

Anticipation of emotionally aversive visual stimuli activates right insula

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Understanding the neural substrates of anticipation is required for a comprehensive model of the ways in which anxiety influences information processing. While it is apparent that the insula and medial frontal cortex are involved in processing anticipation of physical (i.e., painful) stimuli, their role in processing anticipation of aversive affective stimuli has yet to be determined. Twenty-eight healthy non-phobic volunteers observed aversive affective images (spiders

and snakes) that were preceded by an auditory signal. The insula, dorsolateral prefrontal cortex, and parahippocampal gyrus activated during anticipation of aversive affective images. These findings indicate that common neural circuitry is involved in the anticipation of (and, perhaps, the subjective experience of anticipating) aversive affective and noxious physical stimuli. *NeuroReport* 15:2261–2265 © 2004 Lippincott Williams & Wilkins.

Key words: Anticipatory anxiety; fMRI; Insula; Pain

INTRODUCTION

One key component of anxiety is the anticipation of future harm [1]. In phobic individuals anxiety occurs not only during exposure to the specific object or condition of the phobia, but also in anticipation of experiencing the object or condition [2]. Thus, anticipation is a critical aspect of anxiety processing. Previous investigations have shown that subjects with mood and anxiety disorders respond differently than healthy controls to emotionally salient stimuli during the appraisal (i.e., the time when cognitive and emotional reactions to the stimulus occur) [3] and regulation (i.e., the time when the individual modulates his/her reaction to the stimulus) [4] phases. However, the anticipatory phase (i.e., the period of time prior to the presentation of an expected stimulus) has been less well studied. This is somewhat surprising as a key component of anxiety disorders is the sensitized anticipation of harm [5].

In a PET study, transient anticipatory anxiety was induced by the anticipation of an electric shock [6]. Decreased regional cerebral blood flow was observed in the ventral medial prefrontal cortex during anticipatory anxiety relative to an eyes-closed resting condition. It was concluded that the medial prefrontal cortex 'is part of a network... whose activity reflects a dynamic interplay between cognitive task performance and emotion' [6]. Another study found activation in the insula, superior frontal parietal sulcus, fusiform gyrus, and dorsal anterior cingulate during anticipation of an electrical shock [7]. Finally, Ploghaus *et al.* [8] found that the anterior insula appeared active during the anticipation of a noxious thermal stimulus.

Several functional neuroimaging studies have implemented aversive visual stimuli (e.g., images of spiders and

snakes, known to be common phobic stimuli [9]) to probe the neural substrates of anxious processing. Paquette and colleagues [10] studied spider-phobic individuals and found activation in the parahippocampal gyrus and dorsolateral prefrontal cortex. Unlike studies looking at physical pain [7,8,11], involvement of the insula in anxious processing was not observed in this study.

This study aims to identify the neural circuitry involved in anticipating aversive affective stimuli. We hypothesized increased activation in the insula, parahippocampus, and medial prefrontal cortex during the anticipation phase. Moreover, if anxiety-related personality variables are related to the increased anticipation of future harm, one would hypothesize that these should correlate with signal activation during the anticipation phase.

MATERIALS AND METHODS

Subjects: Twenty-eight healthy non-phobic subjects (18 females and 10 males) mean (\pm s.d.) age 28.5 ± 11.9 years (range 18–56) with an average education level of 13.9 ± 1.2 years (range 13–18) without a life-time history of Axis I mental disorders based on a Structured Clinical Interview for DSM-IV [12] participated in this study, which was approved by the University of California, San Diego School of Medicine Human Research Protection Program. All subjects gave written informed consent to participation.

Measures: After completion of the task (described below), individuals rated all 30 images. Twenty-three of 28 subjects completed computerized ratings with standardized instructions based on the IAPS normative ratings technique for

each snake and spider picture [13]. Ratings were acquired in three domains: happy-unhappy, calm-excited, and dominant-submissive.

All subjects were asked to complete the Neuroticism Extraversion Openness Five Factor Inventory (NEO-FFI) [14].

Subjective fear of snakes and spiders was measured using the following two self-ratings (using 7 point Likert scales): "If I saw a snake now, I would feel very panicky" and "If I came across a spider now, I would leave the room." These two items were taken from the Fear of Spiders Questionnaire [15] and the Fear of Snakes Questionnaire (a modified version of the Fear of Spiders Questionnaire). In a non-published dataset of 83 college students we found that scores on the questions above correlated very highly with total score on the Fear of Snakes Questionnaire ($r=0.932$, $p<0.001$) and Fear of Spiders Questionnaire ($r=0.913$, $p<0.001$), respectively.

The anticipation task combined a continuous performance task with the intermittent presentation of aversive affective stimuli (Fig. 1). During the continuous performance task, subjects were asked to press a left mouse button whenever they saw a circle and a right mouse button whenever they saw a square on the screen. Stimuli were presented at a visual angle of 4° at a rate of 0.5 Hz. Simultaneously, a 250 ms 500 Hz tone was presented at a rate of 2 Hz. Subjects were instructed that during the task, the pitch of the tone would change (from 500 to 1000 Hz) 4–6 s prior to the appearance of a picture of a spider or a snake. Thirty images of spiders or snakes, mostly obtained from the International

Affective Picture System (IAPS), were then presented. The total duration of the task was 512 s. Response selection and latency data were collected during the continuous performance task. No response was required when an image of a snake, spider, or fixation cross was presented on the screen.

The continuous performance task provided response accuracy and response latency information for the low tone, high tone, and post-stimulus (Fig. 1b). By subtracting these, we determined the effect of anticipation of (i.e., low minus high tone) and exposure to the aversive visual stimulus (i.e., post-stimulus minus low tone).

fMRI protocol: Blood oxygenation level-dependent contrast was collected using a 1.5T Siemens scanner (T2*-weighted echo planar imaging, TR=2000 ms, TE=40 ms, 64×64 matrix, 20 4 mm axial slices, 256 scans) along with a high resolution T1-weighted image (MPRAGE, TR=11.4 ms, TE=4.4 ms, flip angle=10°, FOV=256 × 256, 1 mm³ voxels) for anatomical reference.

Three regressors were constructed: (1) the low tone regressor, measuring the continuous performance task, (2) the high tone regressor, capturing the anticipatory phase, and (3) the stimulus regressor, for the aversive visual stimuli (as depicted in Fig. 1b). The difference in activation between the high and low tone was interpreted to represent the activation due to anticipation.

Data were preprocessed and analyzed with the Analysis of Functional NeuroImages software package [16].

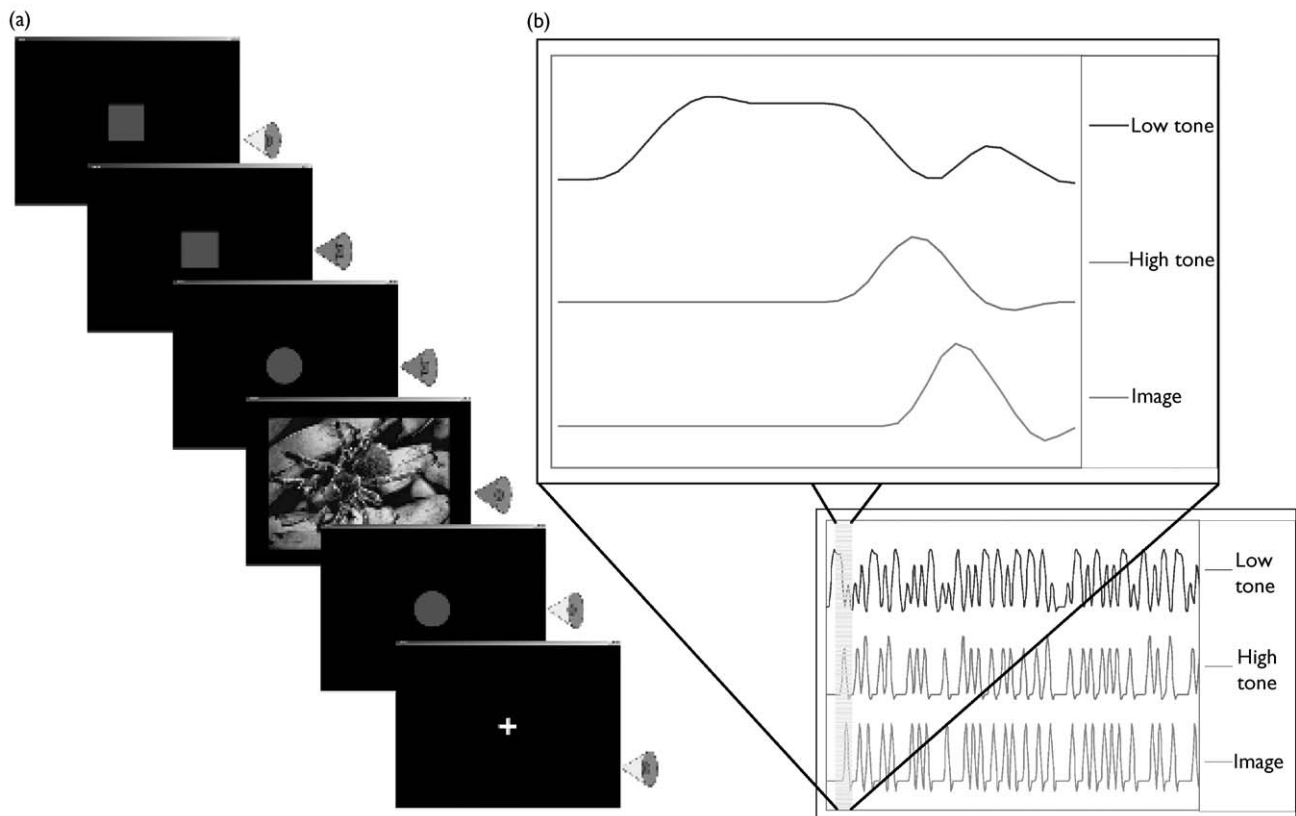


Fig. 1. Task time series design. (a) Visual depiction of task (low and high tones are depicted by light and dark speakers, respectively). (b) Sample of expected hemodynamic response for time series function.

Preprocessed time series data for each individual were analyzed using a multiple regression model consisting of eight regressors. Regressors of interest included three task-related regressors and three movement-related regressors were used to account for residual motion (in the roll, pitch, and yaw direction). Regressors for baseline and linear trends were used to eliminate slow signal drifts. A Gaussian filter with full width-half maximum 6 mm was applied to the voxel-wise percentage signal change data to account for individual variations of the anatomical landmarks. Data of each subject were normalized to Talairach coordinates.

Statistical analyses: Voxel-wise percentage signal change data for the whole brain were entered into a planned mixed model ANOVA with response type as a fixed factor and subjects as a random factor. Contrasts were obtained from the difference between continuous task and high tone regressors. A threshold adjustment method based on Monte-Carlo simulations was used to guard against identifying false positive areas of activation [17]. A prior voxel-wise probability of $p < 0.01$ in a cluster of $512 \mu\text{l}$ resulted in an *a-posteriori* probability of $p < 0.01$. Finally, the average percent signal difference was extracted from regions of activation that were found to survive this threshold/cluster method. All analyses for the behavioral data were carried out with SPSS 10.0 [18].

Brain behavior analyses: First, activation levels acquired from clustered areas of activation during the high tone minus low tone were correlated (using a Pearson product-moment correlation) with average image ratings in the happy, calm, and dominant domains. Second, activation levels acquired from areas of interest (as above) and image ratings were correlated with three NEO-FFI scales: (1) neuroticism, (2) openness, and (3) extraversion (*t*-scores). Third, activation levels acquired from areas of interest (as above) during the high tone and image ratings were correlated with response accuracy and latency data.

RESULTS

Behavioral results: Subjects rated the images of snakes (mean \pm s.d. 3.57 ± 1.68) and spiders (3.46 ± 2.05) similarly ($t(22) = 0.25$, $p = \text{n.s.}$) on a Likert scale of how unhappy (1) to happy (8) the image made them feel. Subjects reported similar ($t(23) = -0.57$, $p = \text{n.s.}$) levels of fear of snakes (2.58 ± 2.04) and spiders (2.92 ± 2.24) on a 7-point Likert scale. This finding supports the idea that, on average, subjects found both types of pictures to have similar emotional valence. Snake image ratings and fear questionnaire responses did not correlate significantly with spider image ratings or fear questionnaire response ($r = 0.279$, n.s. and $r = 0.115$, n.s. , respectively), which indicates that the level of discomfort was stimulus specific.

Response accuracy did not differ significantly between low tone, high tone, and post-stimulus conditions ($F(2,25) = 1.237$, n.s.). However, post-stimulus response latencies were significantly longer than those during the low-tone and high-tone phase ($F(1,26) = 53.83$, $p < 0.001$); the low tone and the high tone phase response latencies did not differ significantly ($t(27) = 0.649$, n.s.).

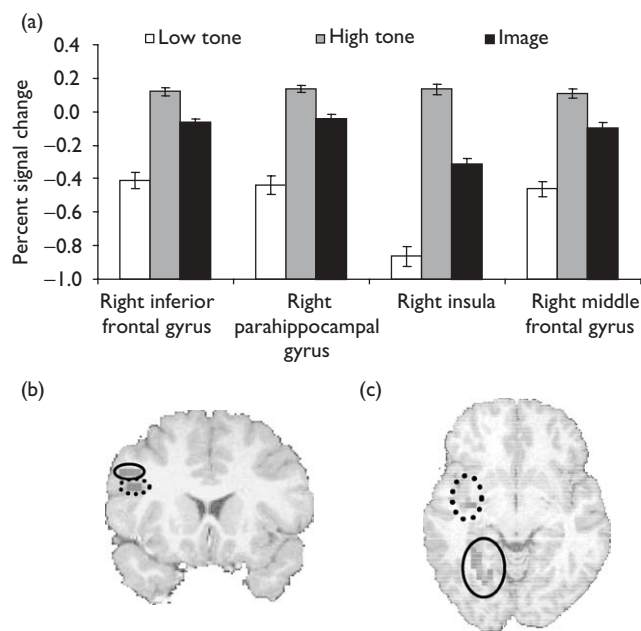


Fig. 2. Activation changes during the high tone–low tone (a) activation for significant clusters in each task condition (b) solid circle: Right BA9; dotted circle: Right BA46; (c) solid circle: BA19; dotted circle: Right Insula. Note x intercept is at -1 percentage signal change.

Table 1. Significant activation clusters for high tone–low tone.

Location (Brodmann Area)	Side	Volume	x	y	z
Inferior frontal gyrus (9)	Right	1536	38	5	28
Parahippocampal gyrus (19)	Right	1152	26	-54	-4
Insula	Right	1152	38	-5	-3
Middle frontal gyrus (46)	Right	576	42	20	20

Functional results: During the high-tone condition relative to the low tone condition, which signifies the anticipation period, activation was observed in right inferior frontal gyrus (BA9), right parahippocampal gyrus (BA19), right insula, and right middle frontal gyrus (BA46) (Fig. 2; Table 1).

Brain–behavior interactions: Increased activation in the right parahippocampal gyrus, including the amygdala, during the anticipation period, was associated with an increased negative appraisal of the spider and snake images (Table 2). Parahippocampal gyrus activation was significantly related to the combined and spider ratings, but not the snake ratings (Fig. 3). However, activation in the four regions activated during the anticipation phase (Table 1) did not correlate significantly with personality measures, phobia questionnaires, or behavioral performance data.

DISCUSSION

This investigation focused on the neural network pertinent to anticipation of aversive affective stimuli and yielded two main results. First, increased activation in the right anterior insula and parahippocampal gyrus was observed when subjects were presented with a stimulus that predicted the

Table 2. Pearson correlations for image rating and functional activation during high tones.

Area of activation	Side	Snake	Spider	Combined
Inferior frontal gyrus (9)	Right	0.313	0.113	0.253
Parahippocampal gyrus (19)	Right	-0.062	-0.564**	-0.421*
Insula	Right	-0.213	-0.114	-0.198
Middle frontal gyrus (46)	Right	0.219	0.072	0.173

$n=23$;

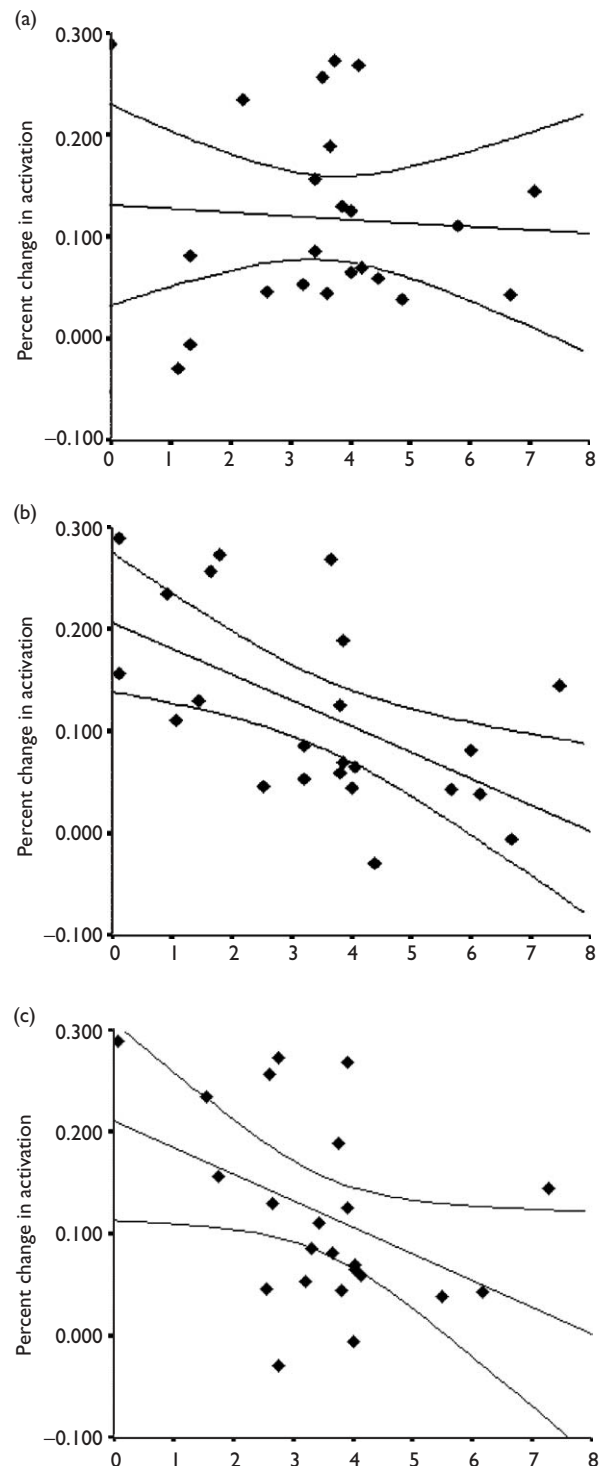
* $p < 0.05$;

** $p < 0.005$.

future onset of aversive emotional stimuli. Second, the degree of activation during the anticipation period in the parahippocampal gyrus was related to the ratings of image aversiveness. These results support the hypothesis that a network of limbic regions including the anterior insula and parahippocampus is critically involved in the anticipation of aversive emotional stimuli.

Previous investigations have shown that the anterior insula is involved in the anticipation of physical pain [8,19]. However, its involvement in anticipating aversive emotional stimuli has not been previously studied. This study provides evidence that the anterior insula is a key structure for anticipating emotionally aversive, as well as physically painful stimuli. Although viewing these images did not result in the subjects rating themselves as more than moderately unhappy, responses to post-task questionnaires indicated that the images were experienced as unpleasant and aversive. Price [20] has suggested that physical pain and emotional distress involve many of the same brain pathways including the anterior insula and anterior cingulate. He suggests that the neural circuitry for physical pain differs from pain-related affect as the former includes sensory-motor and posterior parietal cortex involvement. The current study provides complementary evidence and supports the notion that anticipation of emotionally aversive stimuli involves brain structures that are also implicated in the anticipation of physically painful stimuli. Consistent with these conclusions, a recent study by Eisenberger and colleagues [21] found that social rejection shares similar pathways with physical pain.

Paradigms looking at the reactions of phobic individuals to both spiders and snakes have found activation of the amygdala, insula [22], and anterior cingulate [23]. Paquette and colleagues [10] presented pictures of spiders and butterflies to pretreatment spider phobic patients. They subtracted these two conditions and found the parahippocampal gyrus and dorsal lateral prefrontal cortex activated uniquely to the phobic stimulus. This activation decreased after successful treatment for the phobic condition. This may suggest that the parahippocampal gyrus and dorsolateral prefrontal cortex are activating due to fear or fear conditioning. In the current study, activation in the parahippocampal gyrus correlated significantly with ratings of image adversity (i.e., on the unhappy to happy dimension). This correlation may be due to an interaction between negative appraisal and anticipation, where greater negative appraisal increases the emotional anticipatory effect. Interestingly, personality variables did not appear to relate to levels of activation, suggesting that this system is highly context specific.

**Fig. 3.** Scatterplot of activation in the parahippocampal gyrus by image ratings for (a) snakes, (b) spiders, and (c) all images.

The current study has several limitations. First, the task design did not allow for the subtraction of the activation related to a change in tone from the activation due to anticipation of a negative image. Any directly cued design must have a sensory cue that is tied to the initiation of the anticipatory period, which provides a common confound in

studying anticipation. In the current study, the anticipatory cue, unlike the image, does not appear to be a significant distractor, as the response accuracy and latency do not change during the high tone in contrast to the low tone. A second limitation of the current study was the lack of information about image aversiveness. Individuals were asked to rate their subjective happiness or unhappiness as a result of viewing the image, and not how aversive the images were perceived. By asking about subjective impact on mood, rather than how aversive the images were judged to be, we hoped to more directly capture the affective impact of the image in a way that a rating of aversiveness might not. Future studies in this area should capture measures of image aversiveness as well as the subjective impact of these aversive stimuli on mood.

CONCLUSION

This study provides qualified support for the idea that common neural substrates, including the anterior insula, underlie the anticipation of emotionally aversive and physically painful stimuli.

REFERENCES

1. Eysenck MW. *Anxiety and Cognition: a Unified Theory*. Hove, UK: Psychology Press; 1997.
2. Tillfors M, Furmark T, Marteinsdottir I and Fredrikson M. Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. *Biol Psychiatry* 2002; **52**:1113–1119.
3. Siegle GJ, Steinhauer SR, Thase ME, Stenger VA and Carter CS. Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biol Psychiatry* 2002; **51**:693–707.
4. Beauregard M, Levesque J and Bourgouin P. Neural correlates of conscious self-regulation of emotion. *J Neurosci* 2001; **21**:RC165.
5. Bradley BP, Mogg K and Lee SC. Attentional biases for negative information in induced and naturally occurring dysphoria. *Behav Res Ther* 1997; **35**:911–927.
6. Simpson JR, Jr., Drevets WC, Snyder AZ, Gusnard DA and Raichle ME. Emotion-induced changes in human medial prefrontal cortex: II. During anticipatory anxiety. *Proc Natl Acad Sci USA* 2001; **98**:688–693.
7. Chua P, Krams M, Toni I, Passingham R and Dolan R. A functional anatomy of anticipatory anxiety. *Neuroimage* 1999; **9**:563–571.
8. Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM and Rawlins JN. Dissociating pain from its anticipation in the human brain. *Science* 1999; **284**:1979–1981.
9. Kendler KS, Myers J, Prescott CA and Neale MC. The genetic epidemiology of irrational fears and phobias in men. *Arch Gen Psychiatry* 2001; **58**:257–265.
10. Paquette V, Levesque J, Mensour B, Leroux JM, Beaudoin G, Bourgouin P and Beauregard M. 'Change the mind and you change the brain': effects of cognitive-behavioral therapy on the neural correlates of spider phobia. *Neuroimage* 2003; **18**:401–409.
11. Sawamoto N, Honda M, Okada T, Hanakawa T, Kanda M, Fukuyama H, Konishi J and Shibasaki H. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *J Neurosci* 2000; **20**:7438–7445.
12. First MB, Spitzer RL, Gibbon M and Williams JB. *Structured Clinical Interview for DSM IV Axis I Disorders – Patient edition (SCID-I/P, vers 2.0)*.
13. Lang PJ, Bradley MM and Cuthbert BN. Emotion and motivation: measuring affective perception. *J Clin Neurophysiol* 1998; **15**:397–408.
14. Costa PT and McCrae RR. Normal personality assessment in clinical practice: the NEO Personality Inventory. *Psychol Assess* 1992; **4**:5–13.
15. Szymanski J and O'Donohue W. Fear of Spiders Questionnaire. *J Behav Ther Exp Psychiatry* 1995; **26**:31–34.
16. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers Biomed Res* 1996; **29**:162–173.
17. Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA and Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. *Magn Reson Med* 1995; **33**:636–647.
18. Norusis MJ. *Spss Base System User's Guide*.
19. Porro CA, Baraldi P, Pagnoni G, Serafini M, Facchin P, Maieron M and Nichelli P. Does anticipation of pain affect cortical nociceptive systems? *J Neurosci* 2002; **22**:3206–3214.
20. Price DD. Central neural mechanisms that interrelate sensory and affective dimensions of pain. *Mol Interventions* 2002; **2**:392–403.
21. Eisenberger NI, Lieberman MD and Williams KD. Does rejection hurt? An fMRI study of social exclusion. *Science* 2003; **302**:290–292.
22. Dilger S, Straube T, Mentzel HJ, Fitzek C, Reichenbach JR, Hecht H *et al*. Brain activation to phobia-related pictures in spider phobic humans: an event-related functional magnetic resonance imaging study. *Neurosci Lett* 2003; **348**:29–32.
23. Pissiota A, Frans O, Michelgard A, Appel L, Langstrom B, Flaten MA and Fredrikson M. Amygdala and anterior cingulate cortex activation during affective startle modulation: a PET study of fear. *Eur J Neurosci* 2003; **18**:1325–1331.

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