

# Neural correlates of trait anxiety in fear extinction

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**Background.** Fear conditioning involves the amygdala as the main neural structure for learning fear responses whereas fear extinction mainly activates the inhibitory prefrontal cortex (PFC). In this study we investigated whether individual differences in trait anxiety affect amygdala and dorsal anterior cingulate cortex (dACC) activation during fear conditioning and extinction.

**Method.** Thirty-two healthy subjects were investigated by functional magnetic resonance imaging (fMRI) at 3 T while performing a cued fear-conditioning task. All participants completed the trait version of the State-Trait Anxiety Inventory (STAI-T). Activations of the amygdala and the dACC were examined with respect to the effects of trait anxiety.

**Results.** Analysis of the fMRI data demonstrated enhanced activation in fear-related brain areas, such as the insula and the ACC, during both fear conditioning and extinction. Activation of the amygdala appeared only during the late acquisition phase whereas deactivation was observed during extinction. Regression analyses revealed that highly trait-anxious subjects exhibited sustained amygdala activation and reduced dACC involvement during the extinction of conditioned responses.

**Conclusions.** This study reveals that high levels of trait anxiety are associated with both increased amygdala activation and reduced dACC recruitment during the extinction of conditioned fear. This hyper-responsivity of the amygdala and the deficient cognitive control during the extinction of conditioned fear in anxious subjects reflect an increased resistance to extinct fear responses and may thereby enhance the vulnerability to developing anxiety disorders.

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## Introduction

Fear conditioning is vital for the detection of danger, the initiation of self-protection mechanisms and the survival of a species. The term ‘conditioning’ refers to the process of learning the association between two previously unrelated stimuli (Pavlov, 1927). In a typical differential fear-conditioning design, a previously neutral stimulus (CS+) is associated with an aversive and fear-inducing unconditioned stimulus (US) and becomes intrinsically aversive, while another neutral stimulus remains unpaired (CS−) (Maren, 2001). ‘Extinction’ is defined as the repeated exposure of

the originally neutral stimulus without presenting the aversive stimulus, which gradually eliminates the learned fear reaction (Myers & Davis, 2002).

Fear conditioning has proven to be an extremely robust and rapid experimental approach for studying the neurobiological substrates of fear and anxiety in animals and humans (Lissek *et al.* 2005; Anderson & Insel, 2006). Numerous neuroimaging studies have revealed a core neural network involved in conditioning and extinction, consisting of the amygdala, insula and anterior cingulate cortex (ACC) (for review see Sehmeyer *et al.* 2009). Thus, amygdala activity is associated with variability in the individual fear-conditioning and fear-extinction response. Research on humans and animals has highlighted the medial prefrontal cortex (PFC) and especially the dorsal ACC (dACC) as the principal structures of the brain’s extinction circuitry (Morgan *et al.* 1993; Quirk *et al.* 2003;

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Phelps *et al.* 2004; Lang *et al.* 2009). These areas regulate the expression of fear by inhibiting the amygdala, such that the fear-conditioned stimulus is prevented from causing a conditioned fear response (Gottfried & Dolan, 2004; Phelps *et al.* 2004; Quirk *et al.* 2006; Sotres-Bayon *et al.* 2006).

Psychiatric disorders associated with increased anxiety and fear levels, such as post-traumatic stress disorder, phobias or panic disorder, exemplify how misguided fear conditioning might render originally innocuous stimuli fear-inducing and threatening. Facilitated fear conditioning and impaired extinction of acquired fear are also core symptoms of anxiety disorders (Blecher *et al.* 2007; Michael *et al.* 2007). Accordingly, fear extinction represents the main therapeutic ingredient of exposure-based psychotherapies (Myers & Davis, 2002; Lissek *et al.* 2005; Anderson & Insel, 2006).

Anxiety-related personality traits, such as trait anxiety, which are regarded as stable and biological predispositions (Pujol *et al.* 2002; Omura *et al.* 2005; Rauch *et al.* 2005; Most *et al.* 2006), are closely related to pathological anxiety (Schmidt *et al.* 2008) and may influence the risk of psychiatric disorders (Bienvenu *et al.* 2001). Trait anxiety reflects an individual's general disposition to experience anxiety-relevant feelings or thoughts or to show anxiety-related behaviors (Spielberger, 1979). Trait anxiety is a stable personality trait describing the tendency to respond fearfully to a wide variety of unspecific stressors (Spielberger, 1972) and is regarded as a risk factor for anxiety disorders (Chambers *et al.* 2004). Highly trait-anxious subjects tend to perceive more situations as threatening and experience more frequently intense and sustained anxiety states compared to subjects with low trait anxiety (Spielberger, 1972, 1979, 1983; Mathews & MacLeod, 2005). It is assumed that anxiety-related personality factors are also associated with enhanced conditionability or impaired extinction of learned fear (Hooker *et al.* 2008; Barrett & Armony, 2009). However, the literature on this topic is equivocal (Pineles *et al.* 2009).

An overactive neuronal fear circuitry and reduced recruitment of prefrontal control have been proposed as neural correlates of facilitated fear conditioning and reduced extinction (Bishop *et al.* 2004; Bishop, 2007, 2009; Haas *et al.* 2007; Hooker *et al.* 2008). It has been shown, for example, that high trait anxiety is related to amygdala dysregulation during the processing of aversive and neutral stimuli in healthy volunteers (Bishop *et al.* 2004; Etkin *et al.* 2004; Dickie & Armony, 2008; Kienast *et al.* 2008; Mujica-Parodi *et al.* 2009) or even during the extinction of conditioned fear (Barrett & Armony, 2009). Moreover, anxiety disorders are associated with hypo-activation of the dACC during the regulation of emotions (Etkin & Wager, 2007). As the

relationships between anxiety-related personality traits and increased neurobiological vulnerability for fear acquisition and extinction are not fully understood, they are of specific interest in the current study.

We used event-related functional magnetic resonance imaging (fMRI) to clarify the relationship between amygdala reactivity, the involvement of the medial PFC, especially the dACC, and trait anxiety during the acquisition and extinction of conditioned fear in healthy subjects. We hypothesized that highly trait-anxious subjects exhibit enhanced fear conditioning and reduced extinction of conditioned responses, reflected by the amounts of amygdala and dACC activation.

## Method

### Subjects

Thirty-two healthy volunteers (12 males, 20 females; mean age 23.6 years, *s.d.* = 4.41, range 19–39 years) participated in the study. All were right-handed (Edinburgh Handedness Inventory; Oldfield, 1971) and were recruited by notice or advertisement in the local press. Exclusion criteria were medical, neurological and psychiatric diseases or MRI contraindications. No family history of mental illness or hereditary neurological disorders in first-degree relatives was reported. All participants gave written informed consent in accordance with the guidelines of the ethical standards of the Declaration of Helsinki. All procedures were approved by the local Ethical Review Board. On the day of scanning, participants completed the trait version of the German State-Trait Anxiety Inventory (STAI-T; Laux *et al.* 1981), a self-report scale to determine the level of trait anxiety. To ensure that the top of the range of trait anxiety scores did not represent individuals with undiagnosed anxiety disorders, standardized clinical assessment with the German version of the Structured Clinical Interview (SCID-I) was performed according to DSM-IV criteria (Wittchen *et al.* 1997). No evidence of anxiety disorders or any other current or previous axis I psychiatric disorder was found.

### Experimental paradigm

In a differential conditioning paradigm, pictures of two different neutral male faces selected from the MacArthur–McDonnell face library (NimStim; Tottenham *et al.* 2002) served as conditioned stimuli (CS–, CS+). Stimuli were gray in color, presented for 2 s in the centre of a black screen. A pseudo-randomized order was used with the restrictions that (a) no more than two successive presentations of the

same CS would occur and (b) the CSs were equally distributed within each half of the acquisition period. The two faces were counterbalanced as CS+ between participants. The US consisted of an acoustic white noise burst (duration 100 ms, 95 dB). The experiment was divided into four phases. During habituation, each CS was shown five times without US. Each of the two acquisition phases consisted of 15 CS−, 15 CS+ without US (CS+<sub>unpaired</sub>) and five CS+ with US (CS+<sub>paired</sub>) trials. In this 25% partial reinforcement schedule, the US co-terminated with the presentation of the CS+<sub>paired</sub> (delay conditioning). Subjects were not informed about this CS–US contingency. In the following extinction phase, 25 CS+<sub>unpaired</sub> and 25 CS− trials were presented. Interstimulus intervals ranged from 8.5 to 14.5 s, during which subjects had to look at a white fixation cross on a black screen. After each experimental phase, participants rated the valence and arousal of the CSs by means of a five-point Lickert scale, the Self-Assessment Manikin (SAM; Bradley & Lang, 1994), ranging from 0 = ‘very unpleasant’ to 4 = ‘very pleasant’ and 0 = ‘not arousing’ to 4 = ‘very arousing’. They responded by pressing the response buttons of an MRI-compatible response box with the right index and middle fingers. Prior to scanning, detailed task instructions were given and participants were familiarized with the task. Post-experimentally, participants were debriefed.

### Image acquisition

MRI data were acquired in a 3-T whole-body scanner (Gyrosan Intera T 3.0, Philips, The Netherlands), equipped with master gradients (nominal gradient strength 30 mT/m, maximal slew rate 150 mT/m/ms). A circularly polarized transmit/receive birdcage head coil with a high-frequency (HF) reflecting screen at the cranial end of the scanner was used for spin excitation and resonance signal acquisition. Functional images were acquired during one fMRI run using a T2\*-weighted single shot echo planar (EPI) sequence [echo time (TE) 32 ms, repetition time (TR) 2000 ms, flip angle 90°, slice thickness 3.6 mm without gap, matrix 64 × 64, field of view (FOV) 230 mm × 230 mm, in-plane resolution 3.6 mm × 3.6 mm]. In total, 825 image volumes of 30 transversal slices orientated parallel to the AC–PC line were acquired, resulting in a total scan time of 27 min. After the completion of the functional scans, a high-resolution three-dimensional (3D) T1-weighted structural scan (TE 3.4 ms, TR 7.4 ms, flip angle 9°, 320 0.5 mm sagittal slices, FOV 256 mm × 256 mm, matrix 512 × 512 resulting in isotropic voxels with an edge length of 0.5 mm, scan duration 11:09 min) was acquired for anatomical localization.

### Data analysis

#### Behavioral data

During fMRI scanning, responses of the CS arousal and valence ratings were recorded from all 32 subjects. A repeated-measures analysis of variance (ANOVA) with two within-subject factors phase (four levels: habituation, acquisition I, acquisition II, extinction) and stimulus (two levels: CS+<sub>unpaired</sub>, CS−) within the general linear model as implemented in SPSS version 15 (SPSS Inc., USA) was performed to validate the conditioning effect.

#### fMRI data

Analysis was performed using the Statistical Parametric Mapping software package version 5 (SPM5; Wellcome Department of Cognitive Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned, slice time acquisition corrected, normalized to the Montreal Neurological Institute (MNI) template, and resliced to a voxel size of 2 mm × 2 mm × 2 mm. Data were smoothed with an 8 mm kernel and subsequently filtered with a high-pass filter (cut-off period of 128 s). Event-related blood oxygen level-dependent (BOLD) responses were analyzed in SPM5 using the general linear model with a canonical hemodynamic response function as the basic function, and separately one regressor for each condition in each phase: CS+<sub>unpaired</sub>, CS+<sub>paired</sub> and CS−. Paired CS+ was modeled as one event and included as predictor of no interest in the regression analysis. The six movement parameters of the rigid body transformation determined during realignment were introduced as covariates into the model. To investigate differences between the phases of the conditioning and extinction procedure, the time courses of both conditioning and extinction were split into two subphases each. The first and second conditioning phases were separated by the SAM rating. Each phase consists of 35 trials and has the same length. The extinction section was also divided into two subphases of same length each lasting 5 min and containing 25 trials, referred to here as early and late extinction phases. In a first-level fixed-effects analysis, one statistical parametric map and corresponding contrast images for each subject reflecting the contrasts of interest were derived. The following contrasts of interest were computed: CS+<sub>unpaired</sub> > CS−, CS− > CS+<sub>unpaired</sub> for early and late acquisition and extinction phases. The individual contrast images were entered into a second-level random-effects t test to obtain activation maps across subjects. Based on our prior knowledge about the core areas involved in fear conditioning and extinction, summarized in our review article

**Table 1.** Mean ratings and statistical results for valence and arousal ratings of the CS+ and CS− after the experimental phases

Phase	Valence			Arousal		
	CS+	CS−	Test statistics (two-tailed)	CS+	CS−	Test statistics (two-tailed)
Habituation	1.9 ± 1.0	1.8 ± 0.9	$t_{31} = 0.27, p = \text{N.S.}$	1.2 ± 0.9	0.9 ± 0.7	$t_{31} = 1.7, p = \text{N.S.}$
Acquisition I	1.5 ± 1.0	1.8 ± 0.8	$t_{31} = -1.4, p = \text{N.S.}$	1.7 ± 1.2	0.9 ± 0.9	$t_{31} = 3.3, p < 0.005^*$
Acquisition II	1.3 ± 1.0	2.1 ± 0.8	$t_{31} = -4.0, p < 0.001^{**}$	1.8 ± 1.3	0.9 ± 0.9	$t_{31} = 4.8, p < 0.001^{**}$
Extinction	1.8 ± 0.9	2.0 ± 0.9	$t_{31} = -1.1, p = \text{N.S.}$	1.2 ± 1.1	0.7 ± 0.9	$t_{31} = 3.4, p < 0.005^*$

CS, Conditioned stimulus; N.S., not significant.

CS+ and CS− ratings given as means ± standard deviation.

\*  $p < 0.005$ , \*\*  $p < 0.001$ .

(Sehlmeier *et al.* 2009), region of interest (ROI) analyses focused on the right and left amygdalae and the dACC. The dACC was defined as a sphere with a radius of 8 mm placed in the most posterior part of the ACC (center  $x = 0, y = 2, z = 30$ ) (Dannlowski *et al.* 2009). The left and right amygdala ROIs were defined according to the AAL Atlas (Tzourio-Mazoyer *et al.* 2002). Mean contrast estimates were extracted for left and right amygdalae in each experimental phase. Linear regression analyses were calculated to determine the effect of trait anxiety on amygdala and dACC activations, during the acquisition and extinction phases. These anxiety scores were entered separately as a parametric variable in the analyses (MacCallum *et al.* 2002). To control for multiple statistical testing, we maintained a cluster-level false-positive detection rate at  $p < 0.05$  using a voxel threshold of  $p < 0.05$  with a cluster ( $k$ ) extent determined empirically by Monte Carlo simulations. These were implemented in AlphaSim, which accounted for spatial correlations between BOLD signal changes in neighboring voxels (Forman *et al.* 1995) (48 voxels for each amygdala and 53 voxels for the dACC ROI). As an add-on, an analysis of whole-brain activation during conditioning and extinction was carried out to demonstrate that the ROI analysis did not ignore significant activations outside the ROIs. As the aim was to demonstrate that no relevant activations were ignored, a more liberal threshold of  $p < 0.001$  and  $k > 10$  voxels spatial extent was used.

## Results

### Behavioral data

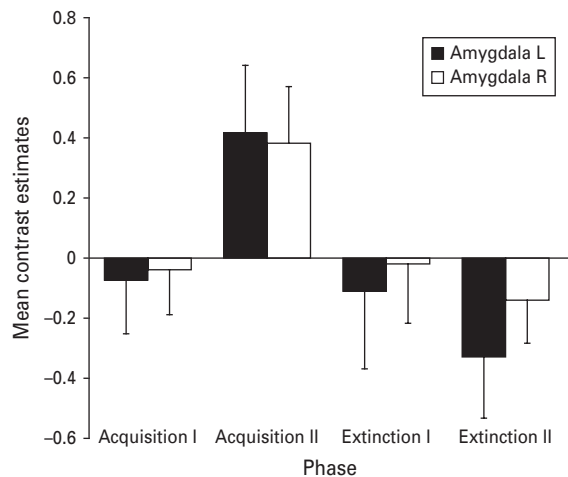
The mean trait anxiety score was 35.2 (S.D. = 7.5), ranging from 22 to 52. The repeated-measures ANOVA yielded significant main effects for stimulus [ $F(3, 93) = 6.99, p < 0.001, \eta_p^2 = 0.18$ ] and phase [ $F(1, 31) = 22.3,$

$p < 0.001, \eta_p^2 = 0.42$ ] and a significant stimulus  $\times$  phase interaction [ $F(2.3, 71.6) = 3.61, p < 0.05, \eta_p^2 = 0.1$ ] for arousal ratings (for details see Table 1). *Post-hoc* tests revealed that arousal ratings were significantly higher for CS+<sub>unpaired</sub> than CS− after both acquisition phases (early:  $t_{31} = 3.30, p < 0.005$ ; late:  $t_{31} = 4.77, p < 0.001$ ) and after extinction ( $t_{31} = 3.42, p < 0.005$ ). The repeated-measures ANOVA yielded a significant stimulus  $\times$  phase interaction [ $F(2.3, 72.3) = 5.7, p < 0.005, \eta_p^2 = 0.15$ ] for valence ratings. As expected, valence ratings differed significantly between CS+<sub>unpaired</sub> and CS− after the late acquisition phase ( $t_{31} = -4.0, p < 0.001$ ). Correlation analysis revealed no significant interactions between trait anxiety and CS ratings.

### fMRI data

#### Fear acquisition

During habituation, the BOLD signal did not differ significantly between CS+<sub>unpaired</sub> and CS−. During the late acquisition phase, ROI analyses yielded larger activation of the left ( $x = -30, y = -4, z = -22, t_{31} = 3.27, k = 96$  voxels,  $p = 0.0057$  corrected) and right amygdalae ( $x = 18, y = 4, z = -18, t_{31} = 2.79, k = 48$  voxels,  $p = 0.0497$  corrected) comparing the presentation of CS+<sub>unpaired</sub> to CS−. A significant increase in BOLD signal was observed in the dACC during early ( $x = 2, y = -2, z = 36, t_{31} = 2.59, k = 97$  voxels,  $p = 0.0073$  corrected) and late conditioning ( $x = -4, y = -2, z = 28, t_{31} = 3.54, k = 202$  voxels,  $p < 0.0001$  corrected). No significant correlations with trait anxiety were found within the ROIs corresponding to the ACC or the amygdalae. Whole-brain analysis revealed conditioning-related (CS+<sub>unpaired</sub> > CS−) neural responses throughout the two acquisition phases outside the ROIs in the following regions: rostral ACC, bilateral insula, thalamus and striatum ( $p < 0.001$  uncorrected,  $k > 10$  voxels) (see Supplementary Table 1 and Supplementary Fig. 1, available online).



**Fig. 1.** ANOVA results for the amygdala regions of interest (ROIs). Mean contrast estimates ( $\pm$  standard error of the mean) ( $CS+_{unpaired} > CS-$ ) for left (L) and right (R) amygdala ROI for early and late acquisition and extinction phases are presented. The ANOVA revealed a significant phase (acquisition, extinction)  $\times$  order (first, second half) interaction for right and left amygdalae ( $p$ 's  $< 0.05$  corrected for multiple comparisons). Activation increased from the first to the second acquisition phase and decreased during the extinction phases.

*Fear extinction*

No significant activation of the amygdala or the dACC could be detected on comparing the presentation of  $CS+_{unpaired}$  to  $CS-$  trails in any of the extinction phases. By contrast,  $CS-$  yielded significantly stronger activation of the left amygdala than  $CS+_{unpaired}$  during the late extinction phase ( $x = -26, y = -2, z = -12, t_{31} = 2.47, k = 81$  voxels,  $p = 0.012$  corrected), suggesting a deactivation of the amygdala during extinction. An additional full-factorial ANOVA with phase (two levels: acquisition, extinction) and order (two levels: first, second) as within-subject factors revealed a significant phase  $\times$  order interaction (left amygdala:  $x = -28, y = -4, z = -12, t_{124} = 2.59, k = 85$  voxels,  $p = 0.0103$  corrected; right amygdala:  $x = 20, y = 6, z = -18, t_{124} = 2.32, k = 63$  voxels,  $p = 0.0272$  corrected). Amygdala activation increased from the first to the second acquisition phase, and decreased during the extinction phases. Contrast estimates of the amygdala activation interaction are shown in Fig. 1. Regression analysis revealed significant positive effects of trait anxiety on amygdala reactivity during the early extinction phase (left amygdala:  $x = -22, y = -8, z = -14, t_{30} = 3.17, r = 0.50, k = 44$  voxels,  $p = 0.059$  corrected; right amygdala:  $x = 20, y = 0, z = -16, t_{30} = 3.25, r = 0.51, k = 114$  voxels,  $p = 0.003$  corrected) (Figs 2a, b and 3a, b). A significant

correlation was also found for the late extinction phase (left amygdala:  $x = -28, y = -4, z = -16, t_{30} = 2.69, r = 0.44, k = 74$  voxels,  $p = 0.0173$  corrected; right amygdala:  $x = 24, y = -4, z = -18, t_{30} = 2.19, r = 0.37, k = 18$  voxels,  $p = 0.2$  corrected). In addition, significant negative effects of trait anxiety on dACC activity were observed during late extinction ( $x = 4, y = -2, z = 28, t_{30} = 3.41, r = -0.53, k = 143$  voxels,  $p = 0.001$  corrected) (Fig. 3c). Trait-anxious subjects showed reduced prefrontal activation during late extinction of conditioned responses. The whole-brain analysis of the extinction phase revealed additional bilateral activation outside the ROIs in the insular cortex and the supplementary motor area for  $CS+_{unpaired}$  in contrast to  $CS-$  ( $p < 0.001$  uncorrected,  $k > 10$  voxels) (see Supplementary Table 1 and Supplementary Fig. 2, online).

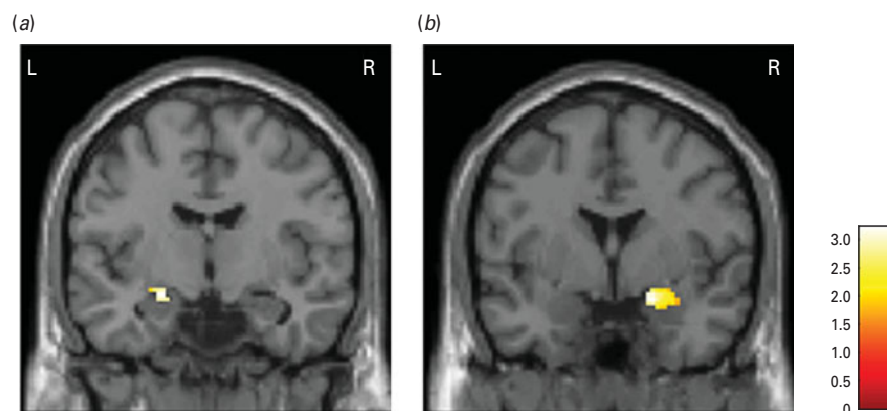
**Discussion**

In the present study, we used fMRI during a cued fear-conditioning design to identify the neural mechanisms of fear learning, and to investigate whether these neural mechanisms are associated with an important personality trait: trait anxiety (Spielberger, 1972). Analysis of the fMRI data revealed significant activation of the amygdala during the late acquisition phase and activation of the dACC during both acquisition phases. During extinction, deactivation of the amygdala was observed. In addition, whole-brain analysis yielded activation of fear-related brain areas such as the insula, striatum and rostral ACC during fear conditioning, and activation of the bilateral insula during extinction, as expected. Of interest, significant correlations between high trait anxiety, enhanced amygdala reactivity and decreased activation of the dACC were apparent during the extinction phase, suggesting that subjects with high trait anxiety show delayed and reduced extinction of conditioned responses.

*Behavioral data*

Behavioral data indicated successful cued conditioning. During acquisition, ratings of negative valence and arousal were increased significantly for  $CS+_{unpaired}$  compared to  $CS-$ . Specifically, valence ratings were significantly different during the late acquisition phase whereas arousal ratings had already differed after the initial acquisition phase. We assume that the evaluation of arousal is more sensitive for conditioning effects than valence rating, which might be confounded by subjects' personal preference, or higher cognitive processes. After extinction,  $CS-$  and  $CS+_{unpaired}$  ratings became equal.





**Fig. 2.** Coronal sections illustrating statistical parametric maps of significant positive relationships between trait anxiety and (a) activity within the left (L) amygdala ( $x = -22$ ,  $y = -8$ ,  $z = -14$ ,  $t_{30} = 3.17$ ,  $r = 0.50$ ,  $p = 0.002$  uncorrected,  $k = 44$  voxels,  $p = 0.059$  corrected) and (b) activation of the right (R) amygdala during the early extinction of conditioned fear ( $x = 20$ ,  $y = 0$ ,  $z = -16$ ,  $t_{30} = 3.25$ ,  $r = 0.51$ ,  $k = 114$  voxels,  $p = 0.001$  uncorrected,  $p = 0.0030$  corrected).

### Neuronal networks involved in fear conditioning and extinction

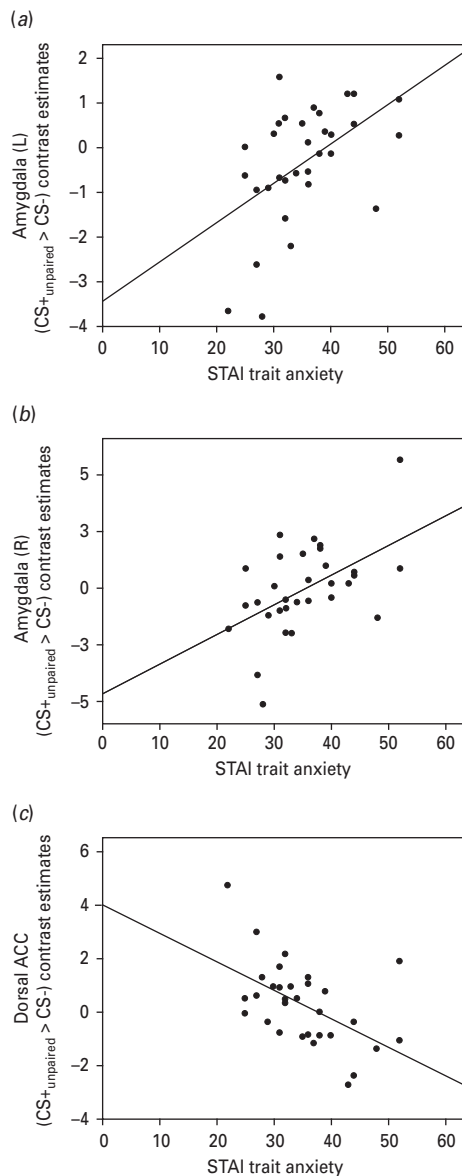
As expected, ROI analyses revealed larger bilateral activation of the amygdalae during the acquisition phase ( $CS + \text{unpaired} > CS -$ ), in accordance with previous studies (LaBar *et al.* 1998; Fischer *et al.* 2000; Phelps *et al.* 2001; Morris & Dolan, 2004; Schiller *et al.* 2008). The only difference to these studies is the occurrence of amygdala activation during the late conditioning phase. The time course of amygdala activation observed here may be due to the small CS-US contingency rate of 25% used in this study, suggesting that the association between CS and US had only been established after a certain number of pairings (Phelps *et al.* 2004; Barrett & Armony, 2009). This pairing rate was used in our study to deliberately avoid habituation effects occurring before the extinction phase. Furthermore, the lower aversiveness of the tone compared to the electrical stimulation presented in most studies may also have contributed to the delayed amygdala response. Consistent with prior studies on human fear conditioning and extinction, the presentation of  $CS + \text{unpaired}$  compared to  $CS -$  during fear conditioning elicited whole-brain activation of fear-related brain areas, such as the insula, ACC and striatum. These areas are known to be involved in emotional processing and are regarded as key areas of pain processing and classical fear conditioning (for review see Sehlmeier *et al.* 2009).

During extinction, the main conditioning effect ( $CS + \text{unpaired} > CS -$ ) was associated with a deactivation of the amygdala. In particular, we found a reversal response in the amygdala, such that the activation was greater for  $CS -$  than for  $CS + \text{unpaired}$  during the late extinction phase. Outside the ROI, activations

of the bilateral insulae and the supplementary motor cortex were detected. These results are also in line with those of other neuroimaging studies (Buchel & Dolan, 2000; Phelps *et al.* 2004). The supplementary motor area (SMA) is a key structure for both preparation and execution of movements (Remy *et al.* 1994; Johnson *et al.* 2001; Wang *et al.* 2007). Activation within the extinction phase may reflect a preparatory action in response to the formerly conditioned stimulus, in terms of avoiding or escaping from the threatening stimulation. The insular cortex is assumed to process emotional contents, such as fear (Phelps *et al.* 2001) or pain (Ploghaus *et al.* 1999; Ostrowsky *et al.* 2002; Lopez-Sola *et al.* 2010). Moreover, the insula is recruited in the context of uncertainty and in anticipation of aversive events (Carlson *et al.* 2010; Sarinopoulos *et al.* 2010), which is particularly the case during the extinction phase. To conclude, our data support the development of activity within the amygdala over the course of conditioning and the decline of amygdala activation during extinction. Surprisingly, we did not observe significant activation within the ROI of the dACC for the main conditioning effect ( $CS + \text{unpaired} > CS -$ ) in any of the extinction phases, although it is preferentially engaged during the inhibition of conditioned responses (Phelps *et al.* 2004; Lang *et al.* 2009).

### Influence of trait anxiety

Significant correlations of trait-anxiety scores with amygdala and dACC activation were revealed. Although the acquisition phases were unaffected by this personality trait, higher levels of trait anxiety were associated with greater sustained conditioned



**Fig. 3.** Scatter plots showing correlations between individual's trait anxiety score and contrast estimates for the CS+<sub>unpaired</sub> presentation compared to CS- within the (a) left (L) amygdala ( $r=0.50$ ) and (b) right (R) amygdala ( $r=0.51$ ) during early extinction, (c) and the dorsal anterior cingulate cortex (dACC) ( $r=-0.53$ ) during late extinction of conditioned fear.

amygdala activation, or rather with less amygdala deactivation ( $r=0.50$ ), mainly during early extinction. These results are remarkably consistent with earlier studies on healthy subjects and anxiety patients that reported a positive interaction of amygdala responses with anxiety during the processing of fearful stimuli (Bishop *et al.* 2004; Etkin *et al.* 2004; Dickie & Armony, 2008), during fear learning (Bremner *et al.* 2005; Hooker *et al.* 2008) and fear extinction (Barrett & Armony, 2009).

Moreover, we were able to show that high levels of trait anxiety are also strongly associated with decreased activation of the dACC, the cognitive part of the ACC (Bush *et al.* 2000), during late extinction ( $r=-0.53$ ). At first glance, this finding seems counter-intuitive as we did not observe activation of the dACC in any of the extinction phases. However, regression analysis yielded a significant correlation between dACC activation and trait anxiety. This finding implies that, without controlling for personality, the dACC is not equally engaged across all subjects to extinguish fear responses. The dACC is instead engaged as a function of trait anxiety. Hence, we conclude that involvement of the dACC during the extinction of fear is modulated by differences in trait anxiety. We assume that the hypo-activation of the dACC reported for anxious subjects results in a deficient inhibition of conditioned amygdala responses, additionally prolonging extinction and exaggerating fear responses. These findings are consistent with previous neuroimaging studies that reported an association of anxiety traits, pathological anxiety and activation of the PFC/ACC during fear extinction (Bremner *et al.* 2005; Rauch *et al.* 2005, 2006). Nevertheless, our study extends the current literature showing that high levels of trait anxiety are associated with both increased amygdala activity and reduced activation of the ACC during the process of extinction. In particular, our results partly confirm and expand the recent findings of Barrett & Armony (2009). Even though different conditioning designs were used, the findings seem to converge on trait anxiety correlating with the amygdala and modulating fear extinction rather than fear conditioning. However, the results vary in the areas of the ACC examined. Whereas Barrett & Armony (2009) observed a relationship between trait anxiety and enhanced subgenual ACC activation, we were able to show an association of trait anxiety and reduced dACC activation, as expected. Our data show that this strong and significant relationship was found exclusively during the late extinction phase. The correlation observed during early extinction failed to show significance. Besides this problem with statistical significance, we assume that activation of the dACC is decreased during the extinction process in anxious subjects, leading to the strong negative correlation during late extinction. This suggests that high and low anxious persons do not differ with respect to the dACC activation at early stages of extinction, but at a later point of time. We suppose that trait-anxious subjects are not able to maintain activation of the inhibitory dACC during the extinction process, which may lead to enhanced vulnerability and risk for relapse.

Taken together, subjects characterized by enhanced trait anxiety show deficits in the extinction of acquired fear. This is reflected not only by sustained amygdala activation during early extinction but also by additional decreased dACC activation during late extinction. Highly trait-anxious subjects fail to adapt to altering circumstances and maintain their anticipatory anxiety even when threat-related stimuli (US) are absent (Chan & Lovibond, 1996), as is the case during extinction. Therefore, we assume that the most prominent feature separating high and low anxious subjects may not be conditionability, but the ability to extinguish conditioned responses.

Our simple and robust paradigm has revealed important findings for the understanding of the relationship between personality and neurobiological vulnerability in the development of anxiety disorders. We have shown that anxious subjects are characterized by both amygdala hyper-activation and dACC hypo-activation. This double impact of increased amygdala reactivity and deficient cognitive control may represent enhanced risk for spontaneous recovery of the extinguished response and pathophysiologically for relapse in anxiety disorders.

### Limitations

A few limitations should be pointed out in the current study. First, in accordance with previous studies (Lissek & Powers, 2003; LaRowe et al. 2006), we used an acoustic, brief and loud noise as unconditioned stimulus to startle subjects. As we did not conduct *a priori* aversiveness rating of the US, we cannot rule out that the tone was low aversive and hence caused the observed delayed amygdala response during the acquisition phase. Second, consistent with most studies, we used the subjective measure of valence and arousal only at the end of the experimental blocks (Gottfried & Dolan, 2004; Straube et al. 2007). The application of a continuous measure would have improved the investigation of conditioning-related changes in CS– ratings. However, we did use only neutral male faces as conditioned stimuli instead of both female and male faces.

### Conclusions

Our study reveals, for the first time, that deficits in the extinction of conditioned fear responses in highly trait-anxious subjects are not only associated with enhanced amygdala reactivity but also related to reduced dACC activation, providing a neurobiological correlate for trait anxiety. Therefore, our findings help to elucidate the enhanced neurobiological vulnerability of anxious subjects to develop and maintain an anxiety disorder.

### Note

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/psm>).

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### Declaration of Interest

None.

### References

- Anderson KC, Insel TR (2006). The promise of extinction research for the prevention and treatment of anxiety disorders. *Biological Psychiatry* **60**, 319–321.
- Barrett J, Armony JL (2009). Influence of trait anxiety on brain activity during the acquisition and extinction of aversive conditioning. *Psychological Medicine* **39**, 255–265.
- Bienvenu OJ, Brown C, Samuels JF, Liang KY, Costa PT, Eaton WW, Nestadt G (2001). Normal personality traits and comorbidity among phobic, panic and major depressive disorders. *Psychiatry Research* **102**, 73–85.
- Bishop SJ (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences* **11**, 307–316.
- Bishop SJ (2009). Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience* **12**, 92–98.
- Bishop SJ, Duncan J, Lawrence AD (2004). State anxiety modulation of the amygdala response to unattended threat-related stimuli. *Journal of Neuroscience* **24**, 10364–10368.
- Blechert J, Michael T, Vriends N, Margraf J, Wilhelm FH (2007). Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behaviour Research and Therapy* **45**, 2019–2033.
- Bradley MM, Lang PJ (1994). Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. *Journal of Behavior Therapy and Experimental Psychiatry* **25**, 49–59.
- Bremner JD, Vermetten E, Schmahl C, Vaccarino V, Vythilingam M, Afzal N, Grillon C, Charney DS (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine* **35**, 791–806.



- Buchel C, Dolan RJ** (2000). Classical fear conditioning in functional neuroimaging. *Current Opinion in Neurobiology* **10**, 219–223.
- Bush G, Luu P, Posner MI** (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences* **4**, 215–222.
- Carlson JM, Greenberg T, Rubin D, Mujica-Parodi LR** (2010). Feeling anxious: anticipatory amygdalo-insular response predicts the feeling of anxious anticipation. *Social Cognitive and Affective Neuroscience*. Published online: 5 March 2010. doi:10.1093/scan/nsq017.
- Chambers JA, Power KG, Durham RC** (2004). The relationship between trait vulnerability and anxiety and depressive diagnoses at long-term follow-up of generalized anxiety disorder. *Journal of Anxiety Disorders* **18**, 587–607.
- Chan CK, Lovibond PF** (1996). Expectancy bias in trait anxiety. *Journal of Abnormal Psychology* **105**, 637–647.
- Dannowski U, Ohrmann P, Konrad C, Domschke K, Bauer J, Kugel H, Hohoff C, Schoning S, Kersting A, Baune BT, Mortensen LS, Arolt V, Zwieterlood P, Deckert J, Heindel W, Suslow T** (2009). Reduced amygdala-prefrontal coupling in major depression: association with MAOA genotype and illness severity. *International Journal of Neuropsychopharmacology* **12**, 11–22.
- Dickie EW, Armony JL** (2008). Amygdala responses to unattended fearful faces: interaction between sex and trait anxiety. *Psychiatry Research* **162**, 51–57.
- Etkin A, Klemenhagen KC, Dudman JT, Rogan MT, Hen R, Kandel ER, Hirsch J** (2004). Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron* **44**, 1043–1055.
- Etkin A, Wager TD** (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *American Journal of Psychiatry* **164**, 1476–1488.
- Fischer H, Andersson JL, Furmark T, Fredrikson M** (2000). Fear conditioning and brain activity: a positron emission tomography study in humans. *Behavioral Neuroscience* **114**, 671–680.
- Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC** (1995). Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. *Magnetic Resonance in Medicine* **33**, 636–647.
- Gottfried JA, Dolan RJ** (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nature Neuroscience* **7**, 1144–1152.
- Haas BW, Omura K, Constable RT, Canli T** (2007). Emotional conflict and neuroticism: personality-dependent activation in the amygdala and subgenual anterior cingulate. *Behavioral Neuroscience* **121**, 249–256.
- Hooker CI, Verosky SC, Miyakawa A, Knight RT, D'Esposito M** (2008). The influence of personality on neural mechanisms of observational fear and reward learning. *Neuropsychologia* **46**, 2709–2724.
- Johnson KA, Cunningham R, Ianssek R, Bradshaw JL, Georgiou N, Chiu E** (2001). Movement-related potentials in Huntington's disease: movement preparation and execution. *Experimental Brain Research* **138**, 492–499.
- Kienast T, Hariri AR, Schlagenhaut F, Wrase J, Sterzer P, Buchholz HG, Smolka MN, Grunder G, Cumming P, Kumakura Y, Bartenstein P, Dolan RJ, Heinz A** (2008). Dopamine in amygdala gates limbic processing of aversive stimuli in humans. *Nature Neuroscience* **11**, 1381–1382.
- LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA** (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* **20**, 937–945.
- Lang S, Kroll A, Lipinski SJ, Wessa M, Ridder S, Christmann C, Schad LR, Flor H** (2009). Context conditioning and extinction in humans: differential contribution of the hippocampus, amygdala and prefrontal cortex. *European Journal of Neuroscience* **29**, 823–832.
- LaRowe SD, Patrick CJ, Curtin JJ, Kline JP** (2006). Personality correlates of startle habituation. *Biological Psychology* **72**, 257–264.
- Laux L, Glanzmann P, Schaffner P, Spielberger CD** (1981). *State-Trait-Angstinventar (Testmappe mit Handanweisung, Fragebogen STAI-G Form X 1 und Fragebogen STAI-G Form X 2)*. Beltz: Weinheim.
- Lissek S, Powers AS** (2003). Sensation seeking and startle modulation by physically threatening images. *Biological Psychology* **63**, 179–197.
- Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS** (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy* **43**, 1391–1424.
- Lopez-Sola M, Pujol J, Hernandez-Ribas R, Harrison BJ, Ortiz H, Soriano-Mas C, Deus J, Menchon JM, Vallejo J, Cardoner N** (2010). Dynamic assessment of the right lateral frontal cortex response to painful stimulation. *NeuroImage* **50**, 1177–1187.
- MacCallum RC, Zhang S, Preacher KJ, Rucker DD** (2002). On the practice of dichotomization of quantitative variables. *Psychological Methods* **7**, 19–40.
- Maren S** (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience* **24**, 897–931.
- Mathews A, MacLeod C** (2005). Cognitive vulnerability to emotional disorders. *Annual Review of Clinical Psychology* **1**, 167–195.
- Michael T, Blechert J, Vriends N, Margraf J, Wilhelm FH** (2007). Fear conditioning in panic disorder: enhanced resistance to extinction. *Journal of Abnormal Psychology* **116**, 612–617.
- Morgan MA, Romanski LM, LeDoux JE** (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neuroscience Letters* **163**, 109–113.
- Morris JS, Dolan RJ** (2004). Dissociable amygdala and orbitofrontal responses during reversal fear conditioning. *NeuroImage* **22**, 372–380.
- Most SB, Chun MM, Johnson MR, Kiehl KA** (2006). Attentional modulation of the amygdala varies with personality. *NeuroImage* **31**, 934–944.
- Mujica-Parodi LR, Korgaonkar M, Ravindranath B, Greenberg T, Tomasi D, Wagshul M, Ardekani B, Guilfoyle D, Khan S, Zhong Y, Chon K, Malaspina D**

- (2009). Limbic dysregulation is associated with lowered heart rate variability and increased trait anxiety in healthy adults. *Human Brain Mapping* **30**, 47–58.
- Myers KM, Davis M** (2002). Behavioral and neural analysis of extinction. *Neuron* **36**, 567–584.
- Oldfield RC** (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113.
- Omura K, Todd Constable R, Canli T** (2005). Amygdala gray matter concentration is associated with extraversion and neuroticism. *NeuroReport* **16**, 1905–1908.
- Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F** (2002). Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. *Cerebral Cortex* **12**, 376–385.
- Pavlov IP** (1927). *Conditioned Reflexes*. Oxford University Press: Oxford.
- Phelps EA, Delgado MR, Nearing KI, LeDoux JE** (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* **43**, 897–905.
- Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M** (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience* **4**, 437–441.
- Pineles SL, Vogt DS, Orr MR** (2009). Personality and fear responses during conditioning: beyond extraversion. *Personality and Individual Differences* **46**, 48–53.
- Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JN** (1999). Dissociating pain from its anticipation in the human brain. *Science* **284**, 1979–1981.
- Pujol J, Lopez A, Deus J, Cardoner N, Vallejo J, Capdevila A, Paus T** (2002). Anatomical variability of the anterior cingulate gyrus and basic dimensions of human personality. *NeuroImage* **15**, 847–855.
- Quirk GJ, Garcia R, Gonzalez-Lima F** (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry* **60**, 337–343.
- Quirk GJ, Likhtik E, Pelletier JG, Pare D** (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *Journal of Neuroscience* **23**, 8800–8807.
- Rauch SL, Milad MR, Orr SP, Quinn BT, Fischl B, Pitman RK** (2005). Orbitofrontal thickness, retention of fear extinction, and extraversion. *NeuroReport* **16**, 1909–1912.
- Rauch SL, Shin LM, Phelps EA** (2006). Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research: past, present, and future. *Biological Psychiatry* **60**, 376–382.
- Remy P, Zilbovicius M, Leroy-Willig A, Syrota A, Samson Y** (1994). Movement- and task-related activations of motor cortical areas: a positron emission tomographic study. *Annals of Neurology* **36**, 19–26.
- Sarinopoulos I, Grupe DW, Mackiewicz KL, Herrington JD, Lor M, Steege EE, Nitschke JB** (2010). Uncertainty during anticipation modulates neural responses to aversion in human insula and amygdala. *Cerebral Cortex* **20**, 929–940.
- Schiller D, Levy I, Niv Y, LeDoux JE, Phelps EA** (2008). From fear to safety and back: reversal of fear in the human brain. *Journal of Neuroscience* **28**, 11517–11525.
- Schmidt NB, Mitchell MA, Richey JA** (2008). Anxiety sensitivity as an incremental predictor of later anxiety symptoms and syndromes. *Comprehensive Psychiatry* **49**, 407–412.
- Sehlmeier C, Schoning 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**, e5865.
- Sotres-Bayon F, Cain CK, LeDoux JE** (2006). Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex. *Biological Psychiatry* **60**, 329–336.
- Spielberger CD** (1972). *Anxiety: Current Trends in Theory and Research*, vol. 1. Academic Press: New York, NY.
- Spielberger CD** (1979). *Understanding Stress and Anxiety*. Harper & Row: London.
- Spielberger CD** (1983). *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press: Palo Alto, CA.
- Straube T, Weiss T, Mentzel HJ, Miltner WH** (2007). Time course of amygdala activation during aversive conditioning depends on attention. *NeuroImage* **34**, 462–469.
- Tottenham N, Borscheid A, Ellertsen K, Marcus DJ, Nelson CA** (2002). Categorization of facial expressions in children and adults: establishing a larger stimulus set. *Journal of Cognitive Neuroscience* **14** (Suppl.), S74.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M** (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* **15**, 273–289.
- Wang MH, Zhu YH, Li JC, Weng XC** (2007). Functional magnetic resonance imaging of whole brain related to motor preparation and execution [in Chinese]. *Zhonghua Yi Xue Za Zhi* **87**, 971–974.
- Wittchen H-U, Wunderlich U, Gruschwitz S, Zaudig M** (1997). *SCID-I. Structured Clinical Interview for DSM-IV* [in German]. Hogrefe: Göttingen.