Supporting Information

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SI Experimental Procedures

Participants. Twenty-five healthy nonphobic volunteers took part in the study. Subjects were rejected if they had any history of neurological damage or psychiatric disorder. After the MRI scan, five participants were excluded as they expressed reservations about whether the study was genuinely in real time during debriefing. This left 20 participants (10 female; mean age, 25.8 ± 3.7 y). Trait anxiety was measured using the Spielberger questionnaire, with a mean score of 40 ± 9.9. These scores are comparable to the published norms for this age group mean (36 ± 10) (1). Subjects were remunerated £30 for time, travel, and inconvenience. All subjects gave informed consent, and the study was approved by the Essex Research Ethics Committee (United Kingdom).

FSQ Measures. During recruitment, subjects were asked if they had any significant fear of spiders, including tarantulas, and given details on the experiment. Those who expressed reservations were not followed up and not included in the study. We also collected FSQ scores on each subject (2). These mean FSQ scores were in medium to low ranges (mean ± SD, 30.7 ± 18.4; range, 14–86.1). Overall, this mean score is significantly lower than that of diagnosed arachnophobic subjects (89.1 ± 19.6) (3).

Spider Stimuli Creation and Validation. We recorded footage of a Brazilian salmon pink tarantula (*L. parahybana*; body size, 22 cm length × 15 cm width; Fig. S1*A*) being placed in each compartment of the imminence box. Video clips were edited to 4 s and a border was added to the footage to decontextualize the environment. Sixty black-and-white 4-s film clips of the tarantula were then presented in the study (12 in each compartment of the imminence box). Each film was prerated for movement of the tarantula on a four-point scale: 1, no movement; 2, very little movement (<25% of the time); 3, movement 25% to 50% of the time; and 4, movement 50% to 100% of the time. Ratings were made by 10 independent observers. No significant differences were found between compartments ($P > 0.05$).

There was also no significant relationship between participants' experienced fear ratings and movement ($r = 211$; $P = 0.105$). In addition, we tested to see if the direction the spider was facing (toward vs. away from the foot) influenced fear ratings. We found no significant correlation between the tarantula's angle of orientation to the foot, from 0° to 180°, and fear ratings ($P > 0.05$). Finally, mindful of likely habituation effects, we pseudorandomized the presentation of the tarantula in each box so as to decorrelate distance from time (Pearson correlation, $r = 0.09$; $P < 0.495$).

Experimental Setup and Paradigm. Immediately before the scanning session, participants were shown a tarantula housed in a distant glass tank, but were not told whether it was real (in fact, it was a robotic model). This robot tarantula was viewed in a box containing soil, bark, and plants, and emitted subtle lifelike movements. After this, subjects were placed supine on the MRI scanner bed (Fig. S1*B*) and placed their foot, with the shoe removed, into the custom-built open-topped imminence box, which contained six compartments separated by sliding partitions (Fig. S1*C*). A curtain was placed at the end of the scanner bore so subjects could not see their foot or the box.

Although they were not explicitly told so, participants believed they could view the imminence box compartments in real time via a live camera feed from above this open-topped box. Via this video feed, participants watched the tarantula placed pseudorandomly in the compartments at distances of approximately 1 to 18 cm, 18– to 36 cm, 36 to 54 cm, 54 to 72 cm, and 72 to 90 cm from their foot (Fig. S1*C*). Although participants believed contact with the spider was possible, the compartment containing the participant's foot was not used. The top of the compartment was open, and there was potential for contact (e.g., there was no physical barrier between the top of the subject's foot and the tarantula). This possibility was drawn to the subjects' attention before scanning. Subjects were first showed the imminence box and told that their foot would be placed in the tall chamber. The experimenter then stated: "The barrier [between the foot chamber and first spider chamber] only covers half of the chamber where your foot will be, so the top of your foot will be exposed to box 5. However, the experimenter will be in the room at all times, and it is unlikely that the spider will have any direct contact with you."

In reality, participants were viewing prerecorded films of the spider in the different compartments as described earlier. To complete the illusion that activity involving the real and unseen imminence box was occurring in real time, the experimenter remained in the scanner room and moved the robot tarantula from compartment to compartment in synchrony with the video footage of the real Tarantula that the participants were actually viewing. Postscan debriefing revealed that participants believed that they were viewing a genuine real-time video feed of a proximate live tarantula.

Each experimental trial commenced with viewing a schematic representation of the imminence box indicating which compartment the tarantula would enter next. Participants then used a VAS to predict how afraid they would feel when subsequently viewing the tarantula in the relevant compartment (i.e., expected fear). The participants next saw the Tarantula in the compartment and directly rated how afraid they felt (i.e. experienced fear; Fig. S2).

Image Acquisition. MRI scanning was conducted at the Medical Research Council Cognition and Brain Sciences Unit on a 3-T Tim Trio MRI scanner (Siemens) by using a head coil gradient set. Whole-brain data were acquired with echoplanar T2*-weighted imaging (i.e., EPI), sensitive to BOLD signal contrast (48 sagittal slices, 3-mm thickness; repetition time, 2,400 ms; echo time, 30 ms; flip angle, 78°; field of view, 192 mm; voxel size, 3 × 3 × 3 mm). To provide for equilibration effects, the first five volumes were discarded. T1-weighted structural images were acquired at a resolution of 1 × 1 × 1 mm.

Image Preprocessing. SPM5 software (www.fil.ion.ucl.ac.uk/spm/) was used for data analysis. The EPI images were sinc interpolated in time for correction of slice timing differences and realignment to the first scan by rigid body transformations to correct for head movements. Field maps were estimated from the phase difference between the images acquired at the short and long TE and unwrapped by using the FieldMap toolbox. Field map and EPI imaging parameters were used to establish voxel displacements in the EPI image. Application of the inverse displacement to the EPI images served the correction of distortions. For each participant the mean EPI was calculated and examined to guarantee that none exhibited excessive signal dropout in insula and ventral striatum. Using linear and nonlinear transformations, and smoothing with a Gaussian kernel of full-width half-maximum 8 mm, EPI and structural images were coregistered and normalized to the T1 standard template in MNI space (International Consortium for Brain Mapping). Moreover, global changes were removed by proportional scaling, and high-pass temporal filtering

with a cutoff of 128 s was used to remove low-frequency drifts in signal.

Statistical Analysis. After preprocessing, statistical analysis was performed using the general linear model. Our regression matrix included the cue periods (1 s), both expectancy (8 s) and outcome VAS (8 s) time periods, and a 4-s period when the spider was shown in the relevant box. Analysis was carried out to establish each participant's voxel-wise activation during the 4-s presentation of the spider. Activated voxels in each experimental context were identified using an event-related statistical model representing each of the experimental contexts, convolved with a canonical hemodynamic response function and mean-corrected.

All subjects had head motion of less than one voxel (3 mm) across the whole session. To correct for possible confounding effects of head motion under different conditions, we included the head motion parameters as regressors of no interest in our subject-specific (i.e. first level) models. Multiple linear regression was then run to generate parameter estimates for each regressor at every voxel. For group analysis, a random-effects model was used with a small volume correction for FWE within a priori areas of interest, including the amygdala (left, −18,−4, −30; right, 10, −6, −18), BNST (left, −9, 0, −12; right, 12, −1, −12), medial orbital frontal cortex (−3, 48, −18), and midbrain PAG (8, −32, −21). These coordinates were taken from previous studies (4–7), and when needed, converted into MNI space. A spherical ROI 6 to 12 mm in diameter was centered on these coordinates, and statistical inferences were corrected for multiple comparisons within this ROI (FWE, $P < 0.05$). This produces statistical inferences that are FWE-corrected centered on the aforementioned independent ROI coordinates. Outside of these areas of interest, we also present results at $P < 0.05$ FWE-corrected for whole-brain multiple spatial comparisons. When false-negative results at these corrected thresholds would be of particular relevance, we also provide results at the exploratory uncorrected threshold of $P < 0.001$ uncorrected. For areas outside our a priori ROIs, we use FWE correction for the whole brain to give strong control of type I error. There are situations in which exploration at more liberal thresholds is likely to be of interest to the reader, especially if type II error is relevant. We therefore do present selected results at this liberal uncorrected threshold. Our principal conclusions, and tests of our hypotheses of interest, do not rely on these uncorrected tests.

Questionnaires. Following the MRI scan, participants were asked to complete the FSQ and to rate how much scarier than expected the spider was. (For example, they were asked, "Was the spider as scary as you thought?") We also asked subjects to complete a memory for tarantula size test to rate how large they thought the tarantula was by using five different sizes of the spider printed on an A3 sheet of paper (Fig. S4). This was administered between 45 and 60 min after the experiment.

1. Spielberger CD (1983) *Manual for the State Trait Anxiety Inventor* (Consulting Psychologists, Palo Alto, CA).
2. Szymanski J, O'Donohue W (1995) The fear of spiders questionnaire. *J Behav Ther Exp Psychiatry* 26:31–34.
3. Muris P, Merckelbach H (1996) A comparison of two spider fear questionnaires. *J Behav Ther Exp Psychiatry* 27:241–244.
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5. Mobbs D, et al. (2007) When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* 317:1079–1083.
6. Hermann A, et al. (2009) Emotion regulation in spider phobia: Role of the medial prefrontal cortex. *Soc Cogn Affect Neurosci* 4:257–267.
7. Mobbs D, et al. (2009) From threat to fear: The neural organization of defensive fear systems in the human brain. *J Neurosci* 29:12236–12243.

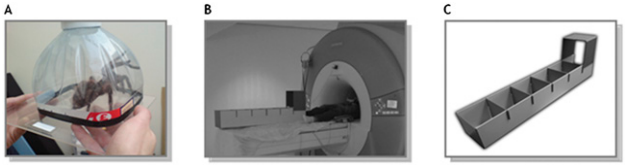

Fig. S1. (*A*) Photograph of the Brazilian pink salmon tarantula (*L. parahybana*) used in the video footage. (*B*) Picture of the experimental setup showing a subject supine in the MRI bore and with his/her foot in the (*C*) custom built imminence box.

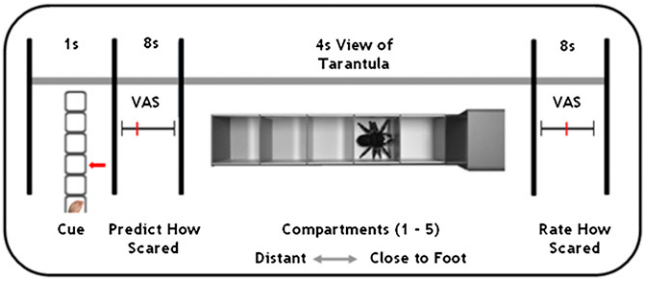

Fig. S2. A schematic representation of the experiment order and timings.

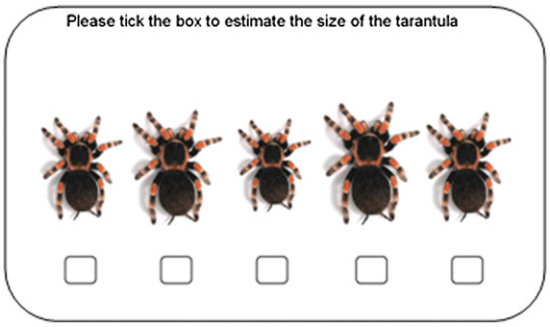

Fig. S3. Changes in fear and expectancy (i.e., underestimation) errors and fear of spiders over time. (*A*) Expectancy errors over time. Red dots signal mean value. The correlations between (*B*) increasing underestimation errors and sustained amygdala activation (−10, −6, −18; $P < 0.039$, svc), and (*C*) FSQ scores and sustained midbrain PAG activation (8, −30, −4; $P < 0.038$, svc) over the course of the experiment.

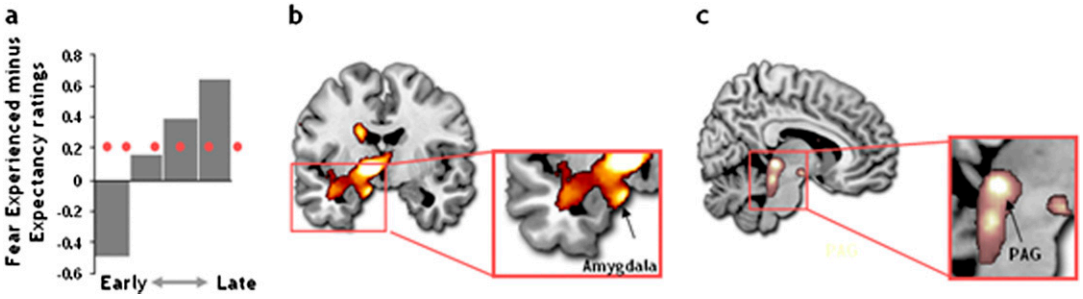

Fig. S4. The memory for tarantula size test (not to scale).

Table S1. Comparison between compartments 5/4 and 1/2 [(compartment 5 + compartment 4) − (compartment 1 + compartment 2)]

Region	MNI coordinates, x/y/z	z value	*P* value
Compartment (5 + 4) − (1 + 2)			
Midbrain	4/−30/−24	3.10	0.028*
Dorsal ACC	−4/28/36	4.13	<0.0005†
Left amygdala	−20/2/−16	2.74	0.030*
Right amygdala	16/8/−14	3.49	0.006*
Compartment (1 + 2) − (5 + 4)			
omPFC	2/52/−6	2.94	0.032*

**P* value small volume corrected using independent coordinates.
†*P* value whole-brain corrected.

Table S2. Parametric modulation weighted by tarantula compartment

Region	MNI coordinates, x/y/z	z value	*P* value
Closer to the foot			
Midbrain	4/−30/−24	2.86	0.028*
Dorsal ACC	28/36/24	4.13	<0.0005†
dlPFC	30/48/30	4.25	<0.0005†
Right insula	44/14/−4	3.47	<0.0005‡
Left insula	−44/2/10	3.52	<0.0005‡
Visual cortex (V1)	2/−66/0	4.83	<0.0005†
Intraparietal cortex	12/−68/64	4.38	<0.0005‡
BNST	12/0/−4	3.14	<0.014*
Further from the foot			
omPFC	−6/54/−16	3.09	0.016*
PCC	−10/−54/20	3.29	<0.001‡
Visual cortex (V1)	−2/−94/12	4.91	<0.0005‡

PCC, posterior cingulate cortex.
**P* value small volume corrected using independent coordinates.
†*P* value whole-brain corrected.
‡*P* value uncorrected.

Table S3. Parametric modulation by tarantula distance correlated with FSQ scores

Region	MNI coordinates, x/y/z	z value	*P* value
Increased FSQ scores			
Midbrain	14/−26/−4	3.30	<0.009*
Right insula	54/−4/8	3.53	<0.0005†
Left insula	−46/−4/4	3.16	<0.001‡
Decreased FSQ scores			
Perigenual ACC	20/44/6	2.99	<0.036*

**P* value small volume corrected using independent coordinates.
†*P* value whole-brain corrected.
‡*P* value uncorrected.

Table S4. Parametric modulation by postexperimental ratings of how much scarier than expected the subjects found the spider

Region with Increased fear of approach scores	MNI coordinates, x/y/z	z value	*P* value*
Midbrain	10/−24/−6	2.94	<0.023
Amygdala	36/−2/−22	2.69	<0.042

*Small volume corrected using independent coordinates.

Table S5. Approach minus retreat independent of distance

Region	MNI coordinates, x/y/z	z value	*P* value
Amygdala	14,/−2/−16	2.90	<0.024*
Right BNST	12/0/−4	2.85	<0.027*
Left BNST	−12/4/−4	3.10	<0.014*
Left insula	−40/16/0	2.94	0.037*
Right insula	40/20/−2	3.68	0.002*
Dorsal ACC	6/34/22	3.37	<0.0005†
Striatum	12/4/4	3.64	<0.003*

All regions were still significant after covarying out FSQ scores.
**P* value small volume corrected using independent coordinates.
†*P* value uncorrected.

Table S6. Parametric modulation by tarantula distance correlated with expectancy error (fear underestimation) scores

Region	MNI coordinates, x/y/z	z value	*P* value*
Left amygdala	−20/0/−18	—	<0.043
Right insula	−30/26/2	3.39	0.013
Underestimate (covarying out FSQ scores)			
Left amygdala	−18/2/−22	2.80	<0.034
Underestimate (covarying out mean fear scores)			
Left amygdala	−26/−6/18	3.88	<0.004

*Small volume corrected using independent coordinates.

Table S7. Habituation for four time bins (T1–T4) over the course of the experiment independent of distance

Region	MNI coordinates, x/y/z	z value	*P* value
Decreased activity			
Midbrain PAG	12/−32/−14	2.81	<0.029*
Hypothalamus	−4/2/−12	3.46	<0.005*
sgACC	8/26/−12	2.84	<0.046*
Amygdala	−24/−2/−14	2.77	<0.030*
Insula	52/26/0	3.22	<0.001†
Increased activity			
MFD	−22/46/0	3.07	<0.001†

**P* value small volume corrected using independent coordinates.
†*P* value uncorrected.

Table S8. Habituation for four time bins over the course of the experiment independent of distance examining the correlation with expectancy errors.

Region increased with errors	MNI coordinates, x/y/z	z value	*P* value
Left amygdala	−10/−/6/−20	3.29	<0.001*
Right amygdala	34/−2/−18	2.73	<0.046†
MFD	16/56/6	3.52	<0.0005*
Mediodorsal thalamus	0/6/−2	3.46	<0.0005*
No significant voxels	—	—	—

**P* value uncorrected.
†*P* value small volume corrected using independent coordinates.

Table S9. Habituation for four time bins over the course of the experiment independent of distance examining the correlation with FSQ scores

Region	MNI coordinates, x/y/z	z value	*P* value
Increased with FSQ			
Midbrain PAG	8/−30/−4	2.71	<0.038*
Parahippocampal gyrus	−38/−20/−20	3.24	<0.001†
Ventral motor cortex	48/16/22	3.18	<0.001†
Decreased with FSQ			
Dorsal caudate	−14/2/24	3.31	<0.0005†
dlPFC	−36/50/18	3.05	<0.001†

**P* value small volume corrected using independent coordinates.
†*P* value uncorrected.