Individual differences in neural correlates of fear conditioning as a function of 5-HTTLPR and stressful life events

Tim Klucken,[1],* Nina Alexander,[2],* Jan Schweckendiek,[1] Christian J. Merz,[1,3] Sabine Kagerer,[1] Roman Osinsky,[4] Bertram Walter,[1] Dieter Vaitl,[1] Juergen Hennig,[5] and Rudolf Stark[1]

[1]Bender Institute of Neuroimaging, Justus Liebig University Giessen, 35394 Giessen, [2]Department of Biological Psychology, Technical University of Dresden, 01069 Dresden, [3]Department of Cognitive Psychology, Ruhr-University Bochum, 44780 Bochum, [4]Department of Psychology I, University of Wuerzburg, 97070 Würzburg and [5]Department of Psychobiology and Behavioral Medicine, Justus Liebig University Giessen, 35394 Giessen

Fear learning is a crucial process in the pathogeneses of psychiatric disorders, which highlights the need to identify specific factors contributing to interindividual variation. We hypothesized variation in the serotonin transporter gene (5-HTTLPR) and stressful life events (SLEs) to be associated with neural correlates of fear conditioning in a sample of healthy male adults ($n = 47$). Subjects were exposed to a differential fear conditioning paradigm after being preselected regarding 5-HTTLPR genotype and SLEs. Individual differences in brain activity as measured by functional magnetic resonance imaging (fMRI), skin conductance responses and preference ratings were assessed. We report significant variation in neural correlates of fear conditioning as a function of 5-HTTLPR genotype. Specifically, the conditioned stimulus (CS^+) elicited elevated activity within the fear-network (amygdala, insula, thalamus, occipital cortex) in subjects carrying two copies of the 5-HTTLPR S′ allele. Moreover, our results revealed preliminary evidence for a significant gene-by-environment interaction, such as homozygous carriers of the 5-HTTLPR S′ allele with a history of SLEs demonstrated elevated reactivity to the CS^+ in the occipital cortex and the insula. Our findings contribute to the current debate on 5-HTTLPR x SLEs interaction by investigating crucial alterations on an intermediate phenotype level which may convey an elevated vulnerability for the development of psychopathology.

Keywords: imaging genetics; classical conditioning; fear; 5-HTTLPR; amygdala

INTRODUCTION

Fear conditioning has been emphasized as a key process in the development of anxiety disorders (Shin and Liberzon, 2010). Thus, current research attempts to identify specific genetic and environmental factors that contribute to individual differences in conditioned fear responses. A well-established method to investigate variation in fear conditioning is the differential conditioning paradigm. This procedure involves pairing of a neutral conditioned stimulus (CS^+) with a salient aversive unconditioned stimulus (UCS), while a second stimulus (CS^-) is never paired with the UCS (non-UCS). After a few trials, the CS^+ elicits conditioned fear responses (CRs) such as increased skin conductance responses (SCRs), changes in subjective ratings and elevated brain activity (Delgado et al., 2006; De Houwer, 2009). Neural correlates of fear conditioning involve activation of a subcortical fear-network comprising the amygdala, the thalamus and the anterior cingulate cortex (ACC) (Hamm et al., 2003; Delgado et al., 2006; Sehlmeyer et al., 2009). The thalamus–amygdala pathway possesses a crucial role for the CS–UCS association process and mediates CRs (Öhman and Mineka, 2001). In addition, recent studies have identified an extended network including the occipital cortex and the insula, which is considered to convey the evaluation of the current CS value and the interoceptive processing of CRs (Sehlmeyer et al., 2009).

Results from twin studies suggesting CRs to be partly heritable (Hettema et al., 2003) stimulated genetic association studies in this field. A functional polymorphism in the promotor region of the serotonin transporter gene (5-HTTLPR) has been considered a promising candidate, given that pharmacological manipulation of serotonergic neurotransmission relates to substantial alterations in fear conditioning (Inoue et al., 2004; Burghardt et al., 2007; Almada et al., 2009). This 43 bp insertion/deletion polymorphism presumably influences transcriptional activity of the serotonin transporter (5-HTT) gene and comprises a low-expressing short (S allele) and a high-expressing long (L allele) variant (Lesch et al., 1996; Stoltenberg et al., 2002). Numerous neuroimaging studies using a variety of different aversive stimuli have suggested the S allele to be associated with increased re-activity of fear-relevant brain structures (Hariri et al., 2002; Bertolino et al., 2005; Hariri et al., 2005; Munafò et al., 2008), whereas neural correlates of fear conditioning have not yet been investigated. However, several studies have addressed the association between 5-HTTLPR and peripheral physiological measures of CR (Garpenstrand et al., 2001; Crişan et al., 2009; Lonsdorf et al., 2009). For instance, in a well-conducted study, Crişan and colleagues (2009) found increased SCRs towards the CS^+ in S allele compared to homozygous L allele carriers. In contrast, Lonsdorf et al. (2009) report increased startle responses in S allele compared to homozygous L allele carriers, whereas no differences in SCRs emerged. Following this line of research, genetic imaging studies are needed to elucidate the underlying neural mechanisms by which 5-HTTLPR genotype biases CRs.

In addition, substantial effort has been undertaken to identify specific combinations of genetic and environmental risk factors which jointly modulate vulnerability to psychiatric disorders. Regarding 5-HTTLPR, the S allele has been suggested to convey an increased risk for depression under conditions of elevated stress (Caspi et al., 2003; Uher and McGuffin, 2008; Caspi et al., 2010). Recently, this issue

has attracted a considerable controversy raised by meta-analyses with conflicting results (Munafò *et al.*, 2008; Risch *et al.*, 2009; Karg *et al.*, 2011). This debate highlights the need to explore biological alterations associated with 5-HTTLPR x life stress (Caspi *et al.*, 2010), which can be discussed as a potential intermediate phenotypes conveying an elevated vulnerability. Following this line of research, prior studies have identified variation in neural and endocrine threat sensitivity as a function of 5-HTTLPR x stressful life events (SLEs). On a neural level, a significant 5-HTTLPR x SLEs interaction has been reported regarding resting state activity and in response to emotional face stimuli within fear-relevant brain structures (Canli *et al.*, 2006; Williams *et al.*, 2009; Lemogne *et al.*, 2011). Furthermore, we have observed significantly elevated cortisol responses to acute stress in subjects homozygous for the S allele with a history of SLEs (S′S′/high SLEs group) in a recently published study (Alexander *et al.*, 2009). The latter results imply that this specific constellation of genetic and environmental risk factors relates to biological alterations associated with elevated threat sensitivity. Thus, a gene-by-environment (G × E) approach might also contribute to a deeper understanding in genetic association studies on fear conditioning, but has not been applied in this field of research so far.

Based on the above mentioned literature, the present study aimed to investigate neural correlates of fear conditioning as a function of 5-HTTLPR genotype and SLEs. We hypothesized homozygous carriers of the low-expressing 5-HTTLPR alleles (S′ group) to demonstrate exaggerated neural activity in the contrast $CS^+ > CS^-$. In addition, we assumed that the association of 5-HTTLPR and neural correlates of fear conditioning is further modulated by SLEs. Based on the observation of elevated endocrine threat/stress reactivity in S′S′/high SLEs subjects (Alexander *et al.*, 2009), we expected this group to demonstrate increased neural reactivity to the CS^+, which would indicate a cross-validation of our previous findings. In order to address these hypotheses, participants were preselected from our stress study regarding 5-HTTLPR genotype and SLEs. This preselection process resulted in a balanced number of participants for different GxE constellations, enhanced statistical power and further ensured sufficient variation regarding the prevalence of SLEs.

METHODS

Participants

Forty-eight subjects (mean age: 26.8; s.d.: 3.0) were recruited from our previously published stress study (Alexander *et al.*, 2009) comprising an ethnically homogenous Caucasian sample of 100 healthy male adults. Current or past mental (assessed by structured clinical interviews, Margraf, 1994), chronic physical problems and consumption of psychotropic drugs or drugs exerting influence on endocrine stress reactivity were defined as exclusion criteria. All subjects were right-handed, had normal or corrected-to-normal vision, and received 40 Euro for their participation. Participants signed an informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the German Psychological Society.

Participants of the present fMRI study were recruited depending on the 5-HTTLPR/rs25531 mini-haplotype and SLEs. Regarding 5-HTTLPR, it has become increasingly common in genetic association studies to additionally account for an A→G single-nucleotide polymorphism (rs25531), which is located upstream of the 5-HTTLPR promoter variant within the greater repeat structure (Nakamura *et al.*, 2000). Prior studies suggested that the L_G allele is associated with a similar reduction in 5-HTT expression as the S allele (Hu *et al.*, 2006; Praschak-Rieder *et al.*, 2007). For enhanced clarity, the terms S′ (S, L_G) and the L′ (L_A) allele are used in the following when

referring to tri-allelic classification of 5-HTTLPR. In previous studies, dominant, co-dominant and recessive models of the 5-HTTLPR S′ allele have been proposed without a clear consensus (Uher and McGuffin, 2008). Since we aimed to explore multiple threat-related biological alterations within the same sample, we based our selection process on a recessive model of the S′ allele which has provided the best fit in our initial stress study (Alexander *et al.*, 2009) and all other previous studies on endocrine stress reactivity (Gotlib *et al.*, 2008; Dougherty *et al.*, 2010; Mueller *et al.*, 2010; Way and Taylor, 2010). Thus, we preselected 24 subjects homozygous for the low-expressing alleles (S′S′ group) and 24 subjects carrying at least one high-expressing allele (L′ group) to participate in the present study. Both the S′S′ and the L′ group comprised 12 subjects with a high number and 12 subjects reporting a low number of SLEs (as defined by median split within the original sample). Due to technical problems, data of one participant (L′/low SLEs) had to be excluded from the analyses.

Assessment of stressful life events

Information on SLEs was obtained using the Life Events Checklist (LEC) developed by the National Centre for Posttraumatic Stress Disorder (PTSD). This 17-item self-report measure showed good psychometric properties in a sample of undergraduate students and has been associated with PTSD symptoms in a clinical sample (Gray *et al.*, 2004). As a unique feature, the LEC provides information on multiple types of exposure to a wide variety of potentially traumatic experiences (e.g. physical and sexual assault, combat, sudden, unexpected death of a loved one). Subjects respond to the items using a 5-point nominal scale (1 = happened to me, 2 = witnessed it, 3 = learned about it, 4 = not sure; 5 = does not apply).

Conditioned stimuli (CS)

Two neutral visual stimuli (two squares; one with continuous lines; one with broken lines) served as CS^+ and CS^-. All stimuli were grey in colour, had identical luminance, and were presented in an 800×600 pixel resolution. The stimuli were projected onto a screen at the end of the scanner (visual field = $18°$) using an LCD projector. The two stimuli were counterbalanced as CS^+ across participants. Pictures were viewed through a mirror mounted on the head coil.

Unconditioned stimuli (UCS)

For the acquisition phase, a set of 20 highly aversive pictures (e.g. mutilations) were presented as UCS. These pictures were successfully used in previous studies, have been rated as highly aversive and have proven to elicit CRs (Libkuman *et al.*, 2007; Klucken *et al.*, 2009a). All pictures were presented in colour and had identical pixel resolution.

Conditioning procedure

The conditioning procedure contained an acquisition and a short extinction phase. Only data from the acquisition phase is discussed here. Subjects were instructed to pay attention to all stimuli and to figure out a possible contingency between the CS and the UCS (e.g. Schiller *et al.*, 2008; Schiller *et al.*, 2010). Prior to the experiment and immediately after the conditioning procedure, participants rated the CS^+, CS^-, and UCS. Detailed methods and results of the ratings are presented in the Supplementary Data. The acquisition phase consisted of 40 trials (20 per CS). The CS duration was 8 s. The UCS appeared immediately after the CS^+ (100% reinforcement) for 4 s. Two training trials were conducted but excluded from the analyses, since learning could not have yet occurred (Phelps *et al.*, 2004; Klucken *et al.* 2012).

For each subject, a pseudo randomized stimulus order was used with the following restrictions: (1) no more than two straight presentations of the same CS, and (2) equal distribution of CS presentations within the first and the second half of the acquisition. In an equally distributed interval of 1–2 s after the UCS offset, participants had to react to a simple distractor task (Goldin *et al.*, 2008; see Schweckendiek *et al.*, 2011 for a detailed description). This procedure was chosen to (1) distract the attention from the aversive pictures during the inter trial interval (ITI) and (2) to enhance overall vigilance. No group differences occurred between the four groups regarding the distractor task (false response rates were below 5% in all four groups). The ITI ranged from 12.5 s to 15 s. Throughout the experiment an MRI-compatible video camera was used to control if subjects watched the stimuli.

Skin conductance measuring

SCRs were sampled simultaneously with MR scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium, placed hypothenar at the non-dominant (left) hand. SCRs were defined in three analysis windows (Prokasy and Ebel, 1967): the maximum response within the time window 1–4 s after the CS (CS^+ or CS^-) onset was counted as the first interval response (FIR), within the time windows 4–8 s as the second interval response (SIR), and within the time window 9–13 s as the unconditioned response (third interval response; TIR). Responses were only registered when the response amplitude was greater than 0.01 µS. Three subjects (2 S′S′/high SLEs, 1 L′/high SLEs) did not show SCRs (no responses to the UCS) and were excluded from SCRs analyses. Statistical analyses were performed via analysis of variance (ANOVA) in a 2 (stimulus-type: CS^+ vs CS^-) × 2 (5-HTTLPR genotype: S′S′ vs L′ group) × 2 (environment: high vs low number of SLEs) experimental design followed by *post hoc* tests in PASW 18 (SPSS Inc., Chicago, IL, USA).

Magnetic resonance imaging

Imaging parameters

Functional and anatomical images were acquired with a 1.5 Tesla whole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition consisted of 160 T1-weighted sagittal images (MPRage, 1 mm slice thickness). For functional images, a total of 505 images were registered using a T2*-weighted gradient echo-planar imaging (EPI) sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice procedure; TR = 2.5 s; TE = 55 ms; flip angle = 90°; field of view 192 × 192 mm; matrix size = 64 × 64). The orientation of the axial slices was paralleled to the orbitofrontal cortex-bone transition in order to minimise susceptibility artefacts in prefrontal areas. Data were analysed using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London UK; 2008) implemented in MATLAB 7.5 (Mathworks Inc., Sherbourn, MA, USA).

Preprocessing and statistical analyses

Prior to all analyses, data were preprocessed. This included realignment and unwarping (b-spline interpolation), slice-time correction, coregistration of functional data to each participant's anatomical image, and normalization to the standard space of the Montreal Neurological Institute (MNI) brain. Spatial smoothing was executed with an isotropic three-dimensional Gaussian filter with a full width at half maximum of 9 mm to allow for corrected statistical inference. The modelled experimental conditions were CS^+, CS^-, UCS, non-UCS (defined as the time window after CS^- presentation corresponding to the time window of UCS presentation after the CS^+; e.g. Stark *et al.*, 2006; Tabbert *et al.*, 2011), the distractor task, and the button

presses, modelled as events. Regressors were convolved with a hemo-dynamic response function (hrf) in the general linear model (GLM). The six movement parameters of the rigid body transformation applied by the realignment procedure were introduced as covariates in the model. The voxel-based time series was filtered with a high pass filter (time constant = 128 s). We further conducted analyses to account for the potential problem of collinearity between the regressors, e.g. in order to ensure that brain activity to the CS^+ can be reliably distinguished from brain activity to the UCS. Results revealed no substantial collinearity between regressors (absolute values of cosine of angles between CS^+ and UCS were: .15; between CS^- and non-UCS: .14), comparable to other previous studies (e.g. Klucken *et al.*, 2009a).

On the first level of analysis, the following contrasts were analysed for each subject: $CS^+ > CS^-$ and UCS > non-UCS. The contrasts were calculated for each subject and introduced as dependent variables in the group analysis (second level analysis). We decided to use full-factorial models in order to avoid potentially biased type I errors in second level analyses due to the use of pooled errors (Boik, 1981; Barcikowski and Robey, 1984). Hence, contrasts from the first level GLM were analysed by full-factorial ANOVAs using partitioned errors (Penny and Henson, 2007). The full-factorial models included the group factors 5-HTTLPR genotype (S′S′ vs L′) and SLEs (low vs high number of SLEs) implemented in SPM8. In detail, four groups were introduced in the ANOVA (S′S′/high SLEs, S′S′/low SLEs, L′/high SLEs, L′/low SLEs). Main effects of 5-HTTLPR genotype, SLEs and interaction effects were analysed for each contrast (e.g. $CS^+ > CS^-$; UCS > non-UCS). Further, appropriate post hoc group comparisons were conducted to specify potential interaction effects. Whole-brain analyses were conducted for the contrast $CS^+ > CS^-$ ($P < 0.001$, uncorrected). Regions of interest (ROI) analyses were performed using the small volume correction in SPM8 ($P < 0.05$ family-wise-error (FWE) corrected; $k > 5$ voxel). The amygdala constitutes a target region, given that fear potentiated startle responses have been closely linked to this structure (Hamm and Weike, 2005; Weike *et al.*, 2005). Further ROIs derive from recent neuroimaging studies suggesting the 5-HTTLPR genotype to be associated with functional and/or structural alterations in other fear-relevant brain regions, comprising the insula, the thalamus and the occipital cortex (Rao *et al.*, 2007; Munafò *et al.*, 2008; Lemogne *et al.*, 2011). The masks for the amygdala, the insula and the thalamus were taken from the 'Harvard-Oxford cortical and subcortical structural atlases' provided by the Harvard Centre for Morphometric Analysis (Fox and Lancaster, 1994; Nielsen and Hansen, 2002). Since no mask for the occipital cortex is available in the Harvard–Oxford cortical and subcortical structural atlases, the mask for the primary visual cortex was taken from the probabilistic cytoarchitectonic maps and functional imaging data of the SPM anatomy toolbox (Eickhoff *et al.*, 2005). Since the masks were based on a probabilistic approach, a 50% cut-off was used for defining the ROI.

Genotyping

DNA was extracted from buccal cells using a standard commercial extraction kit (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany) in a MagNA Pure1 LC System (Roche). Subjects were genotyped for the 5-HTTLPR (and rs25531) by means of polymerase chain reaction (PCR) and gel electrophoresis. A detailed protocol is provided elsewhere (Alexander *et al.*, 2009).

RESULTS

Group characteristics

Within the original sample (Alexander *et al.*, 2009), there was no significant deviation from Hardy–Weinberg-Equilibrium using diallelic

$(x^2_{(1)} = 0.16; P > 0.60)$ or triallelic $(x^2_{(3)} = 30; P > 0.90)$ classification of 5-HTTLPR. Genotype frequencies in the preselected sample of the present fMRI study were $S/S = 21$, $S/L_G = 3$, and $L_G/L_G = 0$ for the S'S' group and $S/L_A = 11$, $L_A/L_G = 1$, $L_A/L_A = 11$ for the L' group. Due to the preselection, we achieved significant differences $(t_{(45)} = 7.54; P < 0.001)$ regarding the number of SLEs in the low $(M = 2.3, s.d. = 1.3)$ and the high $(M = 6.3, s.d. = 2.0)$ SLEs group. As intended, no significant differences between the S'S' and the L' group emerged regarding self-reported SLEs (S'S' group: $M = 3.9$; s.d. $= 2.5$; L' group: $M = 4.6$, s.d. $= 2.9$; $t_{(45)} = 0.9$; $P > 0.05$). Furthermore, the four groups depending on 5-HTTLPR genotype and SLEs did not differ with respect to age $(F(3,44) < 1; P > 0.05)$

UCS results

Skin conductance responses

ANOVA revealed a significant main effect of UCS-type $(F_{(1,41)} = 27.65; P < 0.001)$, which was not modulated by 5-HTTLPR genotype, SLEs or 5-HTTLPR × SLEs interaction. *Post hoc* tests confirmed greater responses to the UCS as compared to the non-UCS both in the S'S' group as well as in the L' group (Figure 1).

Hemodynamic responses

The results showed a significant main effect of task (UCS responses) in all ROIs, including the amygdala, the insula, the thalamus and the occipital cortex. No main effects of 5-HTTLPR genotype and SLEs or 5-HTTLPR × SLEs interactions were observed in the contrast UCS > non-UCS (Table 1).

CS results

Skin conductance responses

We found a significant main effect of CS-type in the FIR $(F_{(1,41)} = 15.70; P < 0.001)$ and in the SIR $(F_{(1,41)} = 20.77; P < 0.001)$. *Post hoc* tests revealed higher responses towards the CS$^+$ as compared to the CS$^-$, thus, providing evidence for successful fear conditioning in all groups. No main effect of 5-HTTLPR genotype, SLEs, or its interaction occurred with regard to conditioned SCRs (Figure 2).

Hemodynamic responses

Main effect of task

With respect to our regions of interest (all FWE-corrected), we found a significant main effect of task in the left thalamus $(z = 3.33; x/y/z = -9/-12/12; P < 0.05)$, the right thalamus $(z = 3.49; x/y/z = 12/-6/12; P < 0.05)$, and the right insula $(z = 3.17; x/y/z = 36/18/0; P < 0.05)$. Trends were found in the left $(z = 3.39; x/y/z = -6/-78/12; P = 0.075)$ and the right $(z = 3.34; x/y/z = 27/-66/9; P = 0.082)$ occipital cortex.

Main effects of 5-HTTLPR genotype and SLEs

Whole-brain analysis (all uncorr.) revealed CS$^+$/CS$^-$ differentiation in the middle frontal gyrus $(z = 3.91; P < 0.0001; x/y/z = -30/41/-15)$ and in the left lateral orbitofrontal cortex $(z = 3.52; P < 0.0001; x/y/z = -30/51/-15)$ in the L' group. In the S'S' group we additionally

Table 1 Neural activations for the main effect of UCS-type for the S'S' group and the L' group separately

Group	Structure	Side	x	y	z	Z_{max}	P_{corr}
S'S' group	Amygdala	L	−18	−6	−12	4.16	0.001
	Amygdala	R	21	−3	−15	3.37	0.010
	Insula	L	−33	21	0	4.60	<0.001
	Insula	R	33	24	−3	5.03	<0.001
	Thalamus	L	−9	−30	−3	5.06	<0.001
	Thalamus	R	6	−15	3	6.11	<0.001
	Occipital cortex	L	−12	−96	−3	6.52	<0.001
	Occipital cortex	R	21	−93	0	7.53	<0.001
L' group	Amygdala	L	−21	−6	−12	4.36	<0.001
	Amygdala	R	27	0	−15	4.26	<0.001
	Insula	L	−33	21	0	5.81	<0.001
	Insula	R	33	24	−3	5.31	<0.001
	Thalamus	L	−9	−30	−3	5.61	<0.001
	Thalamus	R	21	−30	0	6.01	<0.001
	Occipital cortex	L	−12	−99	−6	6.96	<0.001
	Occipital cortex	R	18	−93	0	7.51	<0.001

The threshold was $P < 0.05$ (FWE-corrected; small volume correction according to SPM8). All coordinates are given in MNI space. L: left hemisphere, R: right hemisphere.
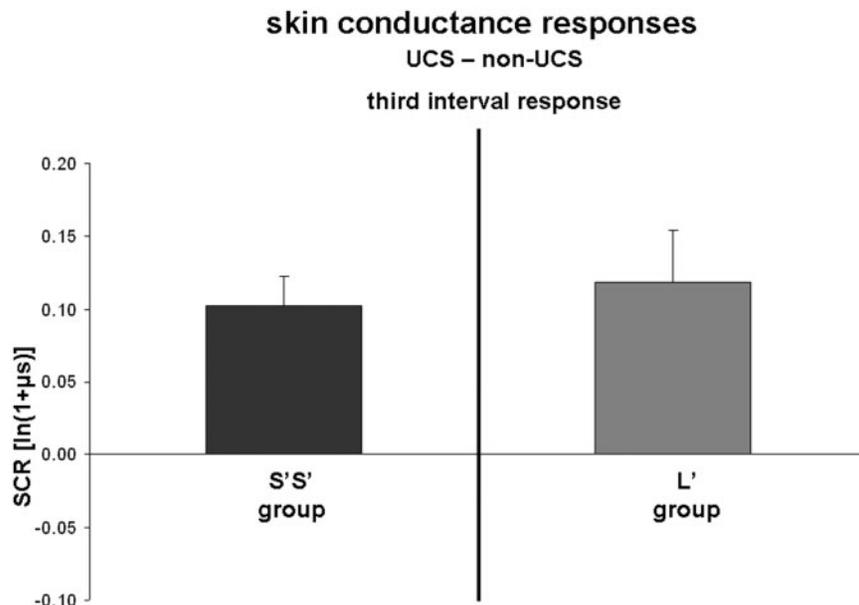
Fig. 1 Mean (± SE) conditioned skin conductance responses (UCS—non-UCS) for the S'S' group and the L' group in the TIR.
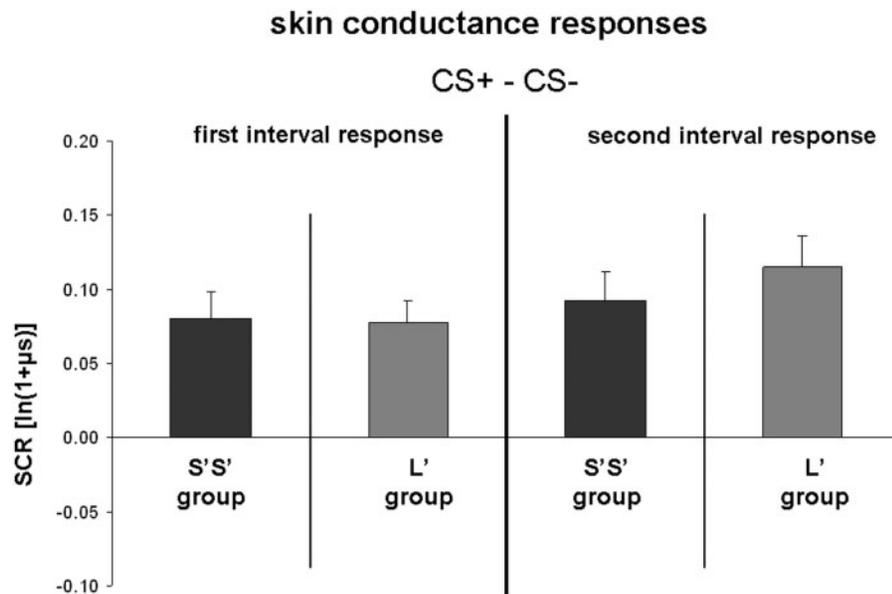
skin conductance responses

CS+ - CS-

Fig. 2 Mean (±SE) of the conditioned skin conductance responses (CS$^+$–CS$^-$) for the S'S' group and the L' group in the FIR and in the SIR.

Table 2 Neural activations for the main effect of 5-HTTLPR genotype (upper part), and the 5-HTTLPR genotype × SLEs interaction effects in the contrast CS$^+$ > CS$^-$ (lower part)

Analyses	Structure	Side	x	y	z	Z_{max}	P_{corr}
Main effect	Amygdala	R	21	0	−18	3.26	0.015
5-HTTLPR	Insula	L	−39	−3	−12	3.15	0.044
genotype S'S' > L'	Insula	R	36	9	−15	4.04	0.002
	Thalamus	L	−9	−27	−3	3.65	0.015
	Occipital cortex	L	−12	−78	6	3.65	0.031
	Occipital cortex	R	21	−96	−3	3.77	0.025
5-HTTLPR	Insula	R	36	0	12	3.37	0.023
genotype × SLEs interaction	Occipital cortex	L	−12	−78	3	3.60	0.036

The threshold was $P < 0.05$ (FWE-corrected; small volume correction; according to SPM8). All coordinates are given in MNI space. L: left hemisphere, R: right hemisphere.

found CS$^+$/CS$^-$ differences in the left precentral gyrus ($z = 6.4$; $P < 0.0001$; $x/y/z = −42/−3/30$) and in the cerebellum ($z = 5.9$; $P < 0.0001$; $x/y/z = −27/−57/−33$). Further, the S'S' group showed significant ROI-activations (all FWE-corrected) in the right amygdala ($z = 3.22$; $x/y/z = 18/−6/−12$; $P < 0.05$), the left insula ($z = 3.33$; $x/y/z = −39/−12/−3$; $P < 0.05$), the right insula ($z = 3.38$; $x/y/z = 39/9/−15$; $P < 0.05$), the left thalamus ($z = 3.93$; $x/y/z = −3/−24/3$; $P < 0.01$), the right thalamus ($z = 4.02$; $x/y/z = 9/−18/0$; $P < 0.01$), the left occipital cortex ($z = 3.59$; $x/y/z = −9/−81/6$; $P < 0.05$), and the right occipital cortex ($z = 3.73$; $x/y/z = 18/−93/−3$; $P < 0.05$).

We observed a significant main effect of 5-HTTLPR genotype in the contrast CS$^+$ > CS$^-$ for each ROI. According to our a priori hypothesis, *post-hoc* tests confirmed significantly greater differentiation in the contrast CS$^+$ > CS$^-$ in the S'S' group as compared to the L' group in the right amygdala, the insula (bilateral), the occipital cortex (bilateral) and the left thalamus (see Table 2; Figure 3 for details). No main effect for SLEs occurred.

Gene x environment interaction

We observed a significant 5-HTTLPR genotype x SLEs interaction effect in the right insula and the left occipital cortex for the contrast CS$^+$ > CS$^-$ (Table 2, Figure 4). According to the a priori hypothesized direction of effects, *post hoc* testing revealed that the S'S'/high SLEs group showed significantly elevated neural responses in the left occipital cortex as compared to the L'/high SLEs ($P = 0.016$) and the S'S'/low SLEs group ($P = 0.019$); whereas no significant difference occurred compared to the L'/low SLEs group ($P > 0.05$). Regarding the right insula activity, *post hoc* tests-tests revealed a similar pattern with S'S'/high SLEs subjects showing highest activity. Specifically, elevated insula activity in the contrast CS$^+$ > CS$^-$ reached significance when comparing the S'S'/high SLEs to the S'S'/low SLEs ($P = 0.011$), to the L'/high SLEs group ($P = 0.002$), and to the L'/low SLEs group ($P = 0.018$). Pairwise comparisons did not reveal any significant differences between the remaining groups (all $P > 0.05$).

DISCUSSION

The present study is the first to investigate neural correlates of fear conditioning as a function of 5-HTTLPR genotype and SLEs. Our results indicate a significant association between 5-HTTLPR and BOLD-responses during fear conditioning in the right amygdala, the left thalamus, the bilateral insula and the bilateral occipital cortex. As hypothesized, S'S' subjects showed increased CS$^+$/CS$^-$ differentiation within these fear-related structures compared to the L' group. Thus, the present study extends previous findings on peripheral physiological measures of CR by linking 5-HTTLPR genotype to individual differences in neural correlates of fear conditioning. Furthermore, our results point to a potential neural mechanism explaining why 5-HTTLPR genotype has been differentially associated with conditioned startle and skin conductance responses. In the study by Lonsdorf and colleagues (2009), S allele carriers showed increased startle potentiation, whereas consistent with our own findings, no such 5-HTTLPR genotype dependent differences appeared with regard to conditioned SCRs. A possible explanation for this selective effect is provided by studies suggesting conditioned startle and skin conductance responses to at least partly involve different neural circuits (Hamm and Weike, 2005; Tabbert *et al.*, 2006). Whereas startle modulation is primarily mediated by connections from the amygdala to the brainstem (Davis and Whalen, 2001; Hamm and Weike, 2005), conditioned SCRs have
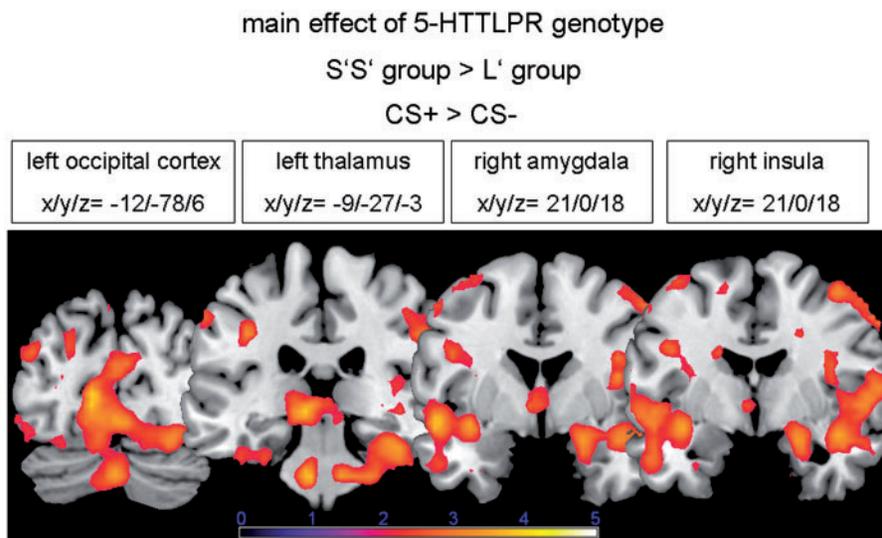
main effect of 5-HTTLPR genotype

S'S' group > L' group

CS+ > CS-

left occipital cortex	left thalamus	right amygdala	right insula
x/y/z= -12/-78/6	x/y/z= -9/-27/-3	x/y/z= 21/0/18	x/y/z= 21/0/18

Fig. 3 Neural activations for the main effect of 5-HTTLPR genotype in the contrast $CS^+ > CS^-$ in the region of interests (also see Table 2). For illustration reasons, the data were thresholded with a T 2.0 (see colour bar for exact t-values).

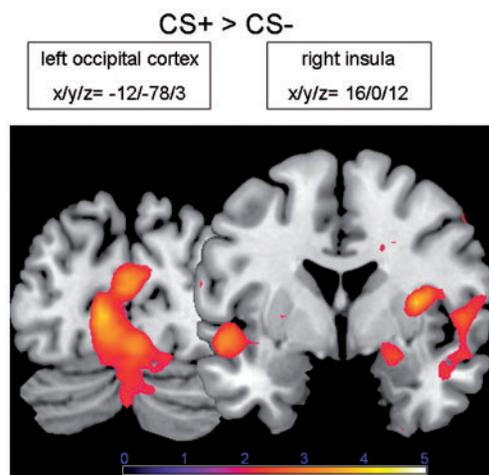**5-HTTLPR genotype x environment interaction effect**

CS+ > CS-

left occipital cortex	right insula
x/y/z= -12/-78/3	x/y/z= 16/0/12

Fig. 4 Neural activations for the 5-HTTLPR genotype × SLEs interaction effect in the contrast $CS^+ > CS^-$ in the region of interests (also see Table 2). For illustration reasons, the data were thresholded with a T 2.0 (see colour bar for exact t-values).

been reported to be largely dissociated from amygdala activity (Weike et al., 2005; Tabbert et al., 2006; Klucken et al., 2009b). Thus, the observation of altered amygdala reactivity to the CS^+ as a function of 5-HTTLPR raises the notion that this genetic variant may predominantly modulate amygdala-dependent CR. However, it has to be acknowledged that associations between 5-HTTLPR and SCRs have been observed in other studies. For example, Crişan et al. (2009) conducted a conditioning paradigm (observational learning) and found increased SCRs towards the CS^+ in S allele carriers. These differences in design and statistical analyses (cf. Garpenstrand et al., 2001) might explain the inconsistencies.

Moreover, the presented results may relate to the consistently reported finding of elevated amygdala reactivity to fearful stimuli in carriers of the 5-HTTLPR S allele (Hariri et al., 2002; Hariri et al., 2005; Munafò et al., 2008). The ability to respond in a fast and adaptive

manner when confronted with an aversive stimulus has been suggested to be a key function of fear conditioning (Domjan, 2005). Thus, it is tempting to speculate that amygdala hyperreactivity observed as a function of 5-HTTLPR genotype may at least partly result from increased fear learning in the past. Even though this potential mechanism is supported by our data, only longitudinal studies will provide insights into the causal relationship between altered fear conditioning and processing in 5-HTTLPR S′ allele carriers.

Furthermore, our study provides only preliminary evidence for a significant 5-HTTLPR genotype × SLEs interaction on neural correlates of fear conditioning, given that effects were not consistently observed across crucial structures of the fear-network. In accordance with the a priori hypothesized direction of effects, the S′S′/high SLEs group appeared to be most reactive, as indicated by increased neural activation to the CS^+ as compared to the CS^- in the insula and the occipital cortex. However, the fact that SLEs were found to selectively moderate the association between 5-HTTLPR and reactivity to the CS^+ as compared to the CS^- in specific brain areas seems unexpected at first and needs further discussion. Besides the amygdala, increased insula activation to the CS^+ is one of the major and most stable results reported in fMRI studies on fear conditioning (Sehlmeyer et al., 2009). Notably, it has been suggested that the insula is not only involved in interoceptive bodily awareness, but also modulates the evaluation of future emotional states (Nitschke et al., 2005; Paulus and Stein, 2006) by conveying a cortical representation of fear to the amygdala (Phelps et al., 2001). Following this line of argumentation, it could be speculated that the effects of SLEs are most pronounced within brain areas crucially involved in fear representations, which have been acquired on the basis of (aversive) experiences in the past. A similar interpretation may apply for the observation of elevated neural reactivity to the CS^+ compared to the CS^- within the occipital cortex in S′S′/high SLEs subjects. Enhanced occipital activation has not solely been observed during acute presentation of emotional material, but also occurs during anticipation of aversive stimuli and has often been referred to as increased motivated attention (Bradley et al., 2003; Ueda et al., 2003). These findings support the assumption that activation within the occipital cortex is not only stimulus driven but at least partly results from altered top-down processes, which may be influenced by environmental adversity in the past.

Moreover, it is interesting to note that S′S′/high SLEs subjects were not only characterized by an increased neural activity to the CS$^+$, but were also found to exhibit markedly elevated cortisol reactivity to psychosocial stress in our previous study (Alexander et al., 2009). Taken together, these findings suggest a broad network of neural and endocrine alterations associated with stress/threat sensitivity within subjects at high risk for psychopathology. Importantly, changes in cortisol reactivity and fear conditioning may very likely reflect interrelated biological processes, given the crucial role of glucocorticoids in fear learning (for review, see Rodrigues et al., 2009). More precisely, several studies indicate stress exposure to significantly alter subsequent fear acquisition in a sex-specific manner. In males, previous exposure to a psychosocial stressor as well as elevated endogen release of glucocorticoids has been repeatedly associated with facilitated fear conditioning, while no such association or the opposite pattern has been observed in women (Jackson et al., 2006; Zorawski et al., 2006 but see Stark et al., 2006; Merz et al., 2010). The latter findings are thus consistent with the coexistent observation of elevated cortisol reactivity (Alexander et al., 2009) and increased neural activation to the CS$^+$ observed within the present study in male S′S′/high SLEs subjects, but also imply that obtained effects may not be generalized to a female population.

Regarding potential clinical implications, our findings add to the discussion whether neural correlates of fear conditioning represent a potential intermediate process bridging the gap from 5-HTTLPR genotype to anxiety-related phenotypes. Elevated levels of anxiety-related personality traits in S allele carriers represent a well-established finding in genetic association studies on 5-HTTLPR, which has been confirmed by several independent meta-analyses (e.g. Munafo et al., 2005; Munafò et al., 2009). As suggested by our findings, enhanced neural reactivity in fear-related brain circuits can be discussed as a potential mechanism mediating this association. Indeed, a recent neuroimaging study reports trait anxiety to be positively correlated with the magnitude of amygdala reactivity to the CS$^+$ (Indovina et al., 2011), once again highlighting the potential role of altered fear conditioning as a possible intermediate phenotype. Moreover, increased neural reactivity to the CS$^+$ as compared to the CS$^-$ in healthy S′S′ subjects observed in our study may represent a premorbid risk factor. In line with this argumentation, a recent longitudinal study identified neuroticism to significantly mediate the relationship between genetic risk factors and symptoms of anxiety and depression (Kendler and Gardner, 2011). Taken together, altered neural responding to the CS$^+$ can be discussed as a possible mechanism conveying elevated levels of trait-anxiety in healthy S′S′ subjects, thereby possibly increasing vulnerability to anxiety and affective disorders. Regarding the moderating role of SLEs, it is of note that evidence for significant 5-HTTLPR × SLEs interaction is not limited to depression susceptibility (Uher and McGuffin, 2008; Caspi et al., 2010), but has also been reported with regard to anxiety-related personality traits (Stein et al., 2008). Consistent with our findings, S′S′/high SLEs were found to exhibit elevated levels of anxiety sensitivity (AS) (Stein et al., 2008), a well-established risk factor for the development of anxiety disorders (Lang et al., 2002; Schmidt et al., 1999). Again, elevated neural fear reactivity within this group suggested by our study may account for this association, given that increased insula activation during the processing of aversive stimuli was also found in subjects with elevated levels of AS (Stein et al., 2007).

In conclusion, our study is the first to report significant variation in neural correlates of fear learning as a function of 5-HTTLPR and SLEs. While associations of 5-HTTLPR genotype and altered reactivity to the CS$^+$ as compared to the CS$^-$ robustly appeared in key structures of the fear network, evidence for a significant GxE interaction is much weaker. Given the limited sample size, our results remain preliminary until independent replication is available, especially with respect to findings on 5-HTTLPR × SLEs interaction. Beside this limitation, our study significantly contributes to the current debate on the role of 5-HTTLPR and SLEs on psychiatric disorders by investigating alterations on an intermediate phenotype level, which may represent a premorbid risk factor that conveys elevated susceptibility for the development of psychiatric disorders.

SUPPLEMENTARY DATA

Supplementary data are available at SCAN online.

Conflict of Interest

None declared.

REFERENCES

Alexander, N., Kuepper, Y., Schmitz, A., Osinsky, R., Kozyra, E., Hennig, J. (2009). Gene-environment interactions predict cortisol responses after acute stress: implications for the etiology of depression. Psychoneuroendocrinology, 34(9), 1294–303.

Almada, R.C., Borelli, K.G., Albrechet-Souza, L., Brandão, M.L. (2009). Serotonergic mechanisms of the median raphe nucleus-dorsal hippocampus in conditioned fear: Output circuit involves the prefrontal cortex and amygdala. Behavioural brain research, 203(2), 279–87.

Barcikowski, R.S., Robey, R.R. (1984). Decisions in single group repeated measures analysis: Statistical tests and three computer packages. The American Statistician, 38(2), 148–50.

Bertolino, A., Arciero, G., Rubino, V., et al. (2005). Variation of human amygdala response during threatening stimuli as a function of 5′HTTLPR genotype and personality style. Biological Psychiatry, 57(12), 1517–25.

Boik, R.J. (1981). A priori tests in repeated measures designs: Effects of nonsphericity. Psychometrika, 46, 241–55.

Bradley, M.M., Sabatinelli, D., Lang, P.J., Fitzsimmons, J.R., King, W., Desai, P. (2003). Activation of the visual cortex in motivated attention. Behavioral Neuroscience, 117(2), 369–80.

Burghardt, N.S., Bush, D.E.A., McEwen, B.S., LeDoux, J.E. (2007). Acute selective serotonin reuptake inhibitors increase conditioned fear expression: blockade with a 5-HT(2C) receptor antagonist. Biological Psychiatry, 62(10), 1111–8.

Canli, T., Qiu, M., Omura, K., et al. (2006). Neural correlates of epigenesis. Proceedings of the National Academy of Sciences of the United States of America, 103(43), 16033–8.

Caspi, A., Sugden, K., Moffitt, T.E., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science, 301(5631), 386–9.

Caspi, A., Hariri, A.R., Holmes, A., Uher, R., Moffitt, T.E. (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. American Journal of Psychiatry, 167(5), 509–27.

Crişan, L.G., Pana, S., Vulturar, R., et al. (2009). Genetic contributions of the serotonin transporter to social learning of fear and economic decision making. Social cognitive and affective neuroscience, 4(4), 399–408.

Davis, M., Whalen, P.J. (2001). The amygdala: vigilance and emotion. Molecular Psychiatry, 6(1), 13–34.

De Houwer, J. (2009). The propositional approach to associative learning as an alternative for association formation models. Learning and Behavior, 37(1), 1–20.

Delgado, M.R., Olsson, A., Phelps, E.A. (2006). Extending animal models of fear conditioning to humans. Biological Psychology, 73(1), 39–48.

Domjan, M. (2005). Pavlovian conditioning: a functional perspective. Annual Review of Psychology, 56, 179–206.

Dougherty, L.R., Klein, D.N., Congdon, E., Canli, T., Hayden, E.P. (2010). Interaction between 5-HTTLPR and BDNF Val66Met polymorphisms on HPA axis reactivity in preschoolers. Biological Psychology, 83(2), 93–100.

Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage, 4(25), 1325–35.

Fox, P.T., Lancaster, J.L. (1994). Neuroscience on the net. Science, 266(5187), 994–6.

Garpenstrand, H., Annas, P., Ekblom, J., Oreland, L., Fredrikson, M. (2001). Human fear conditioning is related to dopaminergic and serotonergic biological markers. Behavioral Neuroscience, 115(2), 358–64.

Goldin, P.R., McRae, K., Ramel, W., Gross, J.J. (2008). The neural bases of emotion regulation: reappraisal and suppression of negative emotion. Biological Psychiatry, 63(6), 577–86.

Gotlib, I.H., Joormann, J., Minor, K.L., Hallmayer, J. (2008). HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. Biological Psychiatry, 63(9), 847–51.

Gray, M.J., Litz, B.T., Hsu, J.L., Lombardo, T.W. (2004). Psychometric properties of the life events checklist. Assessment, 11(4), 330–41.

Hamm, A.O., Weike, A.I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology*, 57(1), 5–14.

Hamm, A.O., Weike, A.I., Schupp, H.T., Treig, T., Dressel, A., Kessler, C. (2003). Affective blindsight: intact fear conditioning to a visual cue in a cortically blind patient. *Brain*, 126(2), 267–75.

Hariri, A.R., Drabant, E.M., Munoz, K.E., et al. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry*, 62(2), 146–52.

Hariri, A.R., Mattay, V.S., Tessitore, A., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science*, 297(5580), 400–3.

Hettema, J.M., Annas, P., Neale, M.C., Kendler, K.S., Fredrikson, M. (2003). A twin study of the genetics of fear conditioning. *Archives of General Psychiatry*, 60(7), 702–8.

Hu, X.-Z., Lipsky, R.H., Zhu, G., et al. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Molecular Psychiatry*, 78(5), 815–26.

Indovina, I., Robbins, T.W., Núñez-Elizalde, A.O., Dunn, B.D., Bishop, S.J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*, 69(3), 563–71.

Inoue, T., Li, X.B., Abekawa, T., et al. (2004). Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. *European Journal of Pharmacology*, 497(3), 311–6.

Jackson, E.D., Payne, J.D., Nadel, L., Jacobs, W.J. (2006). Stress differentially modulates fear conditioning in healthy men and women. *Biological Psychiatry*, 59(6), 516–22.

Karg, K., Burmeister, M., Shedden, K., Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Archives of General Psychiatry*, 68(5), 444–54.

Kendler, K.S., Gardner, C.O. (2011). A longitudinal etiologic model for symptoms of anxiety and depression in women. *Psychological Medicine*, 41(10), 2035–45.

Klucken, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., Stark, R. (2009a). Neural, electrodermal and behavioral response patterns in contingency aware and unaware subjects during a picture–picture conditioning paradigm. *Neuroscience*, 158(2), 721–31.

Klucken, T., Schweckendiek, J., Koppe, G., Merz, C.J., Kagerer, S., Walter, B., et al. (2012). Neural correlates of disgust- and fear-conditioned responses. *Neuroscience*, 201, 209–18.

Klucken, T., Schweckendiek, J., Merz, C.J., et al. (2009b). Neural activations of the acquisition of conditioned sexual arousal: effects of contingency awareness and sex. *The Journal of Sexual Medicine*, 6(11), 3071–85.

Lang, A.J., Kennedy, C.M., Stein, M.B. (2002). Anxiety sensitivity and PTSD among female victims of intimate partner violence. *Depression and Anxiety*, 16(2), 77–83.

Lemogne, C., Gorwood, P., Boni, C., Pessiglione, M., Lehéricy, S., Fossati, P. (2011). Cognitive appraisal and life stress moderate the effects of the 5-HTTLPR polymorphism on amygdala reactivity. *Human Brain Mapping*, 32(11), 1856–67.

Lesch, K.P., Bengel, D., Heils, A., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274(5292), 1527–31.

Libkuman, T.M., Otani, H., Kern, R., Viger, S.G., Novak, N. (2007). Multidimensional normative ratings for the International Affective Picture System. *Behavior Research Methods*, 39(2), 326–34.

Lonsdorf, T.B., Weike, A.I., Nikamo, P., Schalling, M., Hamm, A.O., Ohman, A. (2009). Genetic gating of human fear learning and extinction: possible implications for gene-environment interaction in anxiety disorder. *Psychological Science*, 20(2), 198–206.

Margraf, J. (1994). *Mini-DIPS: Diagnostisches Kurz-Interview bei psychischen Störungen.* Berlin: Springer.

Merz, C.J., Tabbert, K., Schweckendiek, J., et al. (2010). Investigating the impact of sex and cortisol on implicit fear conditioning with fMRI. *Psychoneuroendocrinology*, 35(1), 33–46.

Mueller, A., Brocke, B., Fries, E., Lesch, K.P., Kirschbaum, C. (2010). The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology*, 35(2), 289–96.

Munafo, M.R., Clark, T., Flint, J. (2005). Does measurement instrument moderate the association between the serotonin transporter gene and anxiety-related personality traits? A meta-analysis. *Molecular psychiatry*, 10(4), 415–9.

Munafò, M.R., Brown, S.M., Hariri, A.R. (2008). Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological Psychiatry*, 63(9), 852–7.

Munafò, M.R., Durrant, C., Lewis, G., Flint, J. (2009). Gene X environment interactions at the serotonin transporter locus. *Biological Psychiatry*, 65(3), 211–9.

Nakamura, M., Ueno, S., Sano, A., Tanabe, H. (2000). The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry*, 5(1), 32–8.

Nielsen, F., Hansen, L.K. (2002). Automatic anatomical labeling of Talairach coordinates and generation of volumes of interest via the BrainMap database. *NeuroImage*, 16(2) Presented at the 8th International Conference on Functional Mapping of the Human Brain. Available on CD-Rom. Available at: http://www2.imm.dtu.dk/~fn/ps/Nielsen2002Automatic_poster.ps.gz (date last accessed 28 January 2012).

Nitschke, J.B., Sarinopoulos, I., Mackiewicz, K.L., Schaefer, H.S., Davidson, R.J. (2005). Functional neuroanatomy of aversion and its anticipation. *NeuroImage*, 29(1), 106–16.

Öhman, A., Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review*, 108(3), 483–522.

Paulus, M.P., Stein, M.B. (2006). An insular view of anxiety. *Biological Psychiatry*, 60(4), 383–7.

Penny, W.D., Henson, R. (2007). Analysis of variance. In: Friston, K.J., Ashburner, J.T., Kiebel, S., Nichols, T., Penny, W.D., editors. *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. Amsterdam: Elsevier Acad. Press, pp. 166–78.

Phelps, E.A., O'Connor, K.J., Gatenby, J.C., Gore, J.C., Grillon, C., Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience*, 4(4), 437–41.

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(6), 897–905.

Praschak-Rieder, N., Kennedy, J., Wilson, A.A., et al. (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [(11)C] DASB positron emission tomography study. *Biological Psychiatry*, 62(4), 327–31.

Prokasy, W.F., Ebel, H.C. (1967). Three components of the classically conditioned GSR in human subjects. *Journal of Experimental Psychology*, 73, 247–56.

Rao, H., Gillihan, S.J., Wang, J., et al. (2007). Genetic variation in serotonin transporter alters resting brain function in healthy individuals. *Biological Psychiatry*, 62(6), 600–6.

Risch, N., Herrell, R., Lehner, T., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *The journal of the American Medical Association*, 301(23), 2462–71.

Rodrigues, S.M., LeDoux, J.E., Sapolsky, R.M. (2009). The influence of stress hormones on fear circuitry. *Annual Review of Neuroscience*, 32, 289–313.

Schiller, D., Levy, I., Niv, Y., LeDoux, J.E., Phelps, E.A. (2008). From fear to safety and back: reversal of fear in the human brain. *Journal of Neuroscience*, 28(45), 11517–25.

Schiller, D., Monfils, M.-H., Raio, C.M., Johnson, D.C., LeDoux, J.E., Phelps, E.A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature*, 463(7277), 49–53.

Schmidt, N.B., Lerew, D.R., Jackson, R.J. (1999). Prospective evaluation of anxiety sensitivity in the pathogenesis of panic: replication and extension. *Journal of Abnormal Psychology*, 108(3), 532–7.

Schweckendiek, J., Klucken, T., Merz, C.J., et al. (2011). Weaving the (neuronal) web: fear learning in spider phobia. *NeuroImage*, 54(1), 681–8.

Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., Konrad, C. (2009). Human fear conditioning and extinction in neuroimaging: a systematic review. *PloS one*, 4(6), e5865.

Shin, L.M., Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, 35(1), 169–91.

Stark, R., Wolf, O.T., Tabbert, K., et al. (2006). Influence of the stress hormone cortisol on fear conditioning in humans: evidence for sex differences in the response of the prefrontal cortex. *NeuroImage*, 32(3), 1290–8.

Stein, M.B., Schork, N.J., Gelernter, J. (2008). Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology*, 33(2), 312–9.

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), 318–27.

Stoltenberg, S.F., Twitchell, G.R., Hanna, G.L., et al. (2002). Serotonin transporter promoter polymorphism, peripheral indexes of serotonin function, and personality measures in families with alcoholism. *American Journal of Medical Genetics*, 114(2), 230–4.

Tabbert, K., Merz, C.J., Klucken, T., Schweckendiek, J., Vaitl, D., Wolf, O.T., Stark, R. (2011). Influence of contingency awareness on neural, electrodermal and evaluative responses during fear conditioning. *Social Cognitive and Affective Neuroscience*, 6(4), 496–506.

Tabbert, K., Stark, R., Kirsch, P., Vaitl, D. (2006). Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *NeuroImage*, 32(2), 761–70.

Ueda, K., Okamoto, Y., Okada, G., Yamashita, H., Hori, T., Yamawaki, S. (2003). Brain activity during expectancy of emotional stimuli: an fMRI study. *Neuroreport*, 14(1), 51–5.

Uher, R., McGuffin, P. (2008). The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Molecular Psychiatry*, 13(2), 131–46.

Way, B.M., Taylor, S.E. (2010). The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. *Biological Psychiatry*, 67(5), 487–92.

Weike, A.I., Hamm, A.O., Schupp, H.T., Runge, U., Schroeder, H.W.S., Kessler, C. (2005). Fear conditioning following unilateral temporal lobectomy: dissociation of conditioned startle potentiation and autonomic learning. *Journal of Neuroscience*, 25(48), 11117–24.

Williams, L.M., Gatt, J.M., Schofield, P.R., Olivieri, G., Peduto, A., Gordon, E. (2009). 'Negativity bias' in risk for depression and anxiety: brain-body fear circuitry correlates, 5-HTTLPR and early life stress. *NeuroImage*, 47(3), 804–14.

Zorawski, M., Blanding, N.Q., Kuhn, C.M., LaBar, K.S. (2006). Effects of stress and sex on acquisition and consolidation of human fear conditioning. *Learning and Memory*, 13(4), 441–50.