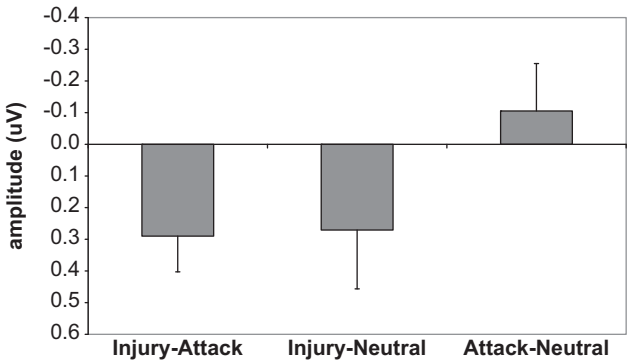

Fig. 3 Mean late N2pc contralaterality scores to the different stimulus pairs recorded at T5/T6 electrodes independent of group. Positive values indicate larger ipsilateral amplitude. Standard errors are marked.

Late N2pc (240–310 ms). The preliminary ANOVA performed with the Contralaterality factor yielded no significant interactions involving the Group factor in this later time window. The significant Contralaterality × Stimulus-Pair interaction ($F(2,46) = 3.37$; $P < 0.04$; $\varepsilon = 0.92$) indicated that an inverted N2pc (significantly larger ipsilaterally than contralaterally) was found for the Injury–Attack pair ($P = 0.05$), whereas no reliable N2pc emerged for the Injury–Neutral ($P = 0.08$) and Attack-Neutral ($P = 0.37$) conditions.

The analyses on the N2pc contralaterality scores showed only a significant Stimulus-Pair effect ($F(2,46) = 3.37$; $P < 0.04$; $\varepsilon = 0.92$), indicating that the N2pc was larger for the Attack–Neutral pair than for the other stimulus pairs, for which mean positive values were actually observed (Figure 3). Post-hoc tests showed a significant difference from the Injury-Attack pair ($P = 0.03$) and a tendency

toward a significant difference from the Injury-Neutral pair ($P = 0.06$).

DISCUSSION

The aim of the present study was to assess whether blood phobics would exhibit a bias in visuospatial attention, by presenting pictures of injuries near fixation, each paired with a disorder-unrelated unpleasant or neutral picture. Participants' attention was allocated to a continuous visual detection task at fixation, and peripheral pictures could be ignored. The N2pc of the ERPs was measured as an index of spatial attentional selection.

Our findings indeed highlighted an attentional bias in blood phobics. Specifically, the results indicate that the bias involves visuospatial selective attention and occurs in early processing stages.

Two separate operational definitions of attentional bias in phobic individuals have been employed in the literature. A 'within-subjects bias' involves a significant difference in attentional processing of phobic relative to neutral stimuli, despite the absence of group differences. A 'between-subjects bias' involves a significant difference between phobics and controls in attentional processing of phobic *vs* non-phobic stimuli (Bar-Haim *et al.*, 2007; Mueller *et al.*, 2009). In our data, the analysis of early N2pc contralaterality scores (obtained by subtracting ipsilateral from contralateral potentials) showed higher amplitudes to the Injury-Neutral than to the Attack-Neutral pair in blood phobics (within-subjects bias). Despite the lack of differences between phobics and controls in the N2pc amplitude to the Injury-Neutral pair (no between-subjects bias), blood phobics demonstrated to orient attention preferentially towards the spatial position of injury rather than other unpleasant stimuli, when these are paired with neutral contents. Moreover, such differential respones was not displayed by controls, for which comparable N2pc amplitudes were found to the Injury-Neutral and Attack-Neutral pairs. This finding suggests an attentional bias that specifically characterizes phobic individuals.

An alternative explanation might be that control participants were more reactive to attack pictures than phobics, as indicated by the larger N2pc contralaterality scores to the Attack-Neutral stimulus pair. This effect could be interpreted as a negative bias due to cumulative exposure to unpleasant stimuli (cf. Bar-Haim *et al.*, 2007). Indeed, no difference in the processing of attack pictures were found between blood phobics and controls during passive viewing when pleasant pictures were also included in the presentation sequence (Buodo *et al.*, 2006). Even if this was true, however, our findings still provide clear evidence of a within-subjects attentional bias towards injuries only in blood phobics.

The preliminary analysis on N2pc amplitude ipsilateral and contralateral to the location of emotional pictures was conducted to specifically verify the existence of a reliable N2pc (cf. Luck and Hillyard, 1994) to each stimulus-pair condition. Analyses on the amplitude of the early N2pc showed significant differences between contralateral and ipsilateral activity in blood phobics for each stimulus-pair. In contrast, an N2pc was not reliably triggered in controls for the Injury-Attack pair, as no significant difference was found between contralateral and ipsilateral activity. This finding suggests that the spatial distribution of attention in blood phobics was biased towards injury pictures presented outside the focus of attention, not only when together with neutral pictures, as expected in both controls and phobics, but also when together with other unpleasant, but phobia-unrelated, cues. On the other hand, in controls the high motivational relevance of both contents possibly promoted a more diffuse attentional state, rather than preferential orienting towards one or the other. Taken together, these data might provide additional information to the analysis on N2pc contralaterality scores. As expected, analyses on difference waveforms revealed that for both groups the early N2pc was substantially smaller when injury competed for attention with an emotionally salient stimulus, namely attack, than with a neutral stimulus. This suggests that whereas emotional stimuli are obviously prioritized over neutrals, the co-occurrence of blood-related stimuli with other unpleasant salient stimuli makes attentional selection less straightforward, reflected by a smaller N2pc. However, despite the lack of significant difference between phobics and controls in the Injury-Attack condition, it is worth noting that in blood phobics the N2pc difference score to Injury-Attack stimuli is the result of a significant difference between contralateral and ipsilateral activity, whereas in controls the same stimuli elicited a statistically insignificant N2pc.

Interestingly, in blood phobics initial orienting was not followed by maintenance of visuospatial attention towards phobic stimuli, as indicated by the lack of enhanced late N2pc to injury pictures. Lateralization of the late N2pc was completely absent both in the Injury-Neutral and Attack-Neutral pairs, suggesting that selection of emotional stimuli occurs early and is not sustained during later processing stages. Most likely, the absence of a reliable late N2pc in the Injury-Neutral and Attack-Neutral pairs indicates that attention was focused back on the fixation cross. Instead, a reversed N2pc was observed for the Injury-Attack pair. Larger late N2pc ipsilateral to the spatial position of injury pictures suggests that after early attentional selection, attention was shifted away from their spatial position, possibly towards the other picture. Considering that in blood phobics the larger early N2pc to injuries in the Injury-Attack pair indicates the initial attentional selection of phobic stimuli, the late N2pc reversal might be interpreted as avoidance of further attending and processing. The vigilance-avoidance model of attentional bias in anxiety disorders (Mogg *et al.*, 2004) proposes that after initial automatic orienting to disorder-related cues (vigilance), individuals with anxiety

disorders direct their attention away (avoidance) as a strategic attempt to reduce the anxiety state elicited by the aversive stimuli. Avoidance might interfere with detailed elaborative processing and habituation, and is thus hypothesized to play a role in the maintenance of anxiety. Following the vigilance-avoidance model, blood phobics appear to initially prioritize the selection of phobic stimuli, but then do not maintain attention on such stimuli, possibly because of a conflict between avoidance and monitoring the environment for possible safety cues (Mogg *et al*., 2004). We do acknowledge that the time course of avoidance reported in the literature starts later, at about 500 ms and beyond (Mogg *et al*., 2004; Mogg and Bradley, 2006). In addition, disengagement from threat-related stimuli in anxious individuals has been found to be delayed until 300 ms at least (e.g. Fox *et al*., 2001, Fox *et al*., 2002). However, the reversal of late N2pc (240–310 ms), that we tentatively interpret as avoidance, occurs in a time window following the offset of stimulus-pairs, which were 200 ms in duration. Therefore, it is likely that avoidance was made possible by the fact that the stimuli were no longer on the screen. It remains undetermined whether with longer stimulus durations blood phobics would shift attention away from phobic stimuli in a later time window, or whether initial attentional selection would be maintained, possibly due to a difficulty in disengagement.

Analyses on behavioral performance at the visual detection task showed that all participants were less accurate in detecting luminance changes of the fixation cross when one of the pictures on the sides was an injury. Indeed, blood phobics were not slower or less accurate than controls in responding to luminance changes concurrent with the onset of an injury picture. This finding does corroborate the results obtained on the N2pc by demonstrating that blood phobics deployed enough attentional resources in the detection task to achieve controls' accuracy and speed, and still their attention was shifted towards blood stimuli, as indicated by larger N2pc contralateral to injuries in both Injury-Neutral and, most notably, in Injury-Attack stimulus-pairs. Thus, the attentional bias found in blood phobics is not attributable to poorer behavioral accuracy than controls.

One could argue that differences related to earlier ERP components may have contributed to the effects obtained for the N2pc. However, it is unlikely that what emerged in the P1 and N1 windows accounts for the observed attentional bias. In the literature, the P1 component has been proved to be modulated by both visuospatial attention (e.g. Eimer, 1998) and stimulus physical features (e.g. Luck and Hillyard, 1994). However, our finding of a larger P1 (80–140 ms) contralateral to neutral pictures suggests an imbalance in sensory energy rather than an automatic capture of attention by the neutral condition. Indeed, specific features (such as spatial frequency, number of elements and colors) of the neutral pictures employed in the present study might have determined greater perceptual complexity than the unpleasant ones, as they depicted a number of different objects and scenes (cf. Bradley *et al*., 2007; Codispoti *et al*., 2007).

For the N1 component (145–195 ms), which is believed to reflect the operation of a visual discrimination mechanism within the focus of attention (e.g. Vogel and Luck, 2000), a different pattern was observed. The N1 was larger contralaterally to the spatial location of unpleasant compared to neutral pictures, indicating early attentional selection of affectively relevant contents. This suggests that in the N1 time-window attentional modulation largely prevailed over the effects of possible encoding biases relating to differences in sensory/perceptual features among pictures as observed in the P1 time window. Moreover, measurements of luminance levels and Michelson contrast calculations revealed that picture categories did not significantly differ from each other. Overall, the N1 was smaller in phobics than in controls, possibly reflecting a superimposed positivity also encompassing the N2pc time-windows. This global greater positivity in blood phobics might be attributed to a sustained hypervigilant state leading to enhanced processing, as they were presented with pictures including their phobic object (cf. Buodo *et al*., 2007). Indeed, scalp positivity has been viewed as a manifestation of consumption of processing resources in a given cortical area, whereas negativity reflects preparation and the amount of 'cerebral potentiality' (Rockstroh *et al*., 1989).

Taken together, our results indicate that the attentional bias revealed by the N2pc in blood phobics is reliably attributable to early visuospatial selection of phobic stimuli. It seems unlikely that it simply reflects the influence of earlier, more general emotional effects related to N1, with which the early N2pc was partially overlapped, or possible imbalance in lower-level perceptual features among stimulus pairs, as highlighted by the P1. Differences between phobics and controls as a function of stimulus pairs only emerged for the early N2pc. Indeed, attentional modulation of the occipital P1 and N1 components and the N2pc component are hypothesized to be under the control of different attentional mechanisms (Eimer, 1998).

To summarize, the present study provided novel findings to the literature on attentional bias in blood phobia by using an experimental paradigm that involved selective processing through the simultaneous presentation of two emotional stimuli outside the focus of attention. It is worth noting that a passive viewing paradigm is effective enough to reveal the attentional bias in other specific phobics (see Miltner *et al*., 2005), but fails to do so in blood phobics (see Buodo *et al*., 2006). Thus, it seems that an attentional bias can emerge in blood phobics only when a true prioritization is needed. This 'forced' selection also prevents healthy controls from showing preferential processing of blood stimuli over other aversive material.

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