Supporting Information (SI)

Supporting Material and Methods

Pre-scanning test for rubber hand illusion

We tested potential participants on the rubber hand illusion in a separate experiment a few days before the planned brain scan. The procedure has been described in a previous paper (1), and involves the participants completing a "rubber hand questionnaire". The nineteen who finally participated in the fMRI experiments all reported that they experienced the rubber hand illusion (see below).

For the pre-scanning test, the potential participants were seated in front of a table. A life-size, realistic, rubber prosthesis of a male or female right hand (gender matched) was placed in front of the participant. The participant's real right hand was hidden behind a screen 20 cm to the right of the rubber hand. The rubber hand was placed in parallel with the participant's real right hand so that it looked like the real hand. After a 60-second period of synchronous or asynchronous brushing of the rubber hand and the hidden real hand (order balanced across participants), the participants were asked to describe what they had experienced (without leading questions), and asked to complete a questionnaire containing five questions. These questions, based on those used by Botvinick and Cohen (2), required a rating of the strength of agreement or disagreement with five perceptual effects. The first two questions were relevant to the illusion ("Feeling that the rubber hand was my hand", "Feeling of touch located on the rubber hand") and the remaining three served as controls for suggestibility and compliance with task demands (2). The participants used a seven-point visual analogue scale to rate the extent to which these statements did or did not apply. On this scale, 1 meant "absolutely certain that it did not apply", 4 meant "uncertain whether or not it applied", and 7 meant “absolutely certain that it applied”. The nineteen volunteers who went on to participate in the fMRI experiment all confirmed that they experienced the rubber hand illusion in these initial tests (i.e. scored 5-7 on the two relevant questions). They all described typical aspects of the rubber hand illusion such as “the rubber hand feels like my hand” and “the rubber hand senses the touch”. Nine participants who denied that they felt the illusion, or were uncertain about it (i.e. scored 1-4 on the relevant questions), were not scanned.

Monitoring of hand movement

The participants were trained to keep their hand and fingers still when the needle approached the rubber hand or the real hand. A video-camera filmed the participant’s real right hand throughout the experiments. Further, the experimenter who applied the brushstrokes to the hands observed the real hand closely. Potential muscular contractions of the index finger were monitored using MR-compatible electromyograms with a set of surface electrodes placed on the right first interosseus muscle (Brain Products GmbH, Munich, Germany; the data were sampled, stored and visualized on a PC using Vision Recorder and Vision Analyser software from the same company).

EPI scanning parameters

The EPI acquisition was optimized to yield the maximal BOLD sensitivity in the orbitofrontal cortex and the amygdala without significantly reducing it in the other regions of interest such as the frontal and parietal lobes (4, 5). The slices were tilted by 20 degrees (in addition to the 20 degrees the head was actually tilted in the head-coil) with respect to the axial plane and the phase-encoding polarity was chosen such that sensitivity losses due to in-plane susceptibility gradients were minimized. In addition, a z-shim compensation gradient prepulse (-1.3 mT/m*ms) was applied prior to the EPI readout to reduce dropouts due to through-plane susceptibility gradients. One functional image volume of the brain was collected every 3.96 seconds (Repetition time (TR)=3960 ms). To minimize the image ghost, the EPI images were reconstructed using a generalized reconstruction method based on the measured EPI k-space trajectory (6).

Small volume correction

Because we had an *a priori* hypothesis that threatening the hand would activate certain areas associated with pain anticipation and anxiety, we used a small volume correction in these regions (spheres with a radius of 16mm). Specifically, we used peaks from previous studies that have shown that the insular cortex (7) (x=-39, y=-3, z=-9; x=39, y=24, z=-3), anterior insular cortex (8) (x=±40, y=26, z=10), the anterior cingulate cortex (7) (x=±6, y=3, z=45), and the medial wall motor areas (9) (x=6, y=-6, z=62; x=10, y=6, z=50) are consistently activated in pain or pain anticipation studies (9, 10). For all other regions we used the threshold of $p<0.05$ after a correction for the number of voxels in the whole brain (Family-Wise-Error correction).

Additional analyses of the fMRI data.

In addition to the three analyses described in the Material and Methods section of the main paper we conducted three additional analyses of the data.

First, because not all participants experienced seeing the needle approaching one's hand as threatening, as indicated by low anxiety scores, we could examine whether there was a linear relationship between the increase in anxiety during the illusion and the augmented neuronal threat response. For this we used a linear regression model (the second-level SPM 2 simple regression model) to relate the increase in anxiety as reported by the participants during the ownership condition as compared with the no ownership condition, with the stronger neuronal threat response during the illusion (*threat during ownership – threat during no ownership*). The results are presented in the Supporting Results section below and in SI Fig. 6.

Second, we examined if there was a significant relationship between activity in the premotor or parietal cortex that reflects the illusion of ownership [1], and activity in the insula that reflects anxiety. Thus we correlated the anxiety response in the insular cortex (the parameter estimates from the contrast; *threat during synchronous – threat during asynchronous*) to the illusion related activity in the bilateral ventral premotor cortex and the left intraparietal cortex (parameter estimates from the regressor corresponding to the ownership condition). For this we used the second level SPM 2 simple regression (correlation) model and searched for voxels that showed a linear relationship with the insular activity across subjects (SI Fig. 7). We restricted this analysis to spheres of 20-mm radius around the peaks taken from our previous study where we identified areas active during the rubber hand illusion (1).

Finally, we looked for areas in which there was activity when we threatened a hand regardless of whether the person experienced the ownership illusion or not. This was done by examining the main effect of needle threat across all runs (*threat during ownership + threat during no ownership + threat during real hand*). The results are presented below and in SI Fig. 9.

Anatomical localization

The anatomical localization of the activations was related to the major sulci and gyri (11), distinguishable on a mean MRI generated from the standardized anatomical MRIs from the nineteen subjects.

Supporting Results

Subjective ratings: additional analyses

There was a significant correlation between the strength of the illusion and the anxiety when the rubber hand was threatened ($p<0.05$; SI Fig 5B). This result indicates a strong link between the feeling of ownership of the rubber hand and the degree of anxiety evoked when it is physically under threat.

Further, the participants who reported the greatest anxiety when their real hand was threatened also tended to report the greatest anxiety when the rubber hand was threatened during the illusion. In other words there was a significant correlation between the anxiety ratings during the real hand and rubber hand ownership conditions ($p<0.05$; SI Fig 5A). These observations highlight the similarities between the experienced anxiety when the real hand and the rubber hand were threatened.

Finally, the anxiety associated with the needle-threat in the ownership condition did not habituate. The participants rated significantly greater anxiety in the illusion condition than in the control condition throughout the fMRI experiments ($p<0.05$ paired 2-way t-test, data not shown).

Monitoring of hand movement

Although some subjects spontaneously reported an urge to remove the hand when it was threatened by the needle, we observed no actual movements of their real hand as evident from visual inspection, video recording analysis, and the inspection of the electromyograms from a index finger muscle (right first interosseus muscle).

Main effect of seeing the needle approach a hand

Finally, we identified activity related to seeing the needle approaching a hand (main effect of threat; *threat during ownership* + *threat during no ownership* + *threat during real hand*; $p<0.05$ corrected). In this analysis we observed activation in the insula bilaterally, ACC, and medial motor areas (SI Fig. 9 and SI Table). This activity presumably reflects the visual processing of an aversive stimulus near a hand regardless of whether the person feels ownership of the hand or not. The insular clusters were centred in the anterior part of the insula and extended into the frontal operculum. The medial wall activation included a distinct peak for the pre-SMA. We also noted conspicuous activations of the bilateral superior and inferior posterior parietal cortex and in the bilateral extrastriate cortex.

Supporting Discussion

It was not surprising that we found activity in these areas when the participants saw the needle in the asynchronous condition without illusion (the main effect of threat). These responses could reflect empathy for pain as the mirror neuron theory suggests, or simply general anxiety triggered by seeing a potentially harmful object (the needle). The crucial finding, however, is that there was additional activity in the ACC, insula and pre-SMA that was specifically related to threat to one's own body (e.g. Fig 3 and Fig 2).

In a recent fMRI study, Lloyd and colleagues (12) reported enhanced parietal, frontal and occipital activation when participants saw a rubber hand being "injured" by a needle. However, in that experiment the feeling of ownership of the hand was not assessed and the occurrence of a rubber hand illusion was probably eliminated by the incongruent tactile and visual stimulation when the needle hit the rubber hand (and not the real hand). In the present study, we also found activity in the posterior parietal cortex and extrastriate areas, and this reflected seeing the needle near the hand (see SI Table). But crucially, this activity was found irrespective of whether the person felt ownership of the hand or not. Thus, it is likely that these activations represent the visual processing of aversive objects, rather than anticipatory anxiety related specifically to one's own body or to an artificial limb during the illusion of ownership.

It could be argued that Armel and Ramachandran have already shown that forceful bending of the finger of the rubber hand elicits anxiety (13). They measured the skin conductance response of participants and reported that it was enhanced during the illusion of ownership. However, our functional imaging study extends these findings in several important ways, because skin conductance responses are unspecific and do not inform us about the activity of particular cortical areas. First, we have shown that the interoceptive system is as strongly engaged when the rubber hand is threatened in the ownership condition as when the real hand is threatened. Second, we have demonstrated that there is activity in the pre-SMA in both these conditions, suggesting the urge for the participants to withdraw the rubber hand. Third, our results reveal a direct positive relationship between the strength of the ownership illusion and the amplitude of the threat-evoked responses in the introceptive areas. Finally, we have shown that the premotor and parietal activity that is related to the illusion correlates with the engagement of the interoceptive system.

References for Supporting Information

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