Brain Imaging and Behavior (2017) 11:829–845
DOI 10.1007/s11682-016-9557-y

ORIGINAL RESEARCH

Neural response patterns in spider, blood-injection-injury and social fearful individuals: new insights from a simultaneous EEG/ECG–fMRI study

Jarosław M. Michałowski[1] · **Jacek Matuszewski**[1,2] · **Dawid Droździel**[1,2] · **Wojciech Koziejowski**[1] · **Andrzej Rynkiewicz**[1] · **Katarzyna Jednoróg**[3] · **Artur Marchewka**[2]

Published online: 14 May 2016

Abstract In the present simultaneous EEG/ECG-fMRI study we compared the temporal and spatial characteristics of the brain responses and the cardiac activity during fear picture processing between spider, blood-injection-injury (BII) and social fearful as well as healthy (non-fearful) volunteers. All participants were presented with two neutral and six fear-related blocks of pictures: two social, two spider and two blood/injection fear blocks. In a social fear block neutral images were occasionally interspersed with photographs of angry faces and social exposure scenes. In spider and blood/injection fear blocks neutral pictures were interspersed with spider fear-relevant and blood/injection pictures, respectively. When compared to healthy controls the social fear group responded with increased activations in the anterior orbital, middle/anterior cingulate and middle/superior temporal areas for pictures depicting angry faces and with a few elevated superior frontal activations for social exposure scenes. In the blood/injection fear group, heart rate was decreased and the activity in the middle/inferior frontal and visual processing regions was increased for blood/injection pictures. The HR decrease for blood/injection pictures correlated with increased frontal responses. In the spider fear group, spider fear-relevant pictures triggered increased activations within a broad subcortical and cortical neural fear network. The HR response for spider fear-relevant stimuli was increased and correlated with an increased insula and hippocampus activity. When compared to healthy controls, all fear groups showed higher LPP amplitudes for their feared cues and an overall greater P1 hypervigilance effect. Contrasts against the fear control groups showed that the increased responses for fear-specific stimuli are mostly related to specific fears and not to general anxiety proneness. The results suggest different engagement of cognitive evaluation and down-regulation strategies and an overall increased sensitization of the fear system in the three fear groups.

Keywords Fear · EEG/fMRI · Spider phobia · Blood-injection-injury (BII) phobia · Social anxiety disorder (SAD)

Electronic supplementary material The online version of this article (doi:10.1007/s11682-016-9557-y) contains supplementary material, which is available to authorized users.

✉ Jarosław M. Michałowski
jmichalowski@psych.uw.edu.pl

[1] Department of Differential Psychology, Faculty of Psychology, University of Warsaw, Stawki 5/7, 00-183 Warsaw, Poland

[2] Laboratory of Brain Imaging, Neurobiology Centre, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[3] Laboratory of Psychophysiology, Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Introduction

The anticipation of threatening events puts an organism in the state of increased vigilance. After the detection of threat this initial hypervigilance shifts into the mode of increased attention towards the source of danger that is accompanied by a cascade of neuronal changes and behavioral adjustments including increases in autonomic arousal (Fredrikson, 1981; Hare, 1973; LeDoux, 1987; Prigatano and Johnson, 1974). This dynamic model of defensive reactivity proposed by Fanselow (1994) and elaborated by Lang, Bradley, and Cuthbert (1997) was supported in previous studies on spider

phobic individuals. For example, in a context when phobia-relevant stimuli are likely to occur spider-phobic individuals presented increased visual scanning (Tolin, Lohr, Lee, & Sawchuk, 1999; Pflugshaupt et al., 2007) and greater brain response, as indexed by increased Event Related Potentials (ERP) amplitudes observed in the early time window between 100 and 200 ms for the phobia-relevant and phobia-irrelevant stimuli (Kolassa, Musial, Kolassa, & Miltner, 2006; Michalowski et al., 2009; Michalowski, Pané-Farré, Löw, & Hamm, 2015; Weymar, Keil, & Hamm, 2014). After detecting fear-relevant cues spider phobics allocated extra attentional resources for their preferential processing, reflected as Early Posterior Negativity (EPN) and Late Positive Potentials (LPP) and a greater activity in several brain structures, such as visual cortex, amygdala, hippocampus, insula and anterior cingulate cortex (ACC) (Dilger et al., 2003; Fredrikson, Wik, Annas, Ericson, & Stone-Erlander, 1995; Lueken, et al., 2014; Paquette et al., 2003; Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005). This preferential processing is also accompanied by increased heart rate (HR) and galvanic skin response (GSR; Hamm, Cuthbert, Globisch, & Vaitl, 1997).

Based on a great number of studies on spider phobics there is a broad consensus on the temporal and spatial characteristics of the brain response in this specific group (see above). However, this knowledge cannot be easily transferred to other phobias because many of them differ from the small animal phobia subtype in several aspects. For example, in contrast to other phobia subtypes blood-injection–injury (BII) phobics respond with an atypical heart rate slowing after the exposure to their fear-relevant pictures (Hamm et al., 1997). Most recent functional magnet resonance imaging (fMRI) studies have failed to report significant increased amygdala, insula and ACC activity in BII phobics during a blocked presentation of feared stimuli (Caseras, et al., 2010a; Hermann et al., 2007; Schienle, Schäfer, Walter, Stark, & Vaitl, 2005). The failure to find enlarged fear circuit activity might have resulted from the employed blocked symptom provocation. When phobia-relevant pictures were infrequently presented between neutral stimuli common pattern of activation was observed in BII and spider phobia participants (Caseras et al., 2010b). However, in this latter experiment BII phobics also differed significantly from spider phobics showing a decreased left amygdala and heart rate responses as well as an enhanced inferior frontal gyrus (IFG) activity. Considering the proposed role of inferior frontal regions in the automatic regulation of emotion these results suggest that the IFG activity observed in BII phobia individuals can be responsible for the reduced brain responses in emotional appraisal regions and decreased heart rate in this group (Caseras et al., 2010b; Etkin, Egner, & Kalisch, 2011; Ochsner & Gross, 2005).

Prefrontal regions have been also observed to be more active in groups of people with social anxiety disorders (SAD) emphasizing the role of emotion regulation strategies in this population (see Brühl, Delsignore, Komossa, & Weidt, 2014, for review). At the same time, however, most previous studies showed that the amygdala, parahippocampal gyrus, right insula, ACC and occipital areas were still more active in SAD than healthy control participants during the phobia-relevant exposure (Evans et al., 2008; Phan et al., 2013; Schneier et al., 2011; Yoon, Fitzgerald, Angstadt, McCarron, & Phan, 2007). These results indicate that the regulatory prefrontal input does not reach the amygdala in SAD patients. Together with previous emotion regulation research showing a decreased recruitment of inferior frontal regions despite the use of reappraisal/cognitive control strategies the results reveal emotion regulation deficits in SAD patients (Brühl et al., 2014).

Considering differences between BII, social and spider fearful individuals the main goal of the present study was to determine the temporal and spatial characteristics of the fearful stimulus processing in these three populations. To the best of our knowledge, no previous study has directly compared the three groups with the same experimental exposure paradigm. With this approach each of the three fear groups can be compared not only to healthy controls but also to the other fear control groups that enables to investigate the specificity of the fear response. Moreover, current work is the first study to simultaneously examine the temporal and spatial characteristics of the brain response and the cardiac response pattern in fearful individuals. In the present work, data were collected using simultaneous electrocardiography (ECG), electroencephalograpy (EEG) and functional magnetic resonance imaging (fMRI) recordings in spider, blood/injection, social fearful and healthy control participants exposed to fear-related and neutral pictures. Comparing brain and autonomic responses between these fear groups could potentially help to understand mechanisms underlying the development and the maintenance of different anxiety disorders and to validate the new evidence–based diagnosis and classification criteria of mental disorders. This has important implications for the selection of appropriate treatment methods and for further research.

Based on previous evidences we predict to find an increased hypervigilance in spider, blood/injection and social fearful participants that would be indexed by enhanced P100 amplitudes for all experimental stimuli (Buodo, Peyk, Junghofer, Palomba, & Rockstroh, 2007; Kolassa et al., 2006; Kolassa et al., 2007; Michalowski et al., 2009, 2015). At the same time, the three fear groups are not expected to differ in the overall amplitude of the P100. Subsequently, indexed by the LPP component (cf., Kolassa et al., 2006; Michalowski et al., 2009, 2015; Miltner et al., 2005), a fear-specific modulation was expected contrasting the processing of spider pictures in spider and healthy groups. Increased LPPs were also expected in BII fearful participants viewing blood/injection pictures. In their previous ERP study Buodo

and collaborators (Buodo, Sarlo, Codispoti, & Palomba, 2006) found out that the LPP was increased for blood/injection when compared to neutral pictures but this effect was similar in the BII phobia and the control groups. However, the number of fear-related stimuli used in this study (N = 12) might have been too small for differentiating the LPPs between the two groups. In the social fear research threatening facial expressions have been conventionally used. A few studies showed that the social fear modulates the LPP for negative facial expressions (Moser, Huppert, Duval, & Simons, 2008; Sewell, Palermo, Atkinson, McArthur, 2008). However, others did not replicate these findings (Mühlberger et al., 2009; Rossignol, Anselme, Vermeulen, Philippot, & Campanella, 2007). To the best of our knowledge, no previous study investigated brain responses for more complex ecologically relevant social exposure scenes (e.g. a frontal view on a conference room or an interview panel) that were also shown to trigger higher subjective fear level in social fearful when compared to other fearful and healthy control individuals (Michalowski et al., under review). Therefore, the present study was designed to determine whether it is possible to observe preferential processing effects for pictures depicting social exposure scenes mentioned above in addition to the images of angry faces.

As to the spatial characteristics of the brain responses, we hypothesized the involvement of amygdale, hippocampus, insula, ACC as well as visual processing areas and prefrontal emotion regulation regions during the fear-related stimulation. However, considering previous data the pattern of neuronal activity may show phobia-specific differences including less emotional appraisal and more inferior frontal and orbitofrontal responses in the BII and social fear groups. With regard to the BII fear group frontal areas may play an important role in inhibiting the cardiac activity for pictures depicting blood scenes (Caseras et al., 2010b; Thayer & Friedman, 2002). Therefore, we wanted to examine the correlations between BOLD and cardiac responses in the present study. We expected to replicate increased HR in spider phobics and decreased HR in BII phobics exposed to their feared materials (Hamm et al., 1997). HR acceleration should correlate with the activity in the emotional appraisal regions, whereas HR deceleration should be accompanied by increased response in frontal inhibition regions (Thayer & Friedman, 2002).

Materials and methods

Participants

49 participants (♀ = 34, ♂ = 15; age mean = 22.8 y , sd = 3.3) were selected based on a questionnaire screening of 496 students from Warsaw Universities. Students were assessed with the Polish versions of the Mutilation Questionnaire (MQ; Kleinknecht & Thorndike, 1990), the Anxiety Subscale of the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987) and the Spider Phobia Questionnaire (SPQ; Klorman et al., 1974). Those scoring more than or equal to one standard deviation above the mean on one of the three fear questionnaires were selected into an appropriate fear group. Those scoring high on more than one fear-specific questionnaire were excluded. 12 participants (10 females) reporting elevated spider fear (SPQ ≥ 16; $\underline{M}$ = 21.2, $\underline{SD}$ = 3.2) were allocated to the spider fear group. 12 participants (6 females) that were afraid of social situations (LSAS Anxiety ≥39; $\underline{M}$ = 44.3, $\underline{SD}$ = 7.1) were selected for the social fear group. 12 participants (11 females) scoring high on the Mutilation Questionnaire (MQ ≥ 20; $\underline{M}$ = 20.9, $\underline{SD}$ = 1.4) were included in the blood fear group. 13 participants (7 females) scoring below the mean on all scales (SPQ < 9; LSAS Anxiety <25; MQ < 13) were classified as healthy (non-fearful) controls. The four groups did not differ with regard to the age and education level, ts < 1, ps > .1, as well as the trait anxiety as assessed with the STAI, ts < 1.6, ps > .1.

Experimental stimuli and procedure

A pool of 966 photographs selected from the Set of Fear Inducing Pictures (SFIP; Michalowski et al., under review)[1] as well as 10 additional pictures from the Warsaw Set of Emotional Facial Expression Pictures (WSEFEP; Olszanowski et al., 2015) were used. There were 736 neutral photographs (incl. Objects, people, mushrooms, plants, faces, landscapes and animals) and 240 fear-relevant pictures divided into three categories: 80 social fear-relevant photographs (40 pictures of angry faces and 40 social exposure scenes incl. Microphones, people sweating, writing an exam or giving a lecture), 80 spider fear-related pictures (60 pictures of spiders and 20 pictures of bugs) and 80 blood/injection pictures (incl. Alive injured or dead human or animal bodies or their isolated parts). This picture set was divided in two equal halves and subjects were asked to view one of the two halves including 488 pictures with 368 neutral photographs and 120 fear-relevant pictures. Stimuli were presented for 4000 ms each in 8 alternating blocks each lasting for about 3 min. There were two neutral blocks including 46 neutral pictures each and 6 fear-relevant blocks: two blood/injection fear blocks (each including 46 neutral and 20 blood/injection pictures), two spider fear blocks (each including 46 neutral images and 20 pictures of spiders/bugs) and two social fear blocks (each

[1] SFIP pictures originate from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2005), Geneva Affective Picture Database (GAPED; Dan-Glauser & Scherer, 2011), Nencki Affective Picture System (NAPS; Marchewka, Żurawski, Jednoróg, & Grabowska, 2014; Riegel et al., 2016) and Warsaw Set of Emotional Facial Expression Pictures (WSEFEP; Olszanowski et al., 2015), freely available non-profit photography stocks or were taken by the coauthors.

including 46 neutral images, 10 social exposure pictures and 10 images of angry faces) (see Fig. 1). During fear-relevant blocks, pictures were presented in a pseudo-random order with the restriction that fear-relevant pictures should occur between 2 or 3 neutral pictures. The order of the blocks was randomized across different participants with the restriction that blocks of the same type should not be presented one after another. Blocks were separated by a colored fixation cross lasting between 11 and 18 s with a pseudorandom order. The color of the fixation cross (black, blue, green or red) was informative in terms of signaling the type of a forthcoming picture block. Assignment of colors to the specific block (neutral, blood/injection, spider, social) was explained prior to the picture viewing session. This procedure allowed examining whether it is possible to observe context effects for blocks that were explicitly told to be fear-irrelevant. Stimuli were presented on an MR-compatible monitor using Presentation software (Neurobehavioral Systems, Inc). The monitor was placed outside the scanner bore just in front of subjects` feet.

On arrival to the laboratory, each participant was briefed on the experimental procedure and signed a written informed consent. Following the attachment of a 64-lead EEG cup (Brain Products GmbH) and an additional ECG electrode on the subjects` upper back participant was instructed about the upcoming experimental phase. After the scanning session, the EEG cup and the ECG electrode were removed and each participant was asked to view and evaluate all pictures outside the scanner. In this part of the experiment, each picture was presented once more for 3 s in a full screen mode. After the offset of the picture, participant was asked to evaluate his/her subjective experience of fear on a scale ranging from 1 (not at all) to 5 (very much) as well as valence and arousal on a computerized version of the Self-Assessment Manikin (Bradley & Lang, 1994). Consecutive presentation of two stimuli from the same fear-relevant category was avoided. The rating session lasted approximately 75 min. An obligatory 5-min break was taken after half of the stimuli had been assessed, during which subjects were asked to leave the experimental room. At the end of experimental session, participants completed the Spielberger Trait and State Anxiety Inventory (STAI-T; Spielberger et al., 1970; Polish version Wrześniewski, Sosnowski, & Matusik, 2002).

Apparatus and data acquisition

Magnetic resonance imaging was carried out using a 3-Tesla TrioTim MRI scanner (Siemens Medical Solutions) equipped with a 12-channel head coil. A high-resolution T1-weighted image (T1w) was acquired using the following acquisition parameters: TR: 2530 ms, TE: 3.32 ms, flip angle: 7°, 176 slices with an in-plane resolution of 1 mm^3, field of view: 256 mm, slice thickness: 1 mm. Functional images were acquired using an echo planar imaging pulse sequence (field of view: 224 mm, matrix: 64 × 64, slice thickness: 3.5 mm, TE: 30 ms, TR: 2000 ms, flip angle: 90°). Thirty-two contiguous, oblique–axial images oriented parallel to the anterior–posterior commissural plane were acquired in ascending interleaved order with a total of 1014 volumes.

EEG signal was recorded with a 64-lead MR-compatible EEG cup (Brain Products GmbH). An additional electrode was located on the subjects upper back in order to monitor the ECG signal. The ECG and EEG channels were referenced to FCz during recording. Scalp impedance was kept below 20 kΩ for each EEG sensor and below 10 kΩ for the reference and ground electrodes as suggested by the manufacturer. ECG and EEG data were continuously recorded in the 0.1–250 Hz frequency range with a sampling rate of 5000 Hz. The digitized signal was transferred to the recording computer via a fiber-optic cables. During the recording session the EEG recording system was synchronized with the scanner's internal clock.

EEG and ECG data processing

EEG and ECG data preprocessing was performed with Brain Vision Analyzer 2.0 (Brain Products GmbH). First, fMRI gradient artifacts were removed using the average artifact subtraction AAS method (Allen, Josephs, & Turner, 2000) implemented in the Vision Analyzer. A sliding average consisting of 21 consecutive MR volumes (TRs) was used for MR gradient

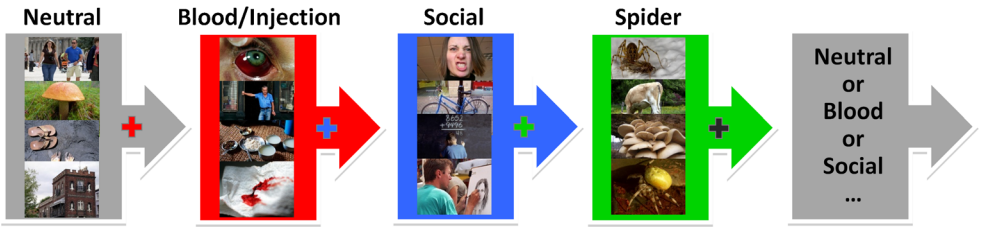

Fig. 1 Illustration of the experimental procedure. Separate blocks of pictures (2 x neutral, 2 x blood/injection and neutral, 2 x social and neutral, 2 x spider fear-related and neutral) were presented in a pseudorandomized order with the restriction that blocks of the same type should not be presented one after another. Blocks were separated by a colored fixation cross lasting between 11 and 18 s. The color of the fixation cross (black, blue, green or red) was informative in terms of signaling the type of a forthcoming picture block. Within blocks, pictures were continuously presented for 4000 ms each and in a pseudorandomized order such that 2 or 3 neutral pictures were always displayed between fear-relevant images

correction, each volume was indicated by a trigger received from the MR system. Corrected EEG and ECG data were down-sampled to 1 kHz. R-wave peaks of the heart signal were identified automatically based on a QRS complex template and then their positions were visually inspected, adjusted when necessary and exported into Matlab R2013 (The Math-Works Inc. Natick, MA, USA). All R-R intervals were calculated in milliseconds. Measures of cardiac activity were computed in terms of mean values of heart rate for 1 s intervals after picture onset. Mean HR values obtained for each fear-relevant picture category were compared to the HR responses elicited by neutral pictures presented within the same block (e.g. blood/injection versus neutral pictures from blood/injection blocks).

The MR-corrected EEG data was initially analyzed with Matlab R2013 and the FMRIB plug-in of the EEGLAB toolbox (version 13 4 4b; Delorme & Makeig, 2004; Niazy, Beckmann, Iannetti, Brady, & Smith, 2005) to perform the OBS correction. Then the EEG data was re-imported into Brain Vision Analyzer 2.0 and filtered between 0.1 and 30 Hz using an Infinity Impulse Response (IIR) filter. The resulting data were down-sampled to 250 Hz. All remaining EEG data channels were then inspected using the Raw Data Inspection tool and signal with maximal absolute value exceeding 250 MicroV within 200 ms interval was marked as an artifact and excluded from the eye movement correction that was next performed with Extended Biased Infomax ICA. After the eye movement correction, stimulus synchronized epochs were extracted beginning 100 ms before to 1200 ms after picture onset and epochs with amplitude difference exceeding 150 MicroV within 200 ms were excluded from further analysis. Finally, the data were transformed to averaged reference and baseline corrected using a 100 ms baseline before picture onset. Epochs pertinent to each picture category were grand-averaged and used for further analyses.

ERP data analyses

Following prior research with analogous experimental paradigms (Schupp, Junghöfer, Weike, & Hamm, 2003; Michalowski et al., 2009), in order to identify the spatial and temporal characteristics of the ERP modulation repeated measures ANOVAs including Picture Category (blood/injection, social exposure, angry faces, spider fear-related and neutral from each type of block), Group (blood/injection fear, spider fear, social fear, controls) and follow-up comparisons between fear-relevant and neutral pictures for each block type were computed for single time points and sensors. To avoid false positives and to assure a more stringent alpha-level adjustment, significant effects were only considered meaningful when observed for at least eight continuous data points (32ms) and two sensors. These statistical waveform analyses as well as visual inspection revealed differences between the fear groups and the non-fearful control group in the time window of the P1 as well as differences in the LPP component between fear-relevant and neutral stimuli. In the second step, mean amplitudes averaged within time windows and sensor clusters that indicated the significant P1 group effects and emotional modulation of the LPP components were included in further statistical analyses. The P1 was scored in posterior sensor clusters (O1 and O2) in the time window from 140 to 188 ms (see Fig. 2). The LPPs were calculated in the time window 630–870 ms in a centro-parietal sensor cluster including CP1, CP2, CPz (angry faces vs neutral, see Fig. 3) and in the time window 530–980 ms in two centro-parietal sensor clusters: C1, C3, CP1 (left hemisphere) and C2, C4, CP2 (right hemisphere) (other fear-relevant vs neutral, Fig. 3).

In order to examine the P1 group effects, planned orthogonal (Helmert) contrasts (cf. Kirk, 1995; Sosnowski & Rynkiewicz, 2008) were calculated to test the hypotheses about a greater overall P1 in the three fear groups when compared to healthy controls and the lack of differences in the P1 amplitude between the three fear groups. To examine the effects of picture category on the P1 amplitude we calculated ANOVAs including Picture Category (blood/injection, social, spider and 4 x neutral [from each block type]). In order to analyze the emotional LPP modulation for each of the four fear-relevant picture categories we conducted four ANOVAs including Picture Category (fear-relevant vs neutral from a given block) as a within-subject factor and Group (blood/injection fear, spider fear, social fear, controls) as a between-subject factor.

fMRI data processing and analysis

Statistical Parametric Mapping (SPM12, Wellcome Trust Center for Neuroimaging, London, UK) running on MATLAB R2013b (The Math-Works Inc. Natick, MA, USA) was used for data preprocessing and statistical analyses. First, functional images were motion corrected. Then, structural (T1w) images from single subjects were co-registered to the mean functional image. High-dimensional Diffeomorphic Anatomical Registration through Exponentiated Lie Algebra (DARTEL) was used to create a group-specific template and flow fields based on segmented tissue from T1w images (Ashburner, 2007). The functional images were normalized using compositions of flow fields and group-specific templates to a default 1.5 mm isotropic voxel size. Finally, the normalized functional images were smoothed with an 8 mm isotropic Gaussian kernel.

At the subject level timings for all fear-relevant and neutral pictures within blocks were entered into the design matrix as well as head movement parameters. The hemodynamic response was modeled using a stick function convolved with the canonical hemodynamic response function for the onset of each picture implemented in SPM software. At the subject level, fear-relevant vs neutral pictures contrasts were

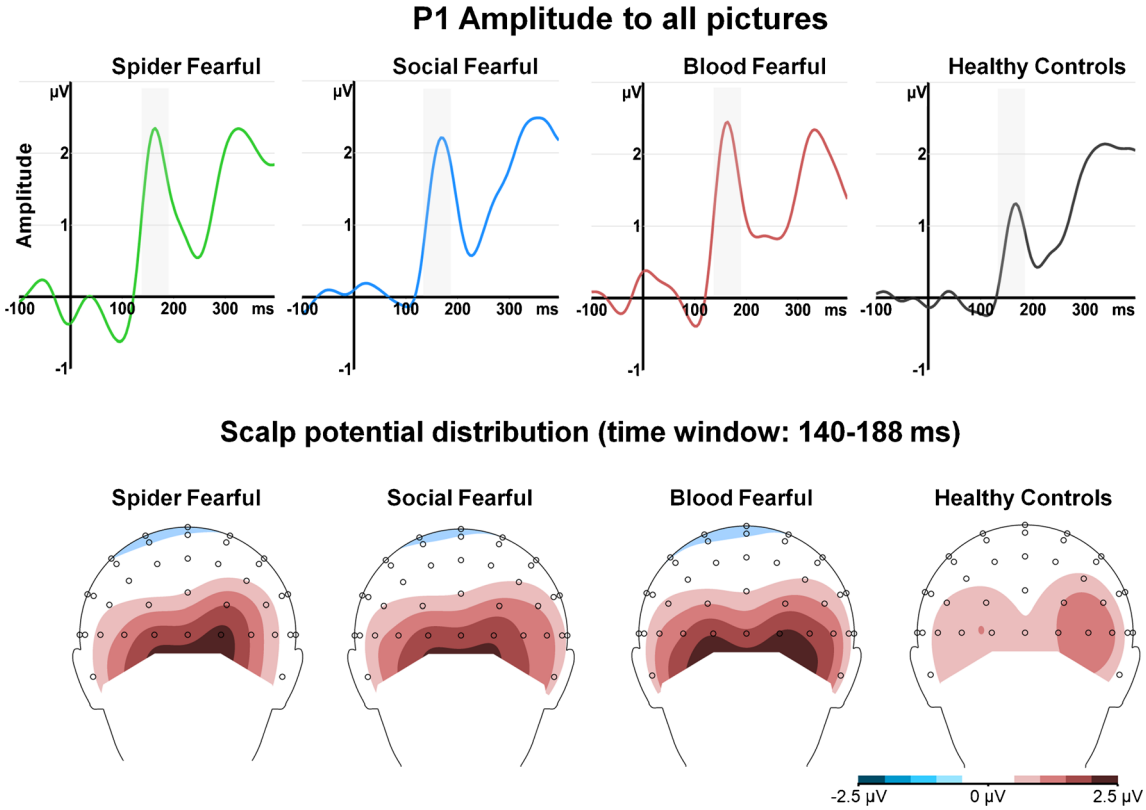

Fig. 2 Illustration of the P1 hypervigilance effect in spider, social and blood fearful individuals. Top: Grand-averaged ERPs elicited over occipital sensors during the exposure to all pictures in the three fear and healthy control groups. Shaded areas are selected in the time interval of the P1. Bottom: scalp potential maps displayed separately for all groups

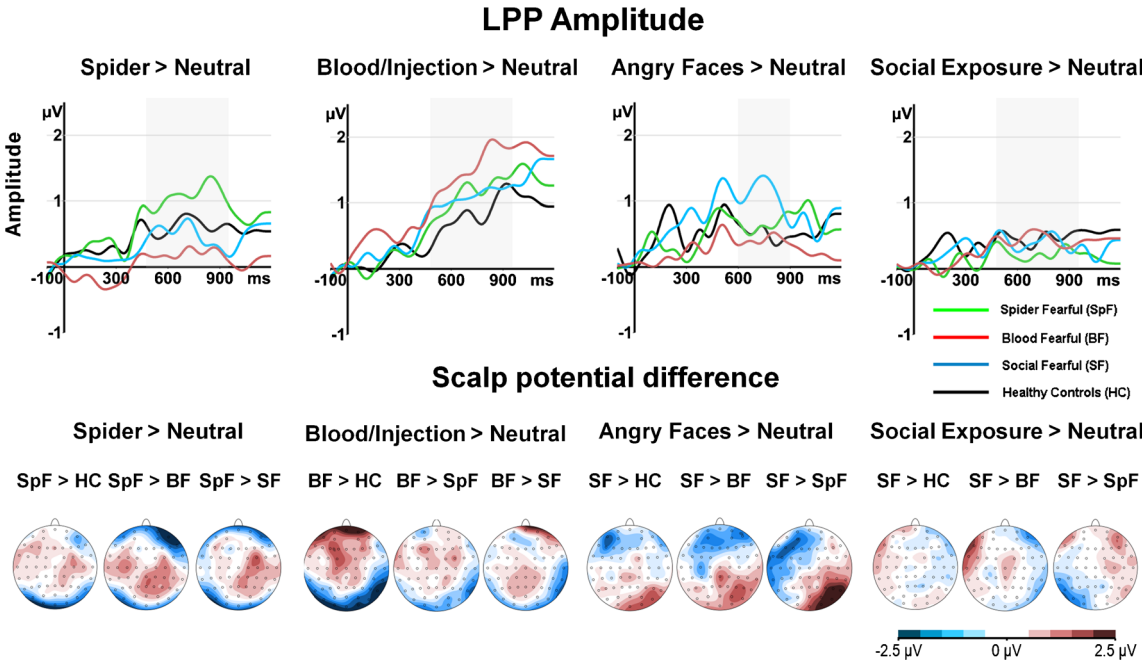

Fig. 3 Illustration of the Picture Category x Group effects as revealed by the LPP component elicited during picture viewing. Top: Grand-averaged ERP waveforms elicited over centro-parietal channels during viewing of the four fear-related (vs. neutral) pictures displayed separately for each group. Shaded areas are selected in the time interval of the LPP. Bottom: topographical ERP difference maps (fear-related minus neutral pictures) displayed as a function of group

calculated for each category (e.g. spider fear-related vs neutral pictures from spider blocks). At the group level, a flexible factorial model was used for all categories of fear relevant pictures (spider, blood, social exposure and angry faces) compared to neutral ones via entering relevant contrasts for each subject. A correction for multiple comparisons ($p < 0.05$) was performed using a cluster extent threshold determined by Monte-Carlo simulation, using a voxel threshold of $p < 0.005$ uncorrected. Coordinates of significant effects are reported in MNI space. XjView software was used to identify the structures within significant clusters (http://www.alivelearn.net/xjview). Additionally, regions of interests (ROIs) analyses were computed for the amygdala, insula, anterior cingulate cortex (ACC), hippocampus as well as inferior, middle and superior frontal gyri and orbitofrontal cortex (OFC). ROIs were localized using the anatomical masks from Aal altas in WFU PickAtlas software (Maldjian, Laurienti, Kraft, & Burdette, 2003). ROI data were extracted via Marsbar SPM toolbox (Brett, Anton, Valabregue, & Poline, 2002). For the whole brain and ROI analyses the fear-relevant vs neutral contrasts were calculated separately for each of the three types of fear-relevant blocks (i.e. spider, blood, social) including corresponding fear-group and the other fear or healthy control groups.

Results

ERP results

P1

We expected that the P1 amplitude should be similar in all fearful individuals and greater in the fear groups than in healthy controls. Thus, we decided to use planned orthogonal (Helmert) contrasts analysis. This method showed that the overall P1 was more pronounced in the three fear groups than in healthy controls, $t(45) = 2.42$, $p < .05$. At the same time, the three fear groups did not differ with regard to the overall P1 amplitude, $ts(45) < 1$, $ps > .1$. Overall ANOVAs including each picture category revealed that there was no significant Picture Category effect, $F(7, 315) = 1.76$, ns.

Late positive potential (LPP)

ANOVAs including Picture Category (spider fear-related and neutral) as a within-subject factor and Group (spider fear, social fear, blood/injection fear and healthy control) as a between-subject factor showed that spider fear-related pictures elicited significantly larger LPPs than neutral contents, $F(1, 45) = 22.73$, $p < .001$, $\eta_p2 = .336$, an effect that was more pronounced in the spider fear group when compared to other groups, Picture Category x Group, $F(3, 45) = 2.62$, $p < .05$, $\eta_p2 = .149$ (see Fig. 3). Pictures from the blood/injection category also elicited larger LPP than neutral pictures, $F(1, 45) = 76.69$, $p < .001$, $\eta_p2 = .643$, an effect that was more pronounced in the blood/injection fear group when compared to controls, Picture Category x Group, $F(1, 23) = 4.99$, $p < .05$, $\eta_p2 = .178$,but not to the other fear groups, Picture Category x Group effects, $Fs < 2.8$, $ps > .1$. Calculating overall ANOVAs including social exposure and neutral pictures as well as all four experimental groups did not result in significant Picture Category and Picture Category x Group effects, $Fs < 1$, $ps > .1$. Overall ANOVA including images of angry faces and neutral pictures as a within-subject factor and all four groups as a between-subject factor revealed greater LPPs for pictures of angry faces than neutral images, Picture Category, $F(1, 45) = 13.02$, $p < .001$, $\eta_p2 = .224$. This effect tended to be more pronounced for the social fear than the healthy control group, Picture Category x Group, $F(1, 23) = 3.54$, $p = .07$, $\eta_p2 = .133$, and it was similar when other groups were compared, Picture Category x Group, $Fs < 2$, $ps > .1$.

fMRI results

Spider fear-relevant photographs

Whole brain analyses revealed that spider fear-related (vs neutral stimuli) evoked stronger responses in spider fearful than in healthy control participants in the bilateral insula, anterior cingulate cortex (ACC), hippocampus, as well as inferior, middle and superior frontal gyri and the inferior temporal visual stream (see Table 1 and Fig. 4, top left). The ROI analyses confirmed these findings and revealed that spider fearful individuals also showed greater bilateral amygdala and orbitofrontal responses for spider fear-related (vs neutral pictures) than the healthy control group (see Table 2). These group differences were very similar when spider and blood fear groups were compared and slightly less pronounced when spider and social fear groups were contrasted (see left panel of the Fig. 4 and supplementary materials).

Blood/injection photographs

The results from the whole brain analyses showed that blood/injection (vs neutral stimuli) evoked stronger responses in blood fearful than healthy control participants in the bilateral supramarginal gyrus, left middle occipital and superior parietal regions as well as left middle frontal gyrus (see Table 1 and Fig. 4, middle panel). The ROI analyses revealed increased left inferior and middle frontal responses in the blood fearful vs healthy control individuals (see Table 2). Contrasting the blood fear and the other fear groups using the whole brain analyses revealed stronger blood fear group responses to blood/injection (vs neutral) stimuli in the left precentral gyrus (BF > SpF) as well as the left middle frontal and right middle occipital gyri (BF > SF) (see middle panel of the Fig. 4 and

Table 1 Whole brain analysis results with activated brain regions, cluster size, cluster peak statistics and MNI Coordinates for fear – relevant > neutral picture contrasts

Brain regions	Side	Voxels	Peak *t*	x	y	z
Spider Pic > Neutral Pic – Spider Fearful vs Healthy Control Group						
		1706	8.1	−39	−3	62
Precentral Gyrus	L					
Middle Frontal Gyrus	L					
Inferior Frontal Gyrus	L					
Superior Frontal Gyrus	L					
		19622	7.4	−24	−62	0
Cuneus	L/R					
Cerebellum Anterior Lobe	L/R					
Posterior Cingulate Gyrus	L/R					
Lingual Gyrus	L/R					
Parahippocampal Gyrus	L					
Fusiform Gyrus	L/R					
Precuneus	L/R					
Insula	L					
Superior Temporal Gyrus	L					
Palladium	L					
Middle Occipital Gyrus	R					
Inferior Occipital Gyrus	R					
Thalamus	L/R					
		7827	7.0	23	−14	66
Middle Cingulate Gyrus	L/R					
Precentral Gyrus	R					
Medial Frontal Gyrus	R					
Middle Frontal Gyrus	R					
Superior Frontal Gyrus	R					
Anterior Cingulate Gyrus	L/R					
Poscentral Gyrus	R					
		4364	6.2	50	27	−3
Insula	R					
Inferior Frontal Gyrus	R					
Superior Temporal Gyrus	R					
		725	6.1	−47	−38	29
Supramarginal Gyrus	L					
Superior Temporal Gyrus	L					
		2474	5.3	−27	32	30
Middle Frontal Gyrus	L					
Superior Frontal Gyrus	L					
Medial Frontal Gyrus	L					
		471	5.1	−20	−27	−20
Parahippocampal Gyrus	L					
Hippocampus	L					
Insula	L					
		714	5.1	47	−35	3
Superior Temporal Gyrus	R					
Middle Temporal Gyrus	R					
Insula	R					
		350	4.9	9	−36	51

Table 1 (continued)

Brain regions	Side	Voxels	Peak *t*	x	y	z
Precuneus	R					
Middle Cingulate Gyrus	R					
		97	4.8	6	−39	71
Paracentral Lobule	R					
		281	4.7	30	50	21
Middle Frontal Gyrus	R					
Superior Frontal Gyrus	R					
		51	4.5	−20	−35	65
Postcentral Gyrus	L					
		68	4.4	−32	−86	−12
Inferior Occipital Gyrus	L					
Lingual Gyrus	L					
Blood Pic > Neutral Pic – Blood Fearful vs Healthy Control Group						
Middle Frontal Gyrus	L	275	4.9	−56	11	38
Middle Frontal Gyrus	L	77	3.5	−47	44	15
Middle Occipital Gyrus	L	92	3.5	−38	−63	32
Supramarginal Gyrus	R	60	3.4	65	−27	45
Supramarginal Gyrus	L	175	3.2	−53	−39	32
Angular Gyrus	L	45	3.1	−45	−57	47
Superior Parietal Lobule	L	50	3.1	−29	−56	42
Social Exposure Pic > Neutral Pic – Social Fearful vs Healthy Control Group						
Superior Frontal Gyrus	R	466	5.1	18	−2	68
Superior Frontal Gyrus	L	155	4.2	−14	−5	68
Superior Frontal Gyrus	L	43	3.8	−24	14	65
Angry Faces > Neutral Pic – Social Fearful vs Healthy Control Group						
Middle Cingulate Gyrus	L	436	4.7	−2	2	27
Precuneus	L	169	3.5	−5	−66	60
Superior Temporal Gyrus	R	67	3.5	47	−5	−23
Middle Temporal Gyrus	R	90	3.3	62	−53	−2
Fusiform Gyrus	R	100	3.1	35	−75	−17
Anterior Orbital Gyrus	L	66	3.0	−29	36	−12

All of the listed brain regions were cluster corrected and met the significance threshold of p = 0.05. L is left; R is right. Cluster size is the number of voxels activated in the regional cluster. The x, y, z coordinates are the MNI coordinates. Only the main peaks of activation within each cluster and their corresponding brain structures are reported

supplementary materials). The ROI analyses of the BF > SpF and BF > SF contrasts did not reveal any other group effects (see supplementary materials).

Photographs of social exposure

The analyses performed for the social exposure (vs neutral) pictures revealed that the social fear group responded with greater bilateral superior frontal gyrus activity than healthy controls (see Table 1 and Fig. 4, top right) as well as stronger left anterior orbital, middle cingulate (whole brain) and anterior cingulate responses (ROI) than the spider fear group (see right panel of the Fig. 4 and supplementary materials). There were no significant group effects when the SF > BF contrast was analyzed.

Photographs of angry faces

As expected, pictures of angry faces (vs neutral stimuli) evoked stronger responses in the social fear group than in healthy controls in the right fusiform gyrus, middle and superior temporal gyri as wel as the left anterior orbital, middle cingulate regions and the left precuneus when analyzed using the whole brain method and an additional left anterior cingulate gyrus (ACC) in the ROI analysis (see Tables 1 and 2 and Fig. 4, bottom right). Social fearful individuals responded with greater superior parietal lobule and occipital pole activity when compared to the spider fear group using whole brain analyses (see supplementary materials). We did not find increased brain responses for the SF > BF contrast.

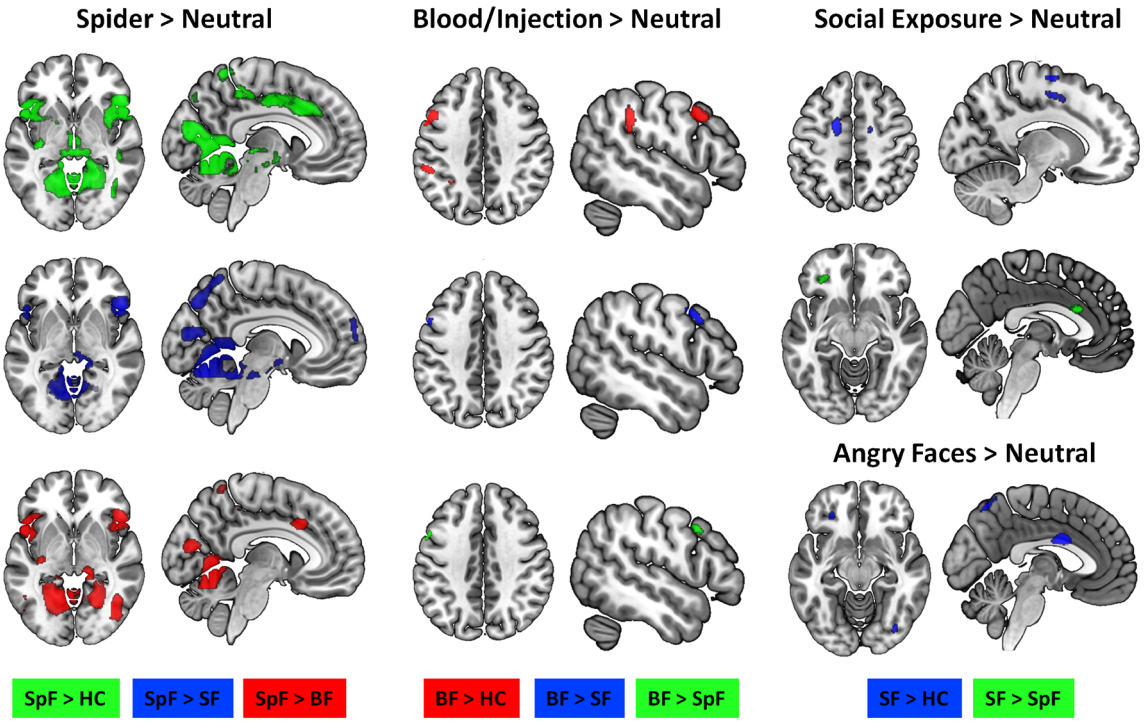

Fig. 4 Whole brain activations for fear groups (vs healthy and fearful control participants) during the presentation of their fear-specific pictures (vs neutral images). Left panel illustrates brain structures with increased activations for spider fear-relevant (vs neutral) stimuli in spider fearful when compared to healthy control (green), social fearful (blue) and blood fearful individuals (red). Middle panel illustrates brain structures with stronger activations for blood/injection pictures in blood fearful than healthy control (red), social fearful (blue) and spider fearful participants (green). Increased activations observed for photographs of social exposure and angry faces in the social fear than the healthy control (blue) and spider fear groups (green) are presented on the right panel. Clusters are significant at $p < 0.05$ (corrected)

Heart rate and HR x BOLD correlations

Spider fear-related photographs

The overall HR analysis calculated for spider and neutral pictures showed a significant Time x Picture Category effect, F (3, 135) = 8.56, $p < .001$, $\eta_p 2 = .160$. Follow-up analyses revealed that compared to neutral slides the HR response for spider fear-relevant pictures was lower at the beginning (1st second) and greater at the end (4th second) of the picture presentation period, Picture Category, t (48) = 2.54, $p < .05$, and t (48) = 3.22, $p < .01$, respectively (see Fig. 5). Other effects including Picture Category and Group were not significant, ts (48) < 1.2, ps > .1. HR delta values (spider fear-relevant minus neutral pictures) calculated for the 1st second of the picture presentation showed negative correlation with the response elicited by spider fear-relevant (vs neutral pictures) in the left amygdale, left insula and left superior frontal gyrus, rs (49) > −.32, ps < .05, and a trend towards negative correlations with the left ACC, r (49) = −.27, $p = .058$, left inferior frontal gyrus, r (49) = −.28, $p = .053$, and right insula, r (49) = −.26, $p = .076$. HR delta values (spider fear-relevant minus neutral pictures) calculated for the 3rd and 4th seconds of the picture presentation period correlated positively with increased bilateral insula activity, and hippocampus responses for spider (vs neutral) pictures, r (49) = .35, $p < .05$, and r (49) = .44, $p < .01$, respectively. There were no other significant correlations between BOLD and HR responses to spider images, rs < .520, ps > .08.

Blood/injection photographs

We observed a heart rate decrease during the exposure to blood/injection when compared to neutral pictures, Picture Category, F (1, 45) = 40.53, $p < .001$, $\eta_p 2 = .474$. There was also significant effect of Picture Category x Group, F (3, 45) =3.33, $p < .05$, $\eta_p 2 = .182$. Overall, HR decrease for blood/injection pictures was more pronounced in the blood fear group when compared to the social fear and the healthy control groups, Picture Category x Group, F (1, 22) = 6.76, $p < .05$, $\eta_p 2 = .235$, and F (1, 23) = 3.43, $p < .077$, $\eta_p 2 = .130$, respectively, and in the spider fear than the social fear group, Picture Category x Group, F (1, 22) = 5.39, $p < .05$, $\eta_p 2 = .197$ (see Fig. 6). We did not observe any other significant effects including Group, Fs < 1.5, ps > .1. HR decrease observed just after the onset of blood/injection (vs neutral) pictures (1st second) correlated with an increased activity in the right middle frontal gyrus, r (49) = −.34, $p < .05$, as well as the right inferior and superior frontal gyri, rs (49) = −.26, ps = .075. An increase in the HR for blood/injection (vs

Table 2 ROI analysis results with activated brain regions, cluster size, cluster peak statistics and MNI coordinates for fear-relevant > neutral stimuli contrasts compared between the appropriate fear group and healthy controls

Brain regions	Side	Voxels	Peak *t*	x	y	z
Spider Pic > Neutral Pic – Spider Fearful vs Healthy Control Group						
Amygdala	L	95	4.3	−27	2	−23
Amygdala	R	26	3.8	29	2	−14
ACC	L	592	5.3	0	11	30
ACC	R	417	5.5	12	20	29
Middle Frontal Gyrus	L	214	6.3	−30	−5	65
Middle Frontal Gyrus	L	3465	5.3	−27	32	30
Middle Frontal Gyrus	R	499	6.1	42	−5	62
Middle Frontal Gyrus	R	867	4.7	30	50	21
Superior Frontal Gyrus	L	342	6.3	−32	−6	66
Superior Frontal Gyrus	L	2049	5.2	−23	38	36
Superior Frontal Gyrus	R	762	7.0	23	−14	66
Superior Frontal Gyrus	R	503	4.5	14	60	24
Hippocampus	L	336	5.1	−35	−15	−18
Hippocampus	L	23	4.4	−12	−39	3
Hippocampus	R	246	5.4	30	−39	−5
Hippocampus	R	180	3.9	27	−9	−21
Inferior Frontal Gyrus	L	1275	6.4	−50	15	−3
Inferior Frontal Gyrus	L	66	4.2	−35	35	26
Inferior Frontal Gyrus	R	1925	6.2	50	27	−3
Inferior Orbital Gyrus	L	800	6.1	−48	15	−6
Inferior Orbital Gyrus	R	754	6.1	48	26	−5
Insula	L	1966	5.8	−45	14	−2
Insula	R	2495	5.8	47	23	−3
Blood Pic > Neutal Pic – Blood Fearful vs Healthy Control Group						
Middle Frontal Gyrus	L	39	4.1	−51	12	39
Middle Frontal Gyrus	L	8	3.5	−47	44	15
Inferior Frontal Gyrus	L	5	3.4	−48	42	14
Social Exposure Pic > Neutral Pic – Social Fearful vs Healthy Control Group						
Superior Frontal Gyrus	L	15	4.0	−15	−3	68
Superior Frontal Gyrus	R	125	5.1	18	−2	68
Angry Faces > Neutral Pic – Social Fearful vs Healthy Control Group						
ACC	L	85	4.7	−2	2	27

neutral) pictures in the second half of the picture presentation period (3rd and 4th seconds) was accompanied by increasing activity in the bilateral amygdale, ACC, middle and superior frontal gyri, rs (49) > .29, ps < .05, as well as bilateral hippocampus, rs (49) > .50, ps < .001. There were no other significant correlations between the BOLD and the HR responses to blood/injection pictures, rs (49) < .25, ps > .08.

Valence, arousal and fear ratings

Overall ANOVAs including Picture Category (blood/injection, social exposure, angry faces, spider fear-relevant, neutral) as a within factor and Group (blood/injection fear, social fear, spider fear, controls) as a between factor revealed that arousal, fear and valence ratings differed as a function of picture category, Fs (4, 172) > 87, ps < .001, $\eta_p 2s$ > .67, and that these effects were modulated by group; Picture Category x Group, Fs (12, 172) > 6, ps < .001, $\eta_p 2s$ > .30 (see Fig. 7). Follow-up analyses showed that stimuli depicting blood/injection, social exposure, angry faces and spiders/bugs were rated as more fear-evoking, arousing and unpleasant by the corresponding fear groups when compared to controls; ts > 3.4, ps < .01.

Discussion

The present study is the first to directly compare the temporal and spatial characteristics of the brain response as well as the

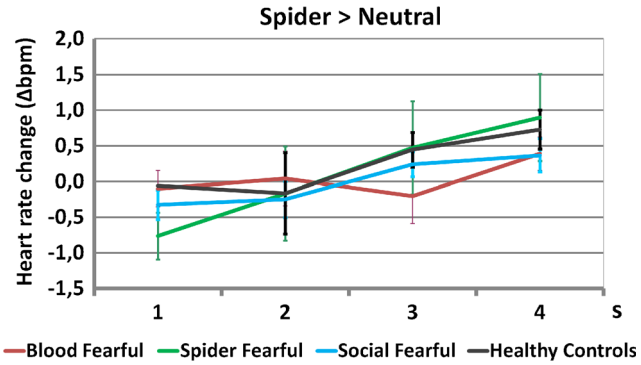

Fig. 5 Top: Average heart rate change waveforms during the 4 s presentation of spider (minus neutral pictures) for blood/injection fear, spider fear, social fear and healthy control groups

cardiac activity during fear-stimulus processing between spider, blood/injection and social fearful as well as healthy (non-fearful) volunteers. As expected, all fearful individuals showed increased vigilance in the fear-relevant context as indexed by overall increased P1 amplitudes. Moreover, increased brain responses to fear-relevant stimuli were observed in fearful when compared to healthy and analogue fear control groups. However, the fear groups participating in the current study showed strong differences in the pattern of cardiac activity and in the strength/spatial characteristics of the brain response to their fear-relevant cues.

Increased vigilance in fearful individuals

Providing a millisecond time resolution of dynamic brain activity, the present ERP data replicated previous findings regarding the P1 hypervigilance effects in fearful participants (Buodo et al., 2007; Kolassa et al., 2006, 2007; Michalowski et al., 2009, 2015). The overall increased P1 was the first ERP component revealing group differences between fearful and non-fearful control participants. The new findings of our research are that the hypervigilance effect was similar in spider, social and blood/injection fearful individuals and it was maintained throughout the whole experiment, even during these experimental blocks that were announced as neutral. These results clearly demonstrate that fearful individuals respond with increased brain activity once they have been put in a context in which their feared stimuli are likely to occur. Even the instruction that a context is safe (neutral blocks) did not reduce this increased brain response. This is in line with animal data showing strong sensitization effects after threat exposure (Rau, DeCola, & Fanselow, 2005; Rau & Fanselow, 2009). These effects might facilitate fear conditioning to other stimuli and promote generalization of fear that is a crucial mechanism in the maintenance of anxiety disorders (see Lissek et al., 2009).

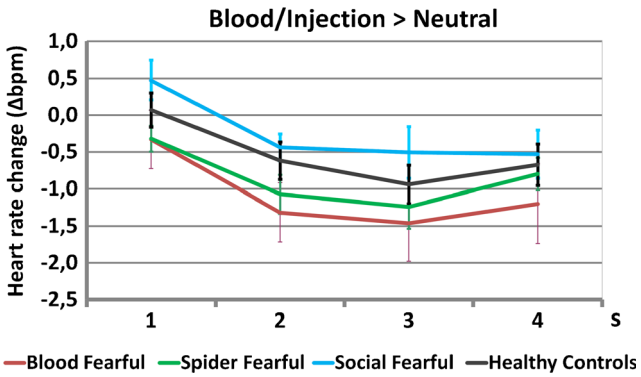

Fig. 6 Top: Average heart rate change waveforms for the 4 s period during viewing of blood/injection (minus neutral images) displayed for blood/injection fearful, spider fearful, social fearful and healthy control participants

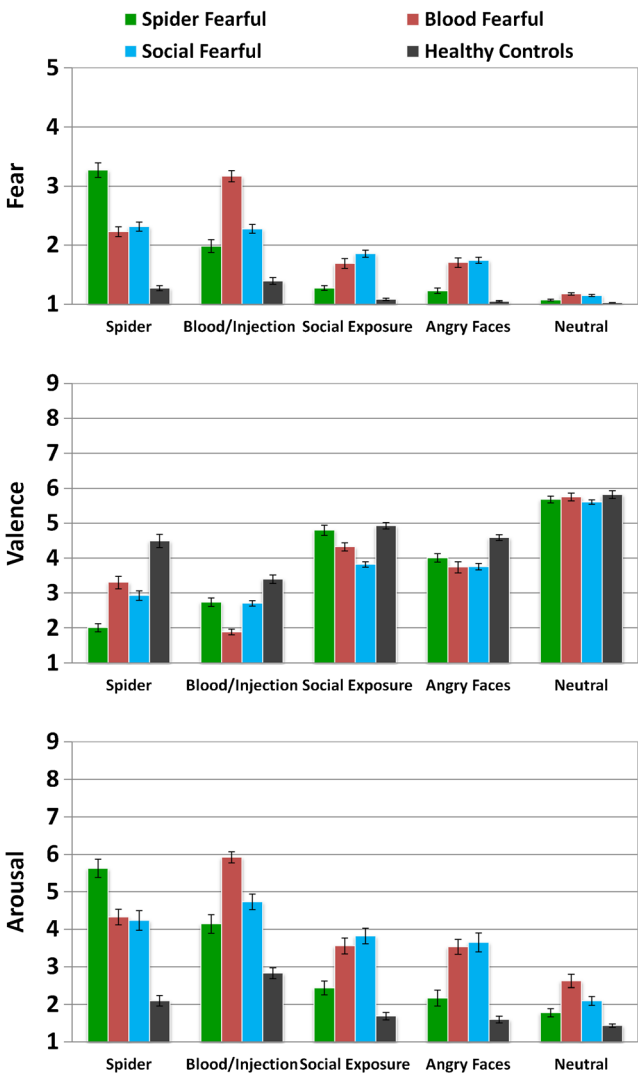

Fig. 7 Subjective fear, valence and arousal ratings. Mean level of fear (top), valence (middle) and arousal (bottom) for different picture categories as assessed by spider, blood and social fear groups as well as healthy controls. SEM is marked by black lines

Preferential processing of fear-relevant pictures

Replicating previous findings (Kolassa et al., 2005; Kopp & Altmann, 2005; Leutgeb Schäfer, & Schienle, 2011; Michalowski et al., 2009, 2015; Miltner et al., 2005; Moser et al., 2008; Sewell et al., 2008) the present study showed that pictures of spiders and angry faces evoke significantly larger LPPs than neutral images and that these effects are more pronounced in the spider and social fear groups, respectively. The observed difference in the LPP topography between images of angry faces and those depicting spider fear-related pictures suggests partly distinct neural substrates involved in the processing of faces and images depicting more complex scenes (see also Moser et al., 2008). In the present study, increased LPP has been also observed for blood/injection than neutral pictures and this effect was significantly stronger in blood/injection fearful than healthy control participants. This phobia specific increase in the LPP amplitude for blood/injection pictures was not reported up to date. Comparable results were revealed for dental treatment scenes presented to participants with dental phobia (Leutgeb et al., 2011). However, dental phobia differs from the classical BII phobia with regard to somatic and cognitive reactions to the symptom provocation (DeJongh, Bongaarts, & Vermeule, 1998; Houtem et al., 2014). In general, the enlarged LPP effects observed in fearful individuals seem to reflect preferential processing of feared stimuli (see e.g. Michalowski et al., 2009).

In agreement with previous research on fear and anxiety (Dilger et al., 2003; Lueken, et al., 2014; Schienle et al., 2005, 2007; Straube et al., 2006; Wendt, Lotze, Weike, Hosten, & Hamm, 2008), in spider fearful people the exposure to spider fear-relevant pictures elicited increased activation within a broad brain network, including amygdala, hippocampus, ACC, insula as well as several frontal and visual areas. The novelty of this study was that the neural responses towards fear-specific stimuli were compared to analogue fear control groups. These comparisons revealed that the increased fear circuit activity observed in the spider fear group for their feared pictures was evident regardless of whether they were compared to healthy or fearful control participants. The specificity of the fear response was also, albeit not so obviously, observed for the other two fear groups (see below). These group-specific effects support the idea that differences in phobic brain responses are related to phobogenic behaviors and go beyond a general fear or anxiety proneness (Kramer, Patrick, Krueger, & Gasperi, 2012; Patrick & Bernat, 2010; Stein, Simmons, Feinstein, & Paulus, 2007; Vaidyanathan, Patrick, & Bernat, 2009).

Analyses performed for spider fear-related pictures revealed that stronger neural activations were accompanied by an increased heart rate response. Specifically, there were significant correlations between the HR increase and the bilateral hippocampus and insula activity confirming the role of these structures in negative emotion processing (Caseras et al., 2010b; Etkin & Wager, 2007). Surprisingly, HR increase observed for spider fear-relevant pictures in spider fearful participants was similar to that observed in fearful and also healthy control groups. This is inconsistent with numerous previous findings (e.g. Caseras et al., 2010b; Hamm et al., 1997). The failure to replicate these previous effects in the present study might be attributed to the specificity of our experimental design. That is, other than in this previous research, fear-relevant pictures were presented more frequently in the present study that might have influenced the heart rate activity and the distribution of attentional resources. Unfortunately, using longer time lags between fearful stimuli exposure in the present study was not recommended from an ethical and methodological point of view.

In contrast to spider phobics, visual processing as well as middle and/or inferior frontal areas were the only regions that showed a greater activity in the blood/injection fear than the healthy and social fear control groups during the presentation of blood/injection pictures. Thus, using time lags in between blood/injection pictures we supported the findings from previous block-design studies that also reported increased frontal responses but no greater amygdala, insula and ACC activity in BII phobics (vs controls) viewing blood/injection pictures (Caseras et al., 2010a; Hermann et al., 2007; Schienle et al., 2003). Importantly, we observed that increased frontal activations correlated with a decrease in the HR observed just after the onset of blood/injection pictures and that the HR decrease was stronger in the blood/injection fear group. To our knowledge, these are the first findings supporting the assumption that frontal regions are responsible for the reduced emotional brain responses and decreased heart rate in BII fearful individuals (Caseras et al., 2010b; Etkin et al., 2011; Ochsner & Gross, 2005).

Subjective ratings demonstrated that pictures of social exposure scenes and angry faces evoked greater fear and arousal in the social fear group than in controls. Interestingly, dissociation of subjective and neural arousal was observed in social fearful participants for social exposure photographs. In this group, greater responses to social exposure scenes were observed only in the bilateral superior frontal areas (vs healthy controls) as well as left anterior orbital and anterior cingulate regions (vs spider fearful controls). These results indicate that the social exposure scenes during fMRI scanning might be insufficient to induce the fear response in social fearful participants. Pictures of angry faces seem to have higher potential for inducing fear response in social fearful people as these stimuli elicited several differential activations between the social fear and the control groups. However, the subjective ratings and the brain responses observed during this symptom provocation in the social fear group were also reduced when compared to those observed in spider fearful subjects. In contrast to the spider fear group, social fearful

participants exposed to images of angry faces did not differ from controls in the activity of several typical fear structures, such as the amygdale, insula and hippocampus. On the other hand, the exposure to angry faces elicited anterior cortical activations in this group, including increased responses in the left anterior orbital and cingulate as well as the right middle/superior temporal regions. Previous studies showed that these anterior regions support the reduction of amygdale activity and play an important role in the emotion regulation process in people experiencing aversive events (Phan et al., 2005). However, the anterior regions might be effective as far as their functional connections with the subcortical emotion system are strong enough (Goldin et al., 2012) and in clinical populations this functional connectivity is often disturbed leading to a pathological fear response (Etkin & Wager, 2007; Phelps, Delgado, Nearing, & LeDoux, 2004). Resting-state studies also found an altered functional connectivity in social anxiety disorder patients indicating a disturbed inhibitory regulation of the OFC onto the ACC and amygdale in this group (Hahn et al., 2011). In the present study we did not include clinical groups but people who reported increased scores on fear specific questionnaires. The results may suggest that our social fearful participants successfully down-regulate their fear response via anterior emotion regulation areas, a mechanism that may facilitate their relatively undisturbed social functioning (Goldin et al., 2012).

The present study indicates that the processing of fear stimuli is organized with the dynamics mirroring the defense cascade. The initial unspecific vigilance in the pre-encounter defense mode (increased P1) is followed by a shift into post-encounter defense, prompted by the identification of a threat signal (LPP). The occurrence of threat leads to neuronal, autonomic and somatic responses consistent with defense preparation (cf. Bradley, 2009; Lang, Davis, & Öhman, 2000). The present data show that these responses differ between the blood-injection and the spider phobia participants. In contrast to the spider fear group, blood-injection fearful individuals responded with a decreased heart rate after the occurrence of their feared photographs. In our study the decreased heart rate for blood/injection pictures was associated with an increased activity in inferior, middle and superior frontal gyri. Considering these findings, we hypothesize that these frontal regions might initiate the down-regulation of the emotional appraisal areas and the HR reduction after the exposure to blood/injection images. An automatic emotion regulation process might also influenced the response observed in the group of social fearful participants in the present study which showed increased anterior orbital and anterior cingulate responses and failed to show typical subcortical responses when images of angry faces or social exposure scenes were presented. In contrast, spider fearful people showed a widespread fear circuit activity indicating a weaker emotion regulation ability of this fear group.

In summary, the present findings suggest different underlying mechanisms in spider, blood and social fear groups. We observed an increased defensive activity in spider phobics who showed elevated activations in key neural networks for fear processing during the exposure to spider fear-relevant photographs. We provided further evidences for a distinct neural response pattern in blood/injection fearful individuals that responded with stronger activity in middle and inferior frontal regions but not in several typical subcortical fear structures when confronted with blood/injection images. The present study also showed that blood/injection images produced a decreased HR response that correlated with increased inferior, middle and superior frontal activity indicating that these frontal regions may play an important role in inhibiting fear responses towards blood/injection pictures. Stronger anterior cortical than subcortical fear system responses were also observed in social fearful individuals for their feared pictures. This finding indicates increased engagement of cognitive evaluation and fear regulation system in the social fear group. Moreover, the analyses performed against fearful control groups showed that the increased fear-specific stimulus processing is largely related to specific fears and not to general anxiety proneness. However, all three fear groups showed increased fear system sensitization that might facilitate further fear conditioning and strengthen the defensive reactivity.

Certain limitations may have influenced the present findings. The generalizability of the observed effects might be limited to the included sample and stimulus materials. The spider fear-relevant picture subset used in our study consisted of photographs of spiders (75%) and bugs (25%). Including photographs of bugs might have reduced the intensity of the fear-specific effects in spider fearful individuals. On the other hand, however, our previous (Michalowski et al., under review) and the present study revealed that this picture subset was still sufficient for eliciting increased fear responses in this group. Due to a greater prevalence of blood/injection and spider fears among women the two groups included higher female rates than the healthy control and the social fear groups. However, higher brain responses and subjective unpleasantness for fear-specific material in blood/injection and spider fearful individuals were observed in the present study independent of whether these groups were contrasted to control samples with similar or different female ratio. We have, therefore, no reason to suspect that our findings are strongly affected by the gender imbalance between groups. Another potential weaknesses of the present study worth mentioning are that the sample included in the study was relatively small and the findings have been obtained with subclinical student participants limiting their direct transfer to anxiety patients. Further studies with larger clinical samples are required to confirm the present results.

Compliance with ethical standards The study involves Human Participants that were briefed on the experimental procedure and signed a written informed consent prior to the beginning of the experiment. The protocol was approved by the Ethics Committee of the Faculty of Psychology at the University of Warsaw in accordance with the Declaration of Helsinki.

Conflict of interest The authors have declared that no competing interests exist.

Grant support The study was supported by grants from the National Science Centre (Narodowe Centrum Nauki, NCN), decision number: DEC-2011/03/D/HS6/05951 and DEC-2011/03/D/HS6/05578. The project was realized with the aid of CePT research infrastructure purchased with funds from the European Regional Development Fund as part of the Innovative Economy Operational Programme, 2007–2013.

Sources of supports We are grateful to Marcin Sińczuk for his support in programming and to Paweł Turnau, who constructed a web-based assessment platform used to collect the subjective ratings.

References

Allen, P. J., Josephs, O., & Turner, R. (2000). A method for removing imaging artifact from continuous EEG recorded during functional MRI. *NeuroImage, 12*, 230–239. doi:10.1006/nimg.2000.059.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage, 38*, 95–113. doi:10.1016/j.neuroimage.2007.07.007.

Bradley, M. M. (2009). Natural selective attention: orienting and emotion. *Psychophysiology, 46*, 1–11. doi:10.1111/j.1469-8986.2008.00702.x.

Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: the self-assessment manikin and the semantic differential. *Journal of Behavior Therapy and Experimental Psychiatry, 25*(1), 49–59. doi:10.1016/0005-7916(94)90063-9.

Brett, M., Anton, J. L., Valabregue, R., & Poline, J. B. (2002). Region of interest analysis using the MarsBar toolbox for SPM 99. *NeuroImage, 16*(2), S497.

Brühl, A. B., Delsignore, A., Komossa, K., & Weidt, S. (2014). Neuroimaging in social anxiety disorder—a meta-analytic review resulting in a new neurofunctional model. *Neuroscience & Biobehavioral Reviews, 47*, 260–280. doi:10.1016/j.neubiorev.2014.08.003.

Buodo, G., Peyk, P., Junghofer, M., Palomba, D., & Rockstroh, B. (2007). Electromagnetic indication of hypervigilant responses to emotional stimuli in blood-injection-injury fear. *Neuroscience Letters, 424*, 100–105.

Buodo, G., Sarlo, M., Codispoti, M., & Palomba, D. (2006). Event-related potentials and visual avoidance in blood phobics: is there any attentional bias? *Depression and Anxiety, 23*, 304–311.

Caseras, X., Giampietro, V., Lamas, A., Brammer, M., Vilarroya, O., Carmona, S., et al. (2010a). The functional neuroanatomy of blood-injection-injury phobia: a comparison with spider phobics and healthy controls. *Psychological Medicine, 40*(1), 125–134. doi:10.1017/S0033291709005972.

Caseras, X., Mataix-Cols, D., Trasovares, M. V., López-Solà, M., Ortriz, H., Pujol, J., et al. (2010b). Dynamics of brain responses to phobic-related stimulation in specific phobia subtypes. *European Journal of Neuroscience, 32*(8), 1414–1422. doi:10.1111/j.1460-9568.2010.07424.x.

Dan-Glauser, E. S., & Scherer, K. R. (2011). The Geneva affective picture database(GAPED): a new 730-picture database focusing on valence and normative significance. *Behavior Research Methods, 43*, 468–477. doi:10.3758/s13428-011-0064-1.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods, 134*(1), 9–21.

DeJongh, A., Bongaarts, G., & Vermeule, I. (1998). Blood–injury-injection phobia and dental phobia. *Behaviour Research and Therapy, 36*, 971–982.

Dilger, S., Straube, T., Mentzel, H. J., Fitzek, C., Reichenbach, H. R., Hecht, H., et al. (2003). Brain activation to phobia-related pictures in spider phobic humans: an event-related functional magnetic resonance imaging study. *Neuroscience Letters, 348*, 29–32.

Etkin, A., Egner, T., & Kalisch, R. (2011). Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends in Cognitive Sciences, 15*(2), 85–93. doi:10.1016/j.tics.2010.11.004.

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *American Journal of Psychiatry, 164*(10), 1476–1488.

Evans, K., Wright, C. I., Wedig, M. M., Gold, A. L., Pollack, M. H., & Rauch, S. L. (2008). A functional MRI study of amygdale responses to angry schematic faces in social anxiety disorders. *Depression and Anxiety, 25*, 496–505.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review, 1*, 429–438. doi:10.3758/BF03210947.

Fredrikson, M. (1981). Orienting and defensive reactions to phobic and conditioned fear stimuli in phobics and normals. *Psychophysiology, 18*, 456–465.

Fredrikson, M., Wik, G., Annas, P., Ericson, K., & Stone-Erlander, S. (1995). Functional neuroanatomy of visually elicited simple phobic fear: additional data and theoretical analysis. *Psychophysiology, 32*, 43–48.

Goldin, P. R., Ziv, M., Jazaieri, H., Werner, K., Kraemer, H., Heimberg, R. G., et al. (2012). Cognitive reappraisal self-efficacy mediates the effects of individual cognitive-behavioral therapy for social anxiety disorder. *Journal of Consulting and Clinical Psychology, 80*(6), 1034. doi:10.1037/a0028555.

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., et al. (2011). Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *NeuroImage, 56*(3), 881–889. doi:10.1016/j.neuroimage.2011.02.064.

Hamm, A., Cuthbert, B. N., Globisch, J., & Vaitl, D. (1997). Fear and the startle reflex: blink modulation and autonomic response patterns in animal and mutilation fearful subjects. *Psychophysiology, 34*, 97–107.

Hare, R. D. (1973). Orienting and defensive responses to visual stimuli. *Psychophysiology, 10*, 453–464.

Hermann, A., Schäfer, A., Walter, B., Stark, R., Vaitl, D., & Schienle, A. (2007). Diminished medial prefrontal cortex activity in blood-injection-injury phobia. *Biological Psychology, 75*(2), 124–130.

Houtem, C. M. H. H., Aartman, I. H. A., Boomsma, D. I., Ligthart, L., Visscher, C. M., & Jongh, A. (2014). Is dental phobia A blood-injection-injury phobia? *Depression and Anxiety, 31*(12), 1026–1034.

Kirk, R. E. (1995). Experimental design: Procedures for behavioral sciences. Pacific Grove, CA: Brooks/Cole Publishing Company.

Kleinknecht, R. A., & Thorndike, R. M. (1990). The mutilation questionnaire as a predictor of blood/injury fear and fainting. *Behaviour Research and Therapy, 28*(5), 429–437.

Klorman, R., Weerts, T. C., Hastings, J. E., Melamed, B. G., & Lang, P. J. (1974). Psychometric description of some specific-fear questionnaires. *Behavior Therapy, 5*(3), 401–409.

Kolassa, I. T., Kolassa, S., Musial, F., & Miltner, W. H. R. (2007). Event-related potentials to schematic faces in social phobia. *Cognition & Emotion, 21*(8), 1721–1744.

Kolassa, I.T., Musial, F., Kolassa, S., & Miltner, W.H.R. (2006). Event-related potentials when identifying or color-naming threatening schematic stimuli in spider phobic and non-phobic individuals. *BMC Psychiatry*, 6–38.

Kolassa, I. T., Musial, F., Mohr, A., Trippe, R. H., & Miltner, W. H. R. (2005). Electrophysiological correlates of threat processing in spider phobics. *Psychophysiology, 42*, 520–530.

Kopp, B., & Altmann, R. (2005). Neurocognitive effects of phobia-related stimuli in animal-fearful individuals. *Cognitive, Affective, & Behavioral Neuroscience, 5*, 373–387.

Kramer, M. D., Patrick, C. J., Krueger, R. F., & Gasperi, M. (2012). Delineating physiologic defensive reactivity in the domain of self-report: phenotypic and etiologic structure of dispositional fear. *Psychological Medicine, 42*(06), 1305–1320.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). Motivated attention: Affect, activation and action. In P. J. Lang, R. F. Simons, & M. T. Balaban (Eds.), *Attention and Orienting: Sensory and Motivational Processes* (pp. 97–135). Hilsdale, NJ: Lawrence Erlbaum Associates, Inc..

Lang, P. J., Bradley, M. M., Cuthbert, B. N. (2005). International affective picture system (IAPS): digitized photographs, instruction manual and affective ratings. Technical report no. A-6. University of Florida: Gainesville.

Lang, P. J., Davis, M., & Öhman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders, 61*, 137–159.

LeDoux, J. E. (1987). Emotion. In F. Plum & V. B. Mountcastle (Eds.), Handbook of physiology: Nervous system V, higher function (pp. 419–460). Washington, DC: American Physiology Society.

Leutgeb, V., Schäfer, A., & Schienle, A. (2011). Late cortical positivity and cardiac responsitivity in female dental phobics when exposed to phobia-relevant pictures. *International Journal of Psychophysiology, 79*(3), 410–416. doi:10.1016/j.ijpsycho.2011.01.003.

Liebowitz, M. R. (1987). *Social phobia (pp. 141–173)*. Basel: Karger Publishers.

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., et al. (2009). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychiatry, 167*, 47–55. doi:10.1176/appi.ajp.2009.09030410.

Lueken, U., Hilbert, K., Stolyar, V., Maslowski, N. I., Beesdo-Baum, K., & Wittchen, H. U. (2014). Neural substrates of defensive reactivity in two subtypes of specific phobia. *Social cognitive and affective neuroscience, nst159., 9*(11), 1668–1675.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage, 19*, 1233–1239.

Marchewka, A., Żurawski, Ł., Jednoróg, K., & Grabowska, A. (2014). The Nencki affective picture system (NAPS): introduction to a novel, standardized, wide-range, high-quality, realistic picture database. *Behavior Research Methods, 46*(2), 596–610. doi:10.3758/s13428-013-0379-1.

Michalowski, J. M., Melzig, C. A., Weike, A. I., Stockburger, J., Schupp, H. T., & Hamm, A. O. (2009). Brain dynamics in spider-phobic individuals exposed to phobia-relevant and other emotional stimuli. *Emotion, 9*(3), 306–315. doi:10.1037/a0015550.

Michalowski, J. M., Pané-Farré, C. A., Löw, A., & Hamm, A. O. (2015). Brain dynamics of visual attention during anticipation and encoding of threat- and safe-cues in spider-phobic individuals. *Social Cognitive and Affective Neuroscience, 10*(9), 1177–1186. doi:10.1093/scan/nsv002.

Miltner, W. H. R., Trippe, R. H., Krieschel, S., Gutberlet, I., Hecht, H., & Weiss, T. (2005). Event-related brain potentials and affective responses to threat in spider/snake-phobic and non-phobic subjects. *International Journal of Psychophysiology, 57*, 43–52.

Moser, J. S., Huppert, J. D., Duval, E., & Simons, R. F. (2008). Face processing biases in social anxiety: an electrophysiological study. *Biological Psychology, 78*, 93–103. doi:10.1016/j.biopsycho.2008.01.005.

Mühlberger, A., Wieser, M.J., Herrmann, M.J., Weyers, P., Tröger, C.,& Pauli, P. (2009). Early cortical processing of natural and artificial emotional faces differs between lower and higher socially anxious persons. Journal of Neural Transmission, 116(6), 735–746. doi: 10.1007/s00702-008-0108-6

Niazy, R. K., Beckmann, C. F., Iannetti, G. D., Brady, J. M., & Smith, S. M. (2005). Removal of MRI environmental artifacts from EEG data using optimal basis sets. *NeuroImage, 28*, 720–737.

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences, 9*(5), 242–249.

Olszanowski, M., Pochwatko, G., Kuklinski, K., Scibor-Rylski, M., Lewinski, P., & Ohme, R. K. (2015). Warsaw set of emotional facial expression pictures: a validation study of facial display photographs. *Frontiers in Psychology, 5*, 1516. doi:10.3389/fpsyg.2014.01516.

Paquette, V., Lévesque, J., Mensour, B., Leroux, J.-M., Beaudoin, G., Bourgouin, P., et al. (2003). « change the mind and you change the brain": effects of cognitive-behavioral therapy on the neural correlates of spider phobia. *NeuroImage, 18*, 401–409.

Patrick, C. J., & Bernat, E. M. (2010). Neuroscientific foundations of psychopathology. In T. Millon, R. F. Krueger, & E. Simonsen (Eds.), *Contemporary directions in psychopathology: Toward the DSM-V* (pp. 419–452). New York: Guilford Press.

Pflugshaupt, T., Mosimann, U. P., Schmitt, W. J., von Wartburg, R., Wurtz, P., Lüthi, M., et al. (2007). To look or not to look at threat? Scanpath differences within a group of spider phobics. *Journal of Anxiety Disorders, 21*, 353–366.

Phan, K. L., Fitzgerald, D. A., Nathan, P. J., Moore, G. J., Uhde, T. W., & Tancer, M. E. (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biological Psychiatry, 57*(3), 210–219.

Phan, K. L., Coccaro, E. F., Angstadt, M., Kreger, K. J., Mayberg, H. S., Liberzon, I., et al. (2013). Corticolimbic brain reactivity to social signals of threat before and after setraline treatment in generalized social phobia. *Biological Psychiatry, 63*, 329–336. doi:10.1016/j.biopsych.2012.10.003.

Phelps, E. A., Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron, 43*, 897–905.

Prigatano, G. P., & Johnson, H. J. (1974). Autonomic nervous system changes associated with a spider phobic reaction. *Journal of Abnormal Psychology, 83*, 169–177.

Rau, V., DeCola, J. P., & Fanselow, M. S. (2005). Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. *Neuroscience and Biobehavioral Reviews, 29*, 1207–1223.

Rau, V., & Fanselow, M. S. (2009). Exposure to a stressor produces a long-lasting enhancement of fear learning in rats. *Stress, 12*(2), 125–133. doi:10.1080/10253890802137320.

Riegel, M., Żurawski, Ł., Wierzba, M., Moslehi, A., Klocek, Ł., Horvat, M., Grabowska, A., Michalowski, J. Jednoróg, K., & Marchewka, A. (2016). Characterization of the Nencki Affective Picture System by discrete emotional categories (NAPS BE). *Behavior Research Methods,* doi:10.3758/s13428-015-0620-1.

Rossignol, M., Anselme, C., Vermeulen, N., Philippot, P., & Campanella, S. (2007). Categorical perception of anger and disgust facial expression is affected by non-clinical social anxiety: an ERP study. *Brain Research, 1132*, 166–176.

Sabatinelli, D., Bradley, M. M., Fitzsimmons, J. R., & Lang, P. J. (2005). Parallel amygdala and inferotemporal activation reflect emotional intensity and fear relevance. *NeuroImage, 24*, 1265–1270.

Schienle, A., Schäfer, A., Hermann, A., Rohrmann, S., & Vaitl, D. (2007). Symptom provocation and reduction in patients suffering from

spider phobia. *European Archives of Psychiatry and Clinical Neuroscience, 257*(8), 486–493.

Schienle, A., Schäfer, A., Stark, R., Walter, B., Kirsch, P., & Vaitl, D. (2003). Disgust processing in phobia of blood-injection-injury. An fMRI study. *Journal of Psychophysiology, 17*, 87–93.

Schienle, A., Schäfer, A., Walter, B., Stark, R., & Vaitl, D. (2005). Elevated disgust sensitivity in blood phobia. *Cognition & Emotion, 19*, 1229–1241.

Schupp, H. T., Junghöfer, M., Weike, A. I., & Hamm, A. O. (2003). Attention and emotion: an ERP analysis of facilitated emotional stimulus processing. *Neuroreport, 14*, 1107–1110.

Sewell, C., Palermo, R., Atkinson, C., & McArthur, G. (2008). Anxiety and the neural processing of threat in faces. *Neuroreport, 19*, 1339–1343. doi:10.1097/WNR.0b013e32830baadf.

Schneier, F. R., Pomplun, M., Sy., M., & Hirsch, J. (2011). Neural response to eye contact and paroxetine treatment in generalized social anxiety disorder. Psychiatry Research, 194, 271–278. doi: 10.1016/j.pscychresns.2011.08.006.

Sosnowski, T., & Rynkiewicz, A. (2008). RUN/EDIT information processing mode and phasic cardiac acceleration. *Psychophysiology, 45*, 1079–1085.

Spielberger, C., Gorsuch, A., & Lushene, R. (1970). *The state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *The American Journal of Psychiatry, 164*(2), 318–327.

Straube, T., Mentzel, H. J., & Miltner, W. H. R. (2006). Neural mechanisms of automatic and direct processing of phobogenic stimuli in specific phobia. *Biological Psychiatry, 59*, 162–170.

Thayer, J. F., & Friedman, B. H. (2002). Stop that! Inhibition, sensitization, and their neurovisceral concomitants. *Scandinavian Journal of Psychology, 43*(2), 123–130.

Tolin, D. F., Lohr, J. M., Lee, T. C., & Sawchuk, C. N. (1999). Visual avoidance in specific phobia. *Behaviour Research and Therapy, 37*, 63–70.

Vaidyanathan, U., Patrick, C. J., & Bernat, E. M. (2009). Startle reflex potentiation during aversive picture viewing as an indicator of trait fear. *Psychophysiology, 46*(1), 75–85.

Wendt, J., Lotze, M., Weike, A. I., Hosten, N., & Hamm, A. O. (2008). Brain activation and defensive response mobilization during sustained exposure to phobia-related and other affective pictures in spider phobia. *Psychophysiology, 45*, 205–215.

Weymar, M., Keil, A., & Hamm, A. O. (2014). Timing the fearful brain: hypervigilance and spatial attention in early visual perception. *Social Cognitive and Affective Neuroscience, 9*(5), 723–729. doi: 10.1093/scan/nst044.

Wrześniewski, K., Sosnowski, T., & Matusik, D. (2002). *Inwentarz Stanu i Cechy Lęku STAI*. PTP: Polska adaptacja STAI. Podręcznik. Warszawa.

Yoon, K. L., Fitzgerald, T. A., Angstadt, M., McCarron, R. A., & Phan, K. L. (2007). Amygdala reactivity to emotional faces at high and low intensity in generalized social phobia: a 4-tesla functional MRI study. *Psychiatry Research, 154*, 93–98.