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ORIGINAL INVESTIGATION

**Roshan Cools · Andy J. Calder · Andrew D. Lawrence · Luke Clark · Ed Bullmore · Trevor W. Robbins**

# Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces

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**Abstract** *Rationale:* In this study we used functional magnetic resonance imaging (fMRI) to examine the effects of acute tryptophan depletion (ATD), a well-recognised method for inducing transient cerebral serotonin depletion, on brain activation to fearful faces. *Objectives:* We predicted that ATD would increase the responsiveness of the amygdala to fearful faces as a function of individual variation in threat sensitivity. *Methods:* Twelve healthy male volunteers received a tryptophan depleting drink or a tryptophan balancing amino acid drink (placebo) in a double-blind crossover design. Five hours after drink ingestion participants were scanned whilst viewing fearful, happy and neutral faces. *Results:* Consistent with previous findings, fearful faces induced significant signal change in the bilateral amygdala/hippocampus as well as the fusiform face area and the right dorsolateral prefrontal cortex. Furthermore, ATD modulated amygdala/hippocampus activation in response to fearful relative to happy faces as a function of self-reported threat sensitivity (as measured with the Behavioral Inhibition Scale; Carver CS, White TL (1994) Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. J Pers Soc Psychol 67:319–333). *Conclusion:* The data support the hypothesis that individual variation in threat sensitivity interacts with manipulation of 5-HT function to bias the processing of amygdala-dependent threat-relevant stimuli.

**Keywords** Serotonin depletion · Fear · Functional imaging · Amygdala · Hippocampus · Faces

R. Cools (✉)
Helen Wills Neuroscience Institute,
University of California–Berkeley,
132 Barker Hall,
Berkeley, CA, USA
e-mail: roshanco@berkeley.edu
Tel.: +1-510-6422839

R. Cools · L. Clark · T. W. Robbins
Department of Experimental Psychology,
University of Cambridge,
Cambridge, UK

A. J. Calder · A. D. Lawrence
MRC Cognition and Brain Sciences Unit,
Cambridge, UK

E. Bullmore
Brain Mapping Unit,
Department of Psychiatry,
Addenbrooke's Hospital,
Cambridge, UK

## Introduction

Enhanced sensitivity to threat-related stimuli is a cardinal feature of anxiety disorders (Richards et al. 2002). These conditions are commonly treated with serotonin-potentiating drugs, such as selective serotonin reuptake inhibitors (SSRIs) (Blier and de Montigny 1999). In healthy volunteers, administration of the SSRI citalopram reduces negative biases in information processing following chronic treatment, although it may actually increase such biases following acute treatment with SSRIs (Harmer et al. 2003a, 2004). The finding that acute SSRI treatment has the reverse effect on threat processing parallels clinical observations of an initial exacerbation of symptoms in the first 2 or 3 weeks following SSRI treatment onset. These somewhat paradoxical effects have been suggested to reflect an initial attenuation of firing activity of 5-HT neurons via activation of somatodendritic 5-$HT_{1A}$ autoreceptors upon treatment onset with a subsequent recovery upon continued treatment due to desensitisation of these 5-$HT_{1A}$ autoreceptors (Blier and de Montigny 1999).

A role for serotonin in threat sensitivity is also supported by animal research (Eison and Eison 1994; Lucki 1998), which demonstrated that 5-HT-enhancing drugs attenuate the aversive effects of brain stimulation (Patkina and Lapin 1976; Smith and Kennedy 2003) and reduce the acquisition of fear conditioning (Burghardt et al. 2004; Inoue et al. 2004; Hashimoto et al. 1996; Groenink et al. 2000). Administration of tryptophan, the precursor of serotonin (5-HT), reduces conditioned freezing in a fear-conditioning paradigm

(Inoue et al. 1996). The amygdala is a key structure for processing of threat-related stimuli in both animals and humans (Adolphs et al. 1994; Fanselow and Kim 1994; LeDoux 1992; Morris et al. 1996) and amygdala responsiveness can be modulated by serotonin. Thus, the amygdala receives dense serotonergic inputs from the dorsal raphe nucleus in the midbrain (Sadikot and Parent 1990) and several 5-HT receptor subtypes are present within the amygdala. 5-HT levels in the amygdala increase during fear conditioning (Inoue et al. 1993) and amygdala neurons, excited by electrical stimulation of glutamatergic inputs from cortex, are inhibited by concurrent iontophoresis of 5-HT, probably via the activation of GABAergic cells through excitatory 5-HT receptors in the amygdala (Stutzmann and LeDoux 1999; Stutzmann et al. 1998). On the basis of this and other evidence, it was suggested that deficient 5-HT function might result in enhanced processing of harmful stimuli due to diminished inhibitory modulation of excitatory sensory afferents, thereby allowing innocuous sensory signals to be processed by the amygdala as emotionally salient (Stutzmann and LeDoux 1999).

Recent evidence highlighted the importance of individual differences on the amygdala response to emotional stimuli (Bishop et al. 2004; Etkin et al. 2004; Fischer et al. 2001; Hariri et al. 2002; Mathews et al. 2004). For example, Bishop et al. (2004) observed that amygdala activation to unattended fearful faces correlated with individual differences in state anxiety, as measured with the Spielberger State–Trait Anxiety Inventory (STAI; Spielberger 1983). Moreover, Etkin et al. (2004) showed that activity in the amygdala to unconscious fearful faces were predicted by individual differences in trait anxiety, again as measured with the STAI. Molecular genetic studies have linked variations in anxiety/fear-related personality traits with variation in the expression of the 5-HT transporter (Sen et al. 2004; Munafo et al. 2004). For example, using fMRI, Hariri et al. (2002) showed that the amygdala response to fearful faces was modulated by a serotonin transporter (5-HTT) polymorphism. Individuals carrying one or two copies of the short allele (*s*-carriers), which has been associated with reduced 5HTT expression and 5-HT function (Bethea et al. 2004; Greenberg et al. 1999; Lesch et al. 1996; Reist et al. 2001; Smeraldi et al. 1998), exhibited greater amygdala activation in response to fearful faces than participants homozygous for the long allele. In keeping with this observation, *s*-carriers of the human 5-HT transporter gene showed stronger amygdala activation as well as greater coupling between the amygdala and the prefrontal cortex during the presentation of aversive but not pleasant pictures (Heinz et al. 2005).

Gray and McNaughton (Gray and McNaughton 2000; McNaughton and Gray 2000) proposed two neural systems, each regulating the individual's sensitivity to one of two classes of motivationally relevant environmental cues. The Behavioral Inhibition System (BIS) was suggested to regulate anxiety sensitivity via monoaminergic projections from the brainstem into the medial temporal lobe. This septo-hippocampal BIS would engage the amygdala to produce fear-related outputs. The behavioural activation system (BAS), of less interest in the present paper, was proposed to regulate the response to appetitive cues via dopaminergic neurotransmission. Consistent with Gray's model, Mathews et al. (2004) recently observed that the fMRI BOLD response in the amygdala and hippocampus to threatening stimuli was qualified by individual variation in threat sensitivity measured with the BIS Scale, which was explicitly designed to measure the BIS construct as described by Gray and McNaughton (Carver and White 1994).

In the present study, we induced the acute depletion of central 5-HT function in young healthy male participants to examine the serotonergic modulation of amygdala activation by threat-relevant stimuli. Serotonin depletion was achieved through the acute tryptophan depletion (ATD) procedure, a well-recognised method for studying the effects of low serotonin on cognitive function in humans. ATD produces a rapid decrease in the synthesis and release of brain 5-HT (Carpenter et al. 1998; Nishizawa et al. 1997; Williams et al. 1999). TRP is depleted via ingestion of an amino acid mixture that does not contain tryptophan but does include other large neutral amino acids (LNAA) (Young et al. 1985). The amino acid load increases protein synthesis in the liver and increases competition for transport across the blood–brain barrier, with both factors decreasing tryptophan availability in the brain.

We predicted that ATD would enhance brain activation in the amygdala and/or hippocampus in response to fearful faces relative to neutral and/or happy faces. Furthermore, we predicted that this effect would be conditioned by individual variation in the personality trait of anxiety sensitivity as measured with the BIS Scale. More specifically, and in keeping with literature suggesting that ATD modulates affect-related information processing primarily in vulnerable individuals (Benkelfat et al. 1994; Young et al. 1985), we hypothesised that ATD would have greater effects in participants who report to be more vulnerable to threat-related cues, i.e. in participants with higher BIS scores than in participants with lower BIS scores.

## Materials and methods

### Participants

Twelve healthy right-handed male volunteers (18–28 years old; mean age of 23.8±SD 2.8) participated in this experiment. The study was approved by the Local Research Ethical Committee in Cambridge and carried out in accordance with the Declaration of Helsinki. Participants were recruited via local advertisements, and screened for psychiatric and neurological disorders and MRI contraindications by means of prescreening questionnaires and interview. All volunteers gave written informed consent, and were paid for their participation. Exclusion criteria were any history of cardiac, hepatic, renal, pulmonary, neurological, psychiatric or gastrointestinal disorder, medication use as well as first-degree family history of psychiatric illness. One participant vomited after ingesting the amino acid mixture and was replaced by a substitute.

## Experimental design

Participants attended two test sessions at least 1 week apart, and were administered either a tryptophan depleting (TRP-) drink or a balancing (BAL) amino acid drink in a double-blind, placebo-controlled, cross-over design (five participants received TRP- and seven received the BAL drink on the first session). Prior to a test session, participants fasted overnight and low-protein food was provided during the test days. Following a resting period of 5 h (4.5 h, SD 35 min in the TRP- condition and 5.0 h, SD 40 min in the balanced condition) to ensure stable and low TRP levels (Riedel 2004; Riedel et al. 1999), participants entered the MRI scanner at the Wolfson Brain Imaging Centre (WBIC). They were scanned whilst viewing fearful, neutral or happy faces and performing a gender categorisation task. Structural scans were obtained at the end of a test session or on a separate session.

## Gender categorisation task

Participants were presented with alternating blocks of happy, fearful and neutral facial expressions, posed by ten models from the Ekman and Friesen (1976) series. Fearful facial expressions act as cues to potential danger and have been shown to share some of the functional properties of intrinsically threat-related, fear stimuli (Lanzetta and Orr 1986). Following previous research (Phillips et al. 1997), neutral-happy morphs containing 75% neutral and 25% happiness were used in place of 100% neutral expressions, which are often perceived as negative (or threatening) and can evoke an amygdala response when faces are unfamiliar (Dubois et al. 1999), especially in high-anxious participants (Somerville et al. 2004). Each of the three facial expression conditions (happy, fearful, and neutral) comprised six blocks of ten trials. Each trial comprised a 960-ms presentation of a face, followed by a 1,440-ms inter-stimulus interval; a total of 24 s per block. Within all blocks, the order of presentation was pseudo-random with respect to the faces' identities. Participants were required to categorise the gender of each face by pressing one of two keys with the index and middle fingers of their right hand. Six fixation periods of 24 s each were interspersed among the 18 blocks of facial expressions. These consisted of a central cross on a blank background, which the participant was instructed to fixate. Blocks were presented in pseudo-random order according to the criteria that the same facial expression category was not shown in consecutive blocks and was separated by more than four intervening blocks (including fixation periods). Fixation periods were separated by a minimum of one and a maximum of four blocks of facial expressions.

## Amino acid mixture

The TRP-deficient amino acid drink (TRP-) contained a total of 75 g of amino acids using the proportions described by Young et al. (1985): 4.1 g L-alanine, 2.4 g glycine, 2.4 g L-histidine, 6.0 g L-isoleucine, 10.1 g L-leucine, 6.7 g L-lysine, 4.3 g L-phenylalanine, 9.2 g L-proline, 5.2 L-serine, 4.3 g L-threonine, 5.2 g L-tyrosine, 6.7 g L-valine, 3.7 g L-arginine, 2.0 g L-cysteine, 3.0 g L-methionine (SHS International Ltd, Liverpool, UK). The balanced mixture contained the same amino acids, plus 3.0 g tryptophan. The drinks were prepared with 200 ml tap water and fruit flavoring to compensate for the unpleasant taste.

## Biochemical measures

Blood samples (10 ml) were taken prior to ingestion of the amino acid mixture and after the fMRI scan (about 6.5 h later) to determine the plasma concentration of tryptophan (TRP) level and the ratio of plasma total tryptophan over the sum of the other large neutral amino acids (TRP/∑LNAA ratio). This ratio is important because the uptake of TRP in the brain is strongly associated with the amounts of other LNAA competing at the blood–brain barrier. Venous samples were taken in lithium heparin tubes, centrifuged and stored at −20°C. Plasma TRP was determined by an isocratic high-performance liquid chromatography (HPLC) method of analysis. Plasma proteins were removed by precipitation with 3% trichlororacetic acid (TCA) and centrifugation at 3,000 rpm, 4°C for 10 min, and then pipetted into heparin aliquots. An aliquot was then diluted in mobile phase before injection into HPLC analysis column. Fluorescence end-point detection was used to identify TRP.

Paired-samples *t* tests were used to compare the two baseline measurements of plasma TRP levels and TRP/∑LNAA ratios, and to compare the measurements of plasma TRP levels and the TRP/∑LNAA ratio in the balanced and TRP- condition. A repeated-measurements ANOVA was performed to look at the effect of ATD on plasma TRP levels and the TRP/∑LNAA ratios.

## Psychological ratings

Participants completed the (self-report) BIS/BAS Scales (Carver and White 1994), developed to measure individual differences in the sensitivity of a behavioural inhibition system (BIS) and a behavioural activation system (BAS), as proposed by McNaughton and Gray (2000). More specifically, the BIS (Carver and White 1994) is a seven-item questionnaire designed to reflect dispositional variation in sensitivity to anxiety-provoking stimuli (e.g., "even if something bad is about to happen to me, I rarely experience fear or nervousness", followed by a 4-point scale (1 indicating strong agreement and 4 indicating strong disagreement) to rate agreement. Carver and White (1994) demonstrated that the BIS was reliable (Cronbach's alpha=0.74), stable (test–retest $r$=0.66), correlated significantly with other measures of anxiety sensitivity (e.g., with the Harm avoidance scale of Cloninger Tridimensional Personality Questionnaire, $r$=0.59), and was a reliable predictor of vulnerability to nervousness as a function of exposure to cues of impending

punishment. In a large community sample, Jorm et al. (1999) used factor analysis to confirm that the BIS items reliably emerged as a unitary factor, was reliable (Cronbach's alpha of 0.76), and correlated significantly with other measures of negative affect (e.g., with the Neuroticism scale of the Eysenck Personality Questionnaire, $r$= 0.64). In our sample of 12 young males, the mean BIS score was 18.5 [standard deviation (SD), 4.0] and ranged from 12 to 26, comparable to those obtained in larger college samples by Carver and White (1994) and Jorm et al. (1999). Visual Analogue Scales (VAS) containing the items drowsy, sad, happy, anxious and nauseous were administered five times during the test day (at roughly 90-min intervals). The Positive and Negative Affect Scale (PANAS; Watson et al. 1988) was completed prior to ingestion and after the scan. Repeated-measures ANOVA was used to investigate the effects of drink treatment (TRP- and balanced) and time (five time points for the VAS, and two for the PANAS) on subjective mood ratings. Greenhouse–Geisser corrections were applied when the sphericity assumption was violated.

### Behavioural data analysis

Proportions of correct (right/left) responses were calculated, arcsin-transformed (2*arcin$\sqrt{x}$), as is appropriate whenever the variance is necessarily proportional to the mean (Howell 1997, p. 328), and analysed using repeated-measures analysis of variance (ANOVA) with affect (happy, fearful and neutral) and treatment (BAL and TRP-) as within-subjects factors. Greenhouse–Geisser corrections were applied when the sphericity assumption was violated.

### Image acquisition

Participants were scanned in a 3-T Bruker Medspec scanner (S300; Bruker, Ettlingen, Germany) with a head coil gradient set at the WBIC. $T_2$*-weighted gradient-echo echo-planar imaging (EPI) data depicting blood oxygenation level-dependent (BOLD) contrast were acquired with TE (echo time)=27 ms. A whole brain acquisition consisted of 21 slices (TR 1.6 s; voxel size before normalisation 1.56× 1.56×5 mm and after normalisation 3×3×3 mm; inter-slice gap 1 mm; matrix size 128×128; bandwidth 100 kHz; oblique axial orientation). The first 12 images in each session were subsequently discarded to avoid T1 equilibrium effects. In addition, for each participant a fast gradient-echo $T_1$-weighted anatomical reference image of the whole brain was also acquired (matrix size 256×220, TR=20.0 s, TE=5 ms) for spatial normalisation.

### Image analysis

Data analysis was performed using SPM99 and SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). Preprocessing procedures consisted of (linear) slice acquisition time correction, within-subject realignment (SPM2), geometric undistortion using fieldmaps (Cusack and Papadakis 2002), spatial normalisation using each individual subject's skull-stripped SPGR (using the Brain Extraction Tool, Smith 2002) and the Montreal Neurological Institute (MNI) skull-stripped structural template (SPM2) and spatial smoothing using a Gaussian kernel (10 mm full width at half-maximum).

A canonical hemodynamic response was used as a covariate in a general linear model and a parameter estimate was generated for each voxel for each block type (fearful, happy, neutral). Fixation was modelled implicitly. Movement parameters were included as covariates of no interest.

For each subject, the following contrasts were computed: (1) fearful faces vs fixation (across TRP- and BAL sessions); (2) fearful faces vs neutral faces (TRP- + BAL); (3) fearful faces vs happy faces (TRP- + BAL); (4) TRP- vs BAL for fearful faces relative to fixation; (5) TRP- vs BAL for fearful faces relative to neutral faces; (6) TRP- vs BAL for fearful faces relative to happy faces. Thus, treatment was modelled as a within-subject variable within each individual's general linear model. Individual contrast images were taken to a second level analysis in which $t$ values were calculated for each voxel treating inter-subject variability as a random affect. Dependency of treatment effects on individual differences in threat sensitivity was assessed by simple regression analyses at the random effects (group) level. More specifically, treatment effects on the response to fearful faces (contrasts 4, 5 and 6) were regressed against BIS scores derived from the BIS Scale (Carver and White 1994). All contrasts of interest were examined for statistical significance at $P$<0.05 (corrected for multiple comparisons at the level of the whole brain, family-wise error rate).

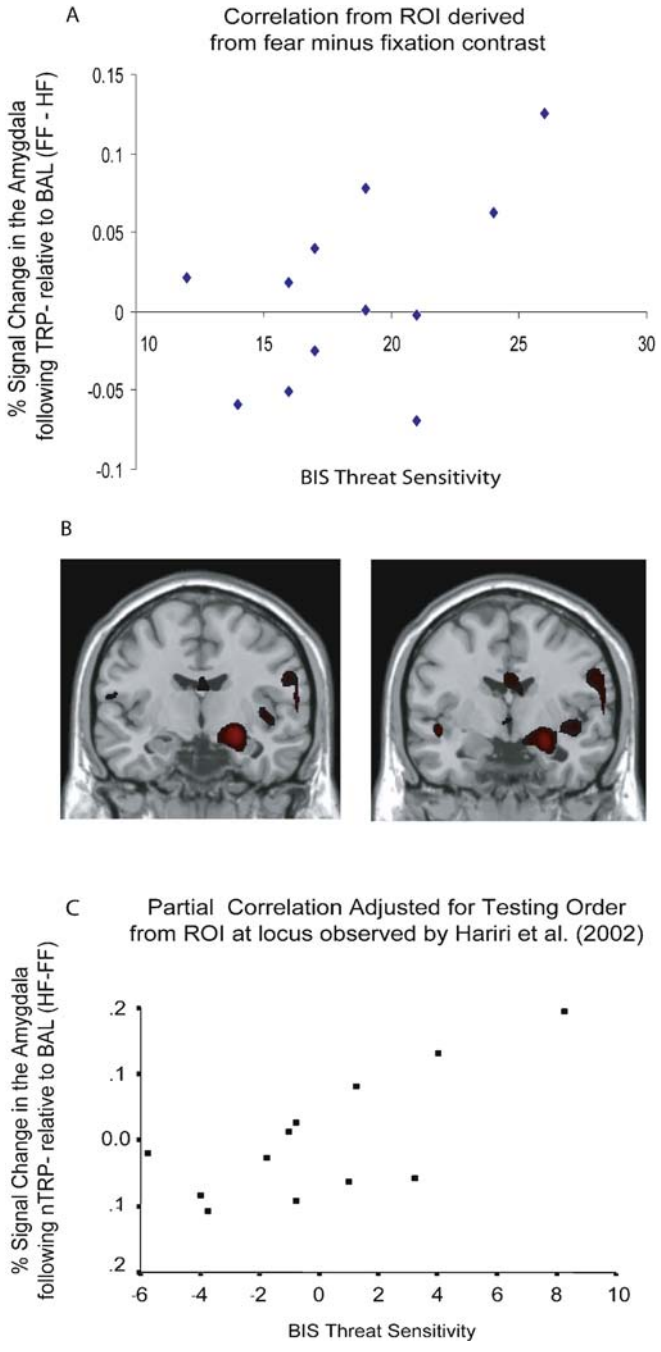
The strong a priori prediction that ATD would enhance brain activation in the amygdala/hippocampus in response to fearful faces enabled us to use region of interest (ROI) analyses. ROIs were defined a priori as all those clusters reaching significance at $P$<0.05 (corrected for multiple comparisons at the whole brain level) as revealed by contrast (1), which compared fearful faces with fixation (across TRP- and BAL sessions). This contrast was orthogonal to our main contrasts of interest, which examined treatment effects.

The MarsBar tool (Brett et al. 2002) was used to average the signal within these independently defined regions of interest (ROI) at the group level. The random effects model was then re-applied to the average signal within these ROIs in order to test the statistical significance of our contrasts of interest (one-sample one-tailed $t$ tests) at the group level. Average signal change was extracted from each ROI and these are the values reported in Figs. 1 and 2. ROI analyses were thresholded at $P$<0.05 (one-tailed).

## Results

### Functional imaging data

All significant effects revealed by contrast (1), reflecting signal change induced by fearful faces relative to fixation,




◀ **Fig. 1** **a** Scatterplot of individual scores on Behavioral Inhibition System scale (BIS threat sensitivity; *x*-axis) vs percent signal changes in right amygdala activation by fearful faces (*FF*) compared with happy faces (*HF*) following acute tryptophan depletion (*ATD*) relative to placebo (*y*-axis). Note that individuals with larger scores on the threat sensitivity trait tend to show greater enhancement of amygdala signal by ATD. **b** The anatomical locations of the association between threat sensitivity and ATD effects on brain activation are shown superimposed on two coronal slices (approximate *y* dimensions in MNI space: −9 and −4) from a single brain T1 template image in MNI space. These images were generated with MRIcro (Rorden and Brett 2000) by overlaying the statistical parametric map (all *t* values>1), representing the significance of the slope of the regression line relating the treatment effect on the amygdala response to BIS scores. **c** Scatterplot of threat sensitivity scores (*x*-axis) vs percent signal changes in right amygdala activation by fearful faces (*FF*) compared with happy faces (*HF*) following acute tryptophan depletion relative to placebo (*y*-axis), after correction (partial correlation) for testing order. These signal change values were extracted from a 5-mm spherical region of interest at Talairach coordinates *x*, *y*, *z*=24, −8, −16 (Hariri et al. 2002)

are shown in Table 1. Consistent with our prediction, fearful faces induced significant signal change in the bilateral amygdalae as well as the fusiform gyrus (commonly known as the fusiform face area; Kanwisher et al. 1997).

## Effects of treatment and individual variation as a function of threat sensitivity

Whole brain and ROI analyses did not reveal any significant effects of treatment on the BOLD response to fearful faces when all participants were collapsed into one group. However, simple regression analyses revealed that individual differences in threat sensitivity, as measured with self-reported BIS scores, significantly predicted the treatment effect on the BOLD response in the right amygdala to fearful faces relative to happy faces (right amygdala ROI: $T$=1.95; $P$=0.039, $R_{12}$=0.53, $R^2$=0.28). This association is shown in Fig. 1a. Post-hoc examination of the statistical parametric map revealed that the peak was localised at MNI coordinates *x*, *y*, *z*=24, −9, −12, bordering the amygdala and hippocampus (Fig. 1b).

The association between threat sensitivity and the amygdala response to fearful faces was not confounded by testing order. In fact, a post-hoc partial correlation analysis showed that the correlation was more robust after correcting for testing order (ROI: $P$=0.013, $R_9$=0.66, $R^2$=0.44).

The coordinates are almost identical to those reported by Hariri et al. (2002; Talairach coordinates *x*, *y*, *z*=24, −8, −16), who found modulation of the right amygdala response

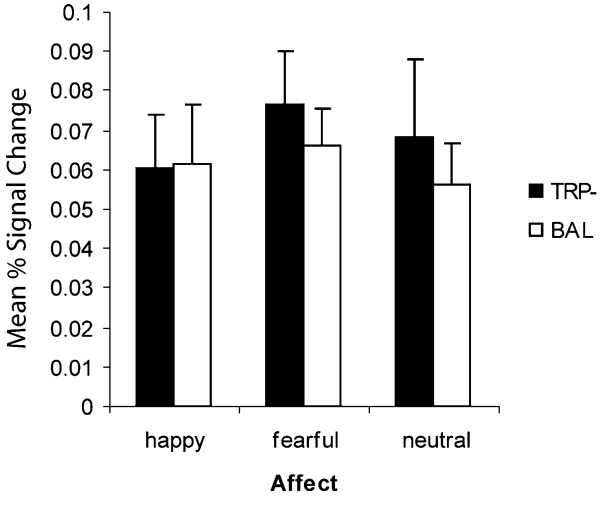



**Fig. 2** Effects of acute tryptophan depletion (TRP-), compared to control amino acid drink (BAL), on mean percent signal change in the right amygdala to happy, fearful and neutral faces. Tryptophan depletion enhanced amygdala activation in response to fearful and neutral faces, but the effects were not significant on average over all participants

**Table 1** Significant effects—local maxima—during fearful faces relative to fixation ($P<0.05$; $t=7.59$)

| Area | *x* | *y* | *z* | *T* | *Z* | Cluster size |
|---|---|---|---|---|---|---|
| Right amygdala/hippocampus | 24 | −12 | −15 | 8.8 | 4.7 | 10 |
| Left amygdala/hippocampus | −18 | −3 | −15 | 8.1 | 4.5 | 5 |
| Right fusiform face area | 39 | −57 | −21 | 10.9 | 5.1 | 71 |
| Prefrontal cortex | 57 | 24 | 18 | 11.2 | 5.2 | 75 |
| Premotor cortex | −54 | 3 | 39 | 8.7 | 4.68 | 4 |
| Supplementary motor area | 3 | 6 | 57 | 8.6 | 4.65 | 5 |
| Occipital cortex | 12 | −90 | −9 | 10.02 | 4.95 | 122 |
| | −30 | −81 | −12 | 9.81 | 4.91 | 68 |
| | −18 | −99 | 9 | 9.4 | 4.83 | 15 |
| | 15 | −99 | 12 | 8.25 | 4.57 | 3 |

to fearful faces by 5-HTTLPR genotype. Supplementary analysis of our effect restricted to a spherical ROI (5 mm) centred on these coordinates confirmed the overlap in locus between the present study and the study by Hariri et al. ($T=2.33$, $P=0.02$, $R_{12}=0.58$, $R^2=0.34$; following partial correction for testing order: $P=0.006$, $R_9=0.72$, $R^2=0.52$; Fig. 1c).

For illustrative purposes, group mean percent signal change scores from our right amygdala ROI are shown in Fig. 2 as a function of treatment and block type. Note that the effects of treatment across the group as a whole did not reach significance level.

There was no association between threat sensitivity and the BOLD response to fearful faces in any of the other ROIs, nor at the whole brain level.

Supplementary analyses revealed that there was also no association between threat sensitivity and the BOLD response to happy faces in any of the ROIs (relative to neutral faces or fixation).[1]

## Behavioural effects of treatment

The interaction between treatment and facial expression did not reach significance level ($F_{2,22}=2.48$, $P=0.13$). There were also no correlations between threat sensitivity, as measured with the BIS scale, and performance changes as a function of treatment (for all comparisons, $P>0.4$; e.g., correlation between BIS scores and ATD-induced performance changes for fearful faces relative to happy faces, $R_{12}=-0.04$, $P=0.9$). Mean proportions of correct responses on the gender categorisation task in each treatment condition are presented in Fig. 3.

[1] In reply to a Reviewer's question, we performed an additional, post-hoc correlation analysis between neuroticism scores obtained from the short Eysenck Personality Questionnaire (Eysenck and Eysenck 1991) and found that these did not correlate with ATD-induced changes in the amygdala response to fearful faces relative to happy faces ($R_{12}=-0.23$, $P=0.24$). Thus, the effect seems specific to anxiety sensitivity as measured by the BIS, rather than to neuroticism/negative emotionality per se, and this latter null result is in keeping with previous research (Canli et al. 2001).

## Biochemical measures

The analysis of amino acid levels revealed that the depletion was successful. At baseline, there were no differences between conditions in terms of plasma TRP level [g/ml, mean (SD): BAL 11.0 (2.3), TRP- 11.7 (3.6)] and ratio TRP/∑LNAA [mean (SD): BAL 0.18 (0.08); TRP- 0.16 (0.06)]. After 6.5 h, plasma TRP and the ratio TRP/∑LNAA were significantly lower in the TRP- condition compared with the balanced condition [TRP levels: BAL 15.9 (4.8), TRP- 4.2 (1.8); $T_{11}=9.96$, $P<0.001$ and ratios: BAL 0.16 (0.09); TRP- 0.04 (0.03); $T_{11}=4.33$, $P=0.001$, respectively]. Following the TRP- drink, plasma TRP was reduced by 64% and the ratio TRP/∑LNAA by 75% relative to baseline. Following the balanced drink, the plasma TRP was increased by 44% and the ratio TRP/∑LNAA was reduced by 11%.

## Subjective effects

No interaction effects were observed between ATD treatment and time on PANAS positive and negative affect scores (positive affect: $F_{1,11}=0.8$, $P=0.4$; negative affect:

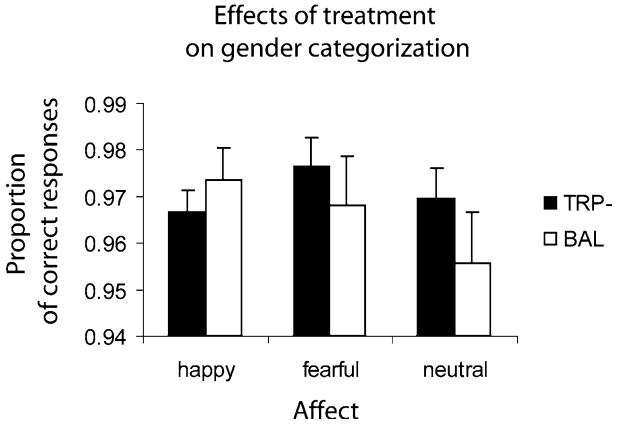



**Fig. 3** Effects of acute tryptophan depletion (TRP-), compared to control amino acid drink (BAL), on the proportion of happy, fearful and neutral faces correctly categorised as male or female. There are no significant behavioural effects of acute tryptophan depletion

$F_{1,11}$=1.0, $P$=0.3), whilst there were significant decreases over time across drink treatment for both scores (positive affect: $F_{1,11}$=6.6, $P$=0.03; negative affect: $F_{1,11}$=5.8, $P$=0.03). Similarly, whilst one main effect of time reached significance for VAS scores (Anxiety decreased over time: $F_{4,24}$=5.4, $P$=0.01), no significant interaction effects between time and drink treatment on VAS scores were observed (Drowsiness: $F_{4,24}$=0.6, $P$=0.6; Sadness: $F_{4,24}$=1.2, $P$=0.3; Happiness: $F_{4,24}$=1.8, $P$=0.2; Anxiety: $F_{4,24}$=2.4, $P$=0.15; Nausea: $F_{4,24}$=1.4, $P$=0.3). For VAS, one set of measures at timepoint 4 was missing for two participants.

## Discussion

The present study revealed no significant overall effect of acute tryptophan depletion on the amygdala/hippocampus response to fearful faces when all participants were collapsed into one heterogeneous group. However, further analyses revealed that the effect of acute tryptophan depletion on the amygdala/hippocampus response varied with individual variation in self-reported threat sensitivity. Thus, tryptophan depletion modulated amygdala/hippocampus activation to fearful faces relative to happy faces as a function of self-report threat sensitivity.

Although the effect observed in the present study extended from the amygdala to the hippocampus (Fig. 1), the coordinates correspond most closely to previously reported amygdala foci (Hariri et al. 2002; Williams et al. 2001). For this reason, we refer to the focus of our effect as 'amygdala' as opposed to 'amygdala/hippocampus' throughout the remainder of the discussion. The validity and interpretation of our findings do not depend on this assumption.

We argue that it is unlikely that the effects reflect global (e.g., vascular) effects on the BOLD signal, since whole brain and ROI analyses did not reveal any significant effects of treatment on the BOLD response to fearful faces when all participants were collapsed into one group. Moreover, the effect was specific to the amygdala and did not extend to other ROIs. Finally, the effects were specific for the fearful–happy contrast and supplementary analyses revealed that there was no modulation of the BOLD response to happy faces in any of the ROIs (relative to neutral faces or fixation). The latter finding also indicates that it is unlikely that the observed effect during fearful relative to happy faces can be accounted for by modulation of activation during happy faces. We argue that the comparison of fearful with neutral faces may not have been sufficiently sensitive to reveal the fear-related effects of ATD, because 'neutral' may not really be perceived as 'neutral', especially in anxious individuals. This latter argument is supported by the previous finding that amygdala activation to neutral faces correlates positively with state anxiety (Somerville et al. 2004). Moreover, it is in keeping with the presently observed behavioural as well as amygdala activation patterns across the group as a whole, suggesting that the neutral faces were perceived as negative despite attempts to reduce this effect by morphing 75% neutral faces with 25% happy faces (see Materials and methods).

The effect of ATD on the BOLD response is also not confounded by performance differences, suggesting that non-specific attentional, perceptual and motivational differences did not contribute to the amygdala change. The performance data trended towards poorer gender categorisation of happy faces and better categorisation of the perhaps more salient fearful and neutral faces following ATD. Whilst concurring with the present amygdala activation profile, this pattern appears inconsistent with findings from Harmer et al. (2003b) and Attenburrow et al. (2003) who observed, respectively, that acute tryptophan depletion reduced and acute tryptophan loading enhanced fear perception in a facial expression recognition task. Those results are paradoxical, because ATD is a model of depression and tryptophan has been widely used as an antidepressant. Critically, however, Harmer et al. (2003b) observed impaired fear recognition in female volunteers only, whilst finding facial expression recognition unchanged in male volunteers. There is considerable variation as a function of gender in terms of both the amygdala BOLD response in general (Hamann and Canli 2004) as well as sensitivity to ATD (Booij et al. 2002). For example, Williams et al. (2003) observed that the 5HTTLPR short allele was associated with higher 5-hydroxyindoleacetic acid (5-HIAA) levels in women but lower levels in men. Therefore, in future research, gender should be taken into account in research evaluating the effects of serotonergic manipulations.

The finding that tryptophan depletion modulates amygdala/hippocampus activation concurs with observations from studies with animals. Thus, the firing of amygdala neurons to stimulus-evoked neuronal activity was inhibited by iontophoresis of 5-HT onto amygdala neurons (Stutzmann and LeDoux 1999). In addition, 5-HT depletion was shown to abolish the inhibition of firing activity of hippocampal neurons during conditioned fear stress-induced freezing behaviour (Tada et al. 2004).

Our finding that high-BIS individuals are more sensitive to the effects of tryptophan depletion on fear-related responses is also in keeping with (neurobiological) models of anxiety (Rosen and Schulkin 1998; McNaughton and Gray 2000) as well as with observations that physiological reactivity to threat-related stimuli is greater in vulnerable (e.g., fearful or threat-sensitive) individuals (Bishop et al. 2004; Mathews et al. 2004; Ohman and Soares 1994). For example, Rosen and Schulkin (1998) proposed a sensitisation model, in which pathological anxiety is conceptualised as an exaggerated fear state and in which hyperexcitability of fear-related brain areas (e.g., the amygdala) is expressed as increased sensitivity to fear-related stimuli. In the Gray and McNaughton (2000) model, anxiety is viewed as a specific state arising from the interaction between the amygdala (which codes for fear and implements heightened arousal) and the septohippocampal behavioural inhibition system, and so heightened anxiety sensitivity would be associated with increased amygdala activity in fear-relevant situations.

The selectivity of the effect of acute tryptophan depletion (ATD) in highly threat-sensitive participants is consistent with previous observations that ATD has its greatest effects in vulnerable populations (Bjork et al. 2000; Cherek and Lane 1999; LeMarquand et al. 1999; Marsh et al. 2002). Thus, ATD induces relapse of depressive symptomatology, but only in individuals with a (family) history of affective illness (Benkelfat et al. 1994; Young et al. 1985).

The present results are most directly relevant to and consistent with the observation that the amygdala response to fearful faces and aversive pictures depends on allelic variation between individuals in the promoter region of the 5-HT transporter (5-HTT) gene (Hariri et al. 2002; Heinz et al. 2005). Hariri et al. (2002) observed greater amygdala activation (at almost identical coordinates) in *s*-carriers than in those participants homozygous for the long allele. *S*-carriers are more likely to express abnormal levels of anxiety (Lesch et al. 1996; Bethea et al. 2004), fear conditioning (Garpenstrand et al. 2001) and mood disorders (Caspi et al. 2003). The short allele of the 5-HTTLPR polymorphism is associated with reduced 5HTT expression, and, whilst one might expect that this would lead to less uptake and more 5-HT in the synaptic cleft, in fact, it is associated with *reduced* 5-HT function. Thus, the short allele is associated with an attenuated prolactin response to SSRI challenge in humans (Reist et al. 2001; Bethea et al. 2004; Whale et al. 2000), lower platelet 5-HT uptake (Greenberg et al. 1999) and lower concentrations of serotonin metabolites in cerebrospinal fluid, at least in Caucasian men (Williams et al. 2003). It has been argued that these effects may result from a lifelong difference in 5HTT gene transcription, leading to long-term neurochemical adaptations (Bethea et al. 2004). In addition, the transporter may not only remove 5-HT from the synapse, but also export 5-HT, thereby increasing extracellular 5-HT (Bethea et al. 2004). Thus, our data are fully consistent with the findings of Hariri et al. (2002) and reinforce the relationship between the amygdala, fear processing, 5-HT function and its dependence on individual differences (Sen et al. 2004; Munafo et al. 2004; Heinz et al. 2005).

Whilst our finding is in keeping with previous studies and current theorising, it should be considered with caution at this stage. In particular, the sample size of the current study was small and included male participants only. Hence confirmation of the reported dependency on threat sensitivity will depend on future experiments.

To conclude, our results indicate that the serotonergic modulation of amygdala/hippocampal activation to fearful vs happy faces varied according to individual differences in threat sensitivity in a manner that conformed to predictions derived from existing theoretical accounts. Future research is necessary to replicate this observation, to assess whether similar amygdala response profiles are observed in people with pathological anxiety, to what extent these may be normalised by 5-HT potentiating drugs and, finally, to determine whether individual differences in gender can account for current discrepancies in the literature.

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