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Unconditioned responses and functional fear networks in human classical conditioning

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Abstract

Human imaging studies examining fear conditioning have mainly focused on the neural responses to conditioned cues. In contrast, the neural basis of the unconditioned response and the mechanisms by which fear modulates inter-regional functional coupling have received limited attention. We examined the neural responses to an unconditioned stimulus using a partial-reinforcement fear conditioning paradigm and functional MRI. The analysis focused on: (1) the effects of an unconditioned stimulus (an electric shock) that was either expected and actually delivered, or expected but not delivered, and (2) on how related brain activity changed across conditioning trials, and (3) how shock expectation influenced inter-regional coupling within the fear network. We found that: (1) the delivery of the shock engaged the red nucleus, amygdale, dorsal striatum, insula, somatosensory and cingulate cortices, (2) when the shock was expected but not delivered, only the red nucleus, the anterior insular and dorsal anterior cingulate cortices showed activity increases that were sustained across trials, and (3) psycho-physiological interaction analysis demonstrated that fear led to increased red nucleus coupling to insula but decreased hippocampus coupling to the red nucleus, thalamus and cerebellum. The hippocampus and the anterior insula may serve as hubs facilitating the switch between engagement of a defensive immediate fear network and a resting network.

Keywords

fMRI; conditioning; psychophysiological interaction; connectivity; insula; PPI

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1.1 Introduction

Classical fear conditioning is used extensively in rodent and human studies to examine the neural mechanisms underlying fear acquisition and expression (Pavlov 1927). In these studies, a neutral stimulus, such as a light, is paired with an intrinsically aversive unconditioned stimulus (US) such as an electric shock. After pairing, the neutral stimulus becomes a conditioned stimulus (CS). Both animal and human neuroimaging studies, focusing on responses to the CS, have found that the CS presentation is associated with increased activity in the amygdala, the brainstem, the insula, and parts of the anterior cingulate cortices (ACC) (see Sehlmeier et al. 2009 for a review). Animal studies indicate that the conditioned response (CR) exhibits considerable temporal specificity, with the peak CR occurring at the time when the US is delivered (Burman & Gewirtz 2004, Davis, Schlesinger, & Sorenson 1989). It is important to note that in temporally predictable fear conditioning, both the perception of the US *and* the immediate expectancy of the shock (“I am supposed to get shocked *now*”) occur almost simultaneously, so that both processes likely result in neural activity changes measurable by fMRI. Similarly, in partial reinforcement paradigms, the US may be expected but not delivered and processes related to both the immediate expectancy (*now*) and the prediction error (“Oh, I guess I did not get shocked”) occur almost simultaneously.

In contrast to the CS and prediction errors (Schiller, Levy, Niv, LeDoux, & Phelps 2008, Schultz, Dayan, & Montague 1997, Seymour et al. 2004), the neural responses to the US have received limited attention (Dunsmoor, Bandettini, & Knight 2008, Knight, Waters, King, & Bandettini 2010) and the functional networks engaged by fear have only recently begun to be addressed (Mobbs et al. 2009).

In these experiments, we first sought to disambiguate brain responses to shock from those related to non-delivery of shock. Second, in order to explore how shock and non-delivered shock responses change over time due to learning, we measured relative changes in responses across conditioning trials. Third, we conducted a psychophysiological interaction analysis to investigate how fear influences inter-regional coupling within the fear processing network.

2 Materials and Methods

2.1 Subjects

Participants were right-handed, had normal or corrected vision, and were screened to exclude psychiatric or neurological problems as determined by a structured clinical interview following DSM-IV diagnostic categories (First, Spitzer, Gibbon, & Williams 1995). Participants largely overlapped with control subjects included in a previous publication on fear extinction (Milad et al. 2009). However, the neuroimaging data in the present study have not previously been reported. After removing individuals with poor fMRI data quality due to excessive head motion, data from twenty-four healthy adults — 13 women and 11 men — were included (mean age and SD 30 ± 11 years). All subjects gave informed consent, and the study was approved by the Partners Healthcare System Human Research Committee and conducted in accordance with the declaration of Helsinki.

2.2 Fear conditioning procedure

We used a fear conditioning paradigm with a 62.5% partial reinforcement schedule, as previously described (Milad et al. 2009, Milad et al. 2007). To habituate subjects to the scanner and procedure, we first did a short run in which all task images were displayed without shocks before beginning the fear conditioning procedure. During fear conditioning, subjects were presented with an image of a room containing an unlit lamp on a desk. In

different trials, the lamp was then switched on to one of three colors (blue, red, or yellow). Two of the colors (CS+) were followed by an electric shock (US) in 62.5% of the cases, and the third color was never followed by a shock (CS−). The fear conditioning procedure entailed 32 lighted lamp presentations, of which 16 were CS− trials, 10 were CS+ trials followed by a shock, and six were CS+ trials not followed by a shock. Between trials, a black screen was displayed with an inter-trial interval of 12 to 18 seconds. The lamp color sequence was counterbalanced across subjects with a pseudo-random presentation order. The CS duration of six seconds was kept constant across trials, providing the participants with high temporal predictability with respect to the US delivery. The 0.5 sec US was an aversive, but not painful, electric shock, delivered immediately after the offset of the CS, with no temporal overlap between the CS and the US. The US was a 500 ms train of electric shocks consisting of 1ms, 600V spikes delivered at 50Hz to the second and third fingers of the right hand with currents ranging from 0.2 to 4 mA. Prior to the experiment the shock current was individually adjusted for each participant so that it would be perceived as highly annoying but not painful. See Figure 1a and b for details.

2.3 Skin conductance

Skin conductance responses (SCR) were measured using a Coulbourn Modular Instruments System (Allentown, PA) Isolated Skin Conductance Coupler (S71-23) with 8 mm (sensor diameter) radiotranslucent Ag/AgCl electrodes (BioPac Systems Inc., Goleta, CA) placed 14 mm apart on the left palm. The SCR for each CS trial was calculated by subtracting the mean skin conductance level measured 2 sec before CS onset from the highest skin conductance level recorded during the 6 sec CS duration. In a similar fashion, SCR responses to the shock (UCR), the non-delivered shock and at the offset of the CS− were also calculated by subtracting the mean skin conductance level recorded 2 seconds immediately after the CS offset from the highest skin conductance level recorded 2 to 5 seconds later. This baseline was chosen to be after the CS to ensure that the UCR was not biased by SCR related to the CS.

2.4 Neuroimaging

2.4.1 Image Acquisition—A Trio 3.0 Tesla whole body, MRI system (Siemens Medical Systems, Iselin, New Jersey) equipped for echo planar imaging (EPI) with a 12-channel head coil was used. Subjects were instructed to lie as still as possible and head movement was restricted with foam cushions. After an automated scout image was obtained and automated shimming procedures were performed, high-resolution, T1-weighted, three-dimensional, magnetization-prepared, rapid acquisition gradient echo (MPRAGE) volumes were collected to facilitate spatial normalization and positioning the subsequent scans. Functional MRI images, sensitive to blood oxygenation level dependent (BOLD) contrast, were acquired with an interleaved gradient echo T2*-weighted sequence (TR= 3000 ms, TE= 30, Flip angle = 90°), collected in 45 coronal oblique slices tilted 30° down from the anterior-posterior commissure line. The voxel size was 3×3×3 mm.

2.4.2 Data analysis

2.4.2.1 Preprocessing: SPM8 (Wellcome Trust Center for Neuroimaging, www.fil.ion.ucl.ac.uk/spm) was used to process all MRI data. Structural images were segmented and spatially normalized to the Montreal Neurological Institute (MNI305) T1 template. Functional images were realigned, corrected for slice timing, spatially normalized to MNI space using transformation parameters obtained from spatial normalization of a coregistered high-resolution T1 image, resampled to 2×2×2 mm, and finally smoothed with an 8 mm FWHM Gaussian kernel to ensure normal distribution of the residual error, reduce spatial noise and to compensate for residual misregistration in the spatial normalization

process. High-pass temporal filtering with a cutoff of 128 seconds was included in the first-level statistical model to remove the effects of low frequency signal drifts. Serial correlations in the fMRI time series were estimated using an autoregressive AR(1) model. Subsequently, activation maxima locations were nonlinearly transformed to Talairach space using the mni2tal algorithm (http://eeg.sourceforge.net/doc_m2html/bioelectromagnetism/mni2tal_matrix.html).

2.4.2.2 First-level model: After preprocessing, each subject's functional time series was modeled using a general linear model with regressors signifying the experimental condition onsets and durations. The conditions were: (1) the 3 second office context, (2) the 6 second CS+, (3) the 6 second CS-, (4) the 0.5 second delivered shock, (5) the 0.5 seconds immediately after the offset of non-reinforced CS+ ("non-delivered shock"), and (6) the 0.5 seconds immediately after the offset of the CS- ("safe-no shock"). Movement parameters derived from the realignment processing step for x,y,z, and roll, pitch and yaw motion were also included in the model to remove residual motion-related noise. Activated voxels in each experimental phase were identified using a statistical model containing boxcar functions representing each of the six experimental conditions, convolved with the SPM8 canonical hemodynamic response function. Since the paradigm was optimized for shock temporal predictability we did not vary the CS duration. Jittering the stimulus duration is sometimes done to increase design efficiency and to avoid multi-collinearity among regressors, as correlated regressors may lead to instability of parameter estimation and a consequent reduction in sensitivity. In the present design the inherent multiple collinearity of the classical conditioning design was not problematic, as the Pearson (r^2) correlations among regressors ranged 0.01 to 0.42, with correlations among regressors in contrasts of interest reported below.

The shock and the non-delivered shock predictors were uncorrelated ($r^2 = 0.004$), allowing accurate separation of the neural shock response from associated expectancy components. In addition, the shock and the non-delivered shock conditions showed low correlations with the "safe-no shock" condition ($r^2 = 0.01$ and $r^2 = 0.006$ respectively). The present study was not optimized to detect differences between cue-induced activations and activity immediately after the termination of the cue, as there was some multiple collinearity between those two regressors ($r^2 = 0.36$).

2.4.2.3 Objective one: Expected and or delivered shocks: First-level contrast images of the delivered shock, the non-delivered shock, and the delivered versus non-delivered shock were obtained for each subject, and modeled in a random effects second level analysis using a repeated measures linear model with subject and task factors. We used a critical cluster threshold of $p < 0.05$ FWE-corrected for multiple comparisons and a size threshold of more than 20 contiguous voxels.

2.4.2.4 Objective two: Changes in shock responses across trials: As the experimental design entailed classical conditioning over several trials, we analyzed responses to the shock and non-delivered shock in a trial-by-trial manner to investigate if responses were sustained or variable across trials. The individual delivered and non-delivered shocks were contrasted to the implicit inter-trial baseline. Trial by trial responses were modeled in a random effects model using repeated measures ANOVA at the second level. Areas with sustained, decreasing, or increasing reactivity across trials were modeled with constant or linearly varying contrasts. A global conjunction of the sustained and the increasing contrasts identified activated areas displaying sustained and/or increasing activation across trials. A global conjunction of the sustained and the decreasing contrast identified areas displaying sustained and/or decreasing activation across trials. We used a critical cluster threshold of

$p < 0.05$ FWE-corrected for multiple comparisons and a size threshold of more than 10 contiguous voxels.

2.4.2.5 Objective three: Regional influences examined with psychophysiological interaction analysis: We next performed a psychophysiological interaction (PPI) analysis (Friston et al. 1997) analysis with three seed regions examining how fear (“non-delivered shock” versus “safe-no shock”) modulated inter-regional coupling. Individual seed selection was based on activity maxima detected in the univariate second-level analyses. The left and right hippocampi were selected as seeds *post hoc* based on observed hippocampus deactivations to non-delivered shock (left hippocampus Talairach_{xyz} = (-26, -16, -13), $Z = 6.21$, $P_{FWE} < 0.001$ right hippocampus (20, -13, -20), $Z = 6.61$, $P_{FWE} < 0.001$). The red nucleus was selected as a *post hoc* seed based on an observed red nucleus response to non-delivered shock, Talairach_{xyz} = (-6, -20, -7), $Z = 6.10$, $P_{FWE} < 0.001$.

For the PPI analysis, the hemodynamically deconvolved signal from the seed brain region time series (Friston et al. 1997, Gitelman, Penny, Ashburner, & Friston 2003), the psychological context, and the interaction between seed region activity and psychological context (PPI) were estimated using a general linear model. We used the contrast “non-delivered shock” versus “safe-no shock” as the psychological context modulation, in order to contrast the presence of shock expectancy to no shock expectancy, without contamination of the BOLD signal response to the effects of the delivered shock.

The PPI design matrix had three columns: the interaction between psychological context and seed brain region time-series, the psychological context modulation parameter (the two conditions of interest), and the physiological parameter (seed region time series). In accordance with the task-related univariate analysis, we also included six estimated movement parameters to account for any residual effects due to inter-scan head motion. The voxel-wise regression of the time-series from the entire brain on the three predictor variables resulted in a first-level PPI contrast for each seed region in every subject. The first level PPI analysis resulted in statistical parametrical maps revealing regions that were differentially modulated by the activity of the seed region, depending on the presence or absence of fear (non-delivered shock versus safe-no shock).

Subsequently, the individual PPI contrast images were modeled in a random effects second-level group analysis using a repeated-measures model with subjects and the three seed regions as factors. Thus, the second level analysis resulted in statistical parametrical maps of regions that were differentially modulated by the activity of the seed region, depending on the presence or absence of shock expectation. Clusters exceeding 20 voxels with interactions at $p < 0.001$, not corrected for multiple comparisons, are reported. The resulting seed $\times$ fear interactions were further decomposed into separate parameter estimates to illustrate how fear altered inter-regional coupling.

3 Results

3.1 Shock levels and skin conductance responses

Participants chose shock levels ranging from 1.7 to 4 mA (mean $\pm$ standard error = 2.2 ± 0.18) at 500V. Fear conditioning led to rapid learning of the CS-US association as evidenced by elevated SCR responses to the CS+ compared to the CS- (mean CS+ SCR $\pm$ std error = $0.14 \pm 0.04 \mu S^{1/2}$, CS- = $-0.01 \pm 0.04 \mu S^{1/2}$, $p = 0.001$). For the unconditioned response, the delivery of the US led to large SCRs of $0.71 \pm 0.07 \mu S^{1/2}$, the non-delivered shock led to SCRs of $0.21 \pm 0.05 \mu S^{1/2}$, and the “safe-no shock” led to SCRs of $0.13 \pm 0.05 \mu S^{1/2}$. The difference between SCRs to the shock and non-delivered shock, as well as between the non-

delivered shock and the “safe-no shock” were significant ($p < 0.001$ and $p = 0.003$ respectively). See Figure 1b for an example of individual SCR responses.

3.2 Objective one: Expected and or delivered shocks

3.2.1 Brain responses to delivered shock—Contrasting “shock” to “safe-no shock” BOLD signal responses identified activity induced by an expected, delivered shock compared to responses to expected, but undelivered shock. We observed BOLD signal increases ipsilaterally (shock was delivered to the right fingertips) below the sensory decussation of the brainstem and in the cerebellum, and contralaterally in the left midbrain, thalamus, amygdala, somatosensory cortex, and middle and dorsal regions of the cingulate cortex. We also observed significant ($P_{\text{FWE}} < 0.05$) bilateral activity increases in anterior and posterior insular cortex and the adjacent dorsal striatum, although the latter cluster failed to reach our cluster threshold. A significant decrease in BOLD signal was observed in the ventromedial prefrontal cortex (vmPFC), as shown in Figure 2 and Table 1.

3.2.2 Brain responses to the non-delivered shock—In association with the non-delivered shock effects, estimated to occur the moment immediately after the presentation of a non-reinforced CS+, when the shock was expected to be delivered but was actually omitted, we identified activity increases largely overlapping the shock-induced activations. Elevated BOLD signal (“non-delivered shock” versus “safe-no shock”) was observed in the left red nucleus of the midbrain, left anterior thalamus, and left dorsal anterior cingulate cortex, and bilaterally in the anterior insular cortex. Decreased BOLD signal was observed in the hippocampus, the right fusiform gyrus, the right medial frontal gyrus, the right pre- and postcentral gyrus, and in the left temporo-occipital transition zone (Figure 2 and Table 1).

3.2.3 Differential brain responses to delivered versus expected shock—Contrasting delivered with non-delivered shock related activity revealed significantly higher BOLD signal following the delivered shock in the left postcentral gyrus, the left precentral gyrus, left thalamus, bilaterally in the posterior insula and in the right culmen of the cerebellum. No regions displayed lower BOLD signal with respect to delivered versus non-delivered shock (Figure 2 and Table 1).

3.3 Objective two: Changes in shock responses across trials

Across the 10 delivered shock trials, we observed progressive diminution of the BOLD response to the shock in the left and right middle and posterior insula, as well as in the somatosensory cortex and in the middle cingulate. Conversely, the left and right anterior insula, the medial frontal gyrus, and the brainstem displayed sustained BOLD signal reactivity throughout the entire experiment. Subregions of the visual cortex displayed both sustained and decreased activity across trials. See Figure 3 and Table 3 for a complete list of regions. Across the six non-delivered shock trials, we observed sustained activity in the left and right anterior insula and frontal operculum, see Table 2.

3.4 Objective three: Regional influences examined with psychophysiological interaction analysis

Based on the results of the univariate analysis we identified three seed regions that were subsequently used in a psycho-physiological interaction (PPI) analysis whose purpose was to identify how the presence (“non-delivered shock”) or absence (“safe-no shock”) of shock expectation modulates the coupling between seed regions and the rest of the brain.

3.4.1 PPI of the hippocampus—The right and left hippocampus displayed decreased BOLD signal during both “non-delivered shock” and “safe-no shock” conditions. Individual time series were extracted using a 6 mm radius spherical ROI centered within 4 mm of (x, y, z) = (−23, −20, −17 and 17, −22, −13) for the left and the right hippocampus respectively. The average numbers of voxels ($\pm$ standard deviation) above threshold in each subject's seed were 43 ± 31 and 43 ± 24 voxels respectively. Mean individual activation thresholds were $p = 0.10$ (range $p < 0.001$ to $p < 0.5$) for the left and $p = 0.14$ (range $p < 0.001$ to $p < 0.5$) for the right hippocampal seeds. Both the right and left hippocampus showed positive PPI effects (i.e. increased functional coherence at fear compared to no fear) with the ventromedial prefrontal cortex ($Z = 4.09$ and 3.24 respectively, $p < 0.001$, uncorrected). The left hippocampus also displayed a positive PPI effect with the right pregenual ACC ($Z = 4.02$, $p < 0.001$, uncorrected). For the right hippocampus, we observed a negative PPI effect (i.e. decreased functional coherence at fear compared to no fear) with the cerebellum, the left insula, the left precentral gyrus and the right parahippocampal gyrus ($p < 0.05$, FWE-corrected). The left hippocampus displayed negative PPI effects ($p < 0.05$, corrected) with the left thalamus and the right cerebellum. See Table 3 and Figure 4a & c for an illustration of the decomposed interaction effects.

3.4.2 PPI of the red nucleus—The midbrain seed (centered in the left red nucleus) was activated during the “non-delivered shock” but not the “safe-no shock” intervals. Individual time series were extracted from a 3 mm radius spherical ROI centered within 4 mm of (x,y,z) = (6, −18, −9). The average ($\pm$ SD) number of voxels above threshold in each subject's seed was 10 ± 5 . Negative PPI effects were observed in the right parahippocampal gyrus ($p < 0.05$, FWE-corrected). A marginally positive PPI effect was observed in the right anterior insula ($Z = 4.19$, $p < 0.001$, uncorrected). See Table 3 and Figure 4b & c for an illustration of the decomposed interaction effects.

4 Discussion

We set out to investigate (1) the effects of an unconditioned stimulus that was expected and was, or was not, delivered, (2) how unconditioned responses change across conditioning trials, and (3) how shock expectation influenced inter-regional coupling within the fear network. We report three principal findings. First, the delivery of a non-painful, but aversive, shock activates a large network of brain regions commonly implicated in pain processing (Apkarian, Bushnell, Treede, & Zubieta 2005). When shock was expected but not delivered, only a subset of these regions, including the anterior insula and the dorsal anterior cingulate cortex, were activated, thereby distinguishing the sensory and the emotional components of the shock, in accordance with the pain literature (Rainville, Duncan, Price, Carrier, & Bushnell 1997). Second, during successive presentations of the shock, sensory-related regions including the primary somatosensory cortex and the posterior insula displayed linear decreases in activity. Conversely, the anterior insula and the midbrain exhibited sustained reactivity to both the delivered and non-delivered shock throughout the experiment. Thirdly, our connectivity analysis showed that fear, in the form of shock expectation, was associated with increased coherence between the hippocampus and the vmPFC, and increased coherence between the red nucleus and the anterior insula. Shock expectation also resulted in decreased coherence between the hippocampus and the red nucleus, suggesting that a switch occurred between engagement of a safety/resting network as contrasted with a fear network.

4.1. Insula

Our results highlight the importance of the anterior insula in fear learning and threat prediction. Classical conditioning studies examining CS-induced activity report anterior

insula activations (reviewed in Sehlmeier et al. 2009). The anterior insular also displays elevated activity in association with prediction errors (Seymour et al. 2004). Furthermore, unlike the amygdala and the anterior cingulate, the insula increases its activity during partial reinforcement conditioning, where there is a high uncertainty with respect to the CS-US contingency (Dunsmoor, Bandettini, & Knight 2007). The present results extend these findings by showing that the anterior insula displayed elevated activity during both shock presentation and at the end of CS+ trials when the shock was expected to be delivered. Further, unlike sensory responses, these anterior insula responses do not diminish over time. The anterior insula has been theorized to function as a re-representation region where anticipated interoceptive emotions can be simulated in advance (Craig 2009). Thus, the anterior insula activity observed in the present study may represent imagining and preparing for an impending aversive event. This idea is supported by the observation that the anterior insula exhibited sustained reactivity throughout the experiment. In the differential contrast between delivered and expected shocks, we observed selective activity increases in the posterior insula to delivered shocks. This is consistent with the posterior insula involvement in detecting pain (Brooks, Zambreanu, Godinez, Craig, & Tracey 2005) and also with recent findings indicating posterior insula involvement in subjective pain experiences (Isnard, Magnin, Jung, Mauguire, & Garcia-Larrea 2011). Of note, the posterior insula displayed a marked decrease in activation over time.

4.2 Red nucleus

The red nucleus (RN) was also activated following the omitted shock, replicating previous reports of RN involvement in conditioned fear (Buchel, Morris, Dolan, & Friston 1998, Hitchcock & Davis 1986). A novel observation from our study was that the brainstem (possibly the RN) displayed high and sustained reactivity throughout all trials. The functional and structural connections of the RN have only recently been characterized in humans using diffusion tensor imaging and resting state fMRI. The RN receives extensive projections from the neocortex, and has dense subcortical connections to the basal ganglia (Habas & Cabanis 2007). Nioche and colleagues (2009) found that the RN displays functional coherence with the hippocampus and the anterior insula at rest, suggesting that the RN may participate in functions such as salience and executive control. In the present study, the regional influence of the hippocampus on the red nucleus was disrupted by fear, whereas the RN's coherence with the anterior insula was increased by fear. While precise localization of brainstem nuclei is at the limit of the spatial resolution of our fMRI analysis, the location of the RN was verified using each individual's structural anatomy. To verify this localization, further studies utilizing high resolution brainstem imaging (Cahill & Stroman 2011) are warranted.

4.3 General discussion and integration of results

We show that neuronal responses in the sensory pain network markedly diminished over time (Figure 3). One possibility for this finding is that knowledge of the impending shocks led participants to successfully down-regulate the perceptual consequences of the shock. This hypothesis is in accordance with studies that have shown that fear conditioning can produce analgesia by influencing the periaqueductal gray (Helmstetter & Tershner 1994). More specifically, the coupling between the anterior insula and the periaqueductal gray is related to perceived stimulus painfulness (Ploner, Lee, Wiech, Bingel, & Tracey 2010). In the present study we found that the red nucleus, adjacent to the PAG, showed elevated activity following the non-delivered shock. Thus, down-regulation of shock sensation may result from increased inter-regional coupling between the anterior insula and brainstem nuclei.

Importantly, we found that the hippocampus exhibited reduced activity during the non-delivered shock interval. The posterior hippocampus has been previously found to be functionally coupled with the medial prefrontal cortex during acquisition of context conditioning in humans (Lang et al. 2009). Here, we further specify this functional coupling in two ways: showing that it is present in cue conditioning, and more specifically, that hippocampus to vmPFC coupling is higher during fear than during safety. Note also that both regions reduced their activity at the threat condition. Mobbs and colleagues have shown that physically proximal threats, in the “circa-strike” phase (Fanselow 1994), leads to activation of the midbrain, thalamus, striatum, insula and the dorsal ACC (Mobbs et al. 2009, Mobbs et al. 2007). This pattern of increased activity is largely overlapping with that observed during the non-delivered shock interval in the present study, suggesting that there is a similar circa-strike fear network engaged at the moment in time when a shock is most expected. The fear induced shifts in functional coherence are consistent with preparation for a “hard-wired” defense reaction executed by the midbrain and motor regions. Taken together, it is conceivable that the hippocampus and the anterior insula are involved in switching between engagement of defensive fear and safety/resting networks.

4.5 Limitations and alternate interpretations

We have interpreted the neuronal responses and connectivity modulations related to the non-delivered shock as reflecting expectancy (“I am going to get shocked *right now*”). It could be argued, however, that these results also represent a prediction error signal (“Oh, I guess I did not get shocked”). While our experimental design and analysis strategy does not allow us to dismiss this alternative, we believe that the prediction error interpretation is less likely for several reasons. First, no regions were more active during the non-delivered shock than during the delivered shock, and all brain regions activated by the omitted shock were also activated to shock delivery, when the shock was expected and delivered, and therefore no expectancy violation occurred. Second, the omitted shock led to skin conductance responses larger than those induced by the offset of the CS-. If the subjects primarily experienced relief from escaping shock, skin conductance would not be expected to rise. Moreover, we did not observe activity increases in the striatum and the dorsolateral prefrontal cortex—regions that previously have been associated with prediction error (McClure, Berns, & Montague 2003, O’Doherty, Dayan, Friston, Critchley, & Dolan 2003, Ploghaus et al. 2000, Schultz et al. 1997, Seymour et al. 2004). Finally, to in an effort to ensure that the observed effects were driven by anticipatory processes, we conducted a complimentary analysis where the “non-delivered shock” regressor was moved back 0.5 sec. in time to signify the moment just *prior* to the offset of the CS+, i.e. before any prediction error could have occurred. This analysis yielded largely identical ACC, anterior insula, thalamus and midbrain activations (results not reported), suggesting that the present findings are most likely driven by anticipatory mechanisms rather than by prediction error. However, further studies with higher temporal resolution designed specifically to dissociate immediate fear processing from prediction error effects are warranted.

A limitation of the current design, and indeed of several fear conditioning designs, is the degree of multi-collinearity between the timing of the conditioned and the unconditioned cue intervals. By design, the conditioned cue has to be predictive of the unconditioned cue, and to achieve maximal temporal predictability, the cue should be of a fixed, predictable length. Both these factors will introduce multi-collinearity among the predictor variables, which can result in unstable, but still unbiased parameter estimates, limiting the efficiency of the fMRI design. Although one alternative approach to task timing is to introduce some variation in the CS duration to reduce the temporal predictability of the shock, this modification will potentially change the nature of the associated conditioning.

4.6 Conclusions

We show that non-painful, but aversive, unconditioned stimuli produce regional responses similar to those reported in previous pain studies. When shock stimuli were expected but not delivered, anterior regions of this network increased their activity, consistent with anticipatory anxiety. The sensory activity responses to the US diminished over time, whereas the anterior insula and the red nucleus exhibited sustained activity. We interpret these non-delivered shock responses as “circa-strike” fear responses (Fanselow 1994, Mobbs et al. 2009), and demonstrate that fear leads to dramatically altered regional influences among the brain regions involved in predicting safety and danger. In particular, the hippocampus and the anterior insula may serve as hubs in switching between engagement of defensive fear and safety/resting networks.

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Figure 1.

a) Time lines and visual stimuli presented during the fear conditioning paradigm

We focused on three different phases of the experiment — delivered shock, non-delivered shock and safe-no shock conditions — each marked in hashed grey. In addition to the above stimuli, a stimulus with a yellow light, like the red light above, was also used for conditioning. Images are adapted from Milad et al, 2007.

b) Conditioning paradigm and galvanic skin conductance responses

The fear conditioning paradigm entailed 32 trials of three types: 10 conditioned cue presentations ending with a shock (delivered shock), 6 conditioned cue presentations ending without a shock (non-delivered shock) and 16 safe cue presentations never ending with a shock (safe-no shock). Normalized skin conductance responses from a single representative subject in three trials are indicated. Skin conductance rose slightly at the onset of the context, continued rising at the presentation of the cue (conditioned response), and displayed a prominent peak after the delivery of the shock (unconditioned response).

c) Unconditioned skin conductance responses to the delivered shock (red) and non-delivered shock (black) across the 16 CS+ trials.

Figure 2. Activity related to shock, non-delivered shock, and shock versus non-delivered shock
 Group (n=24) BOLD activity increases and decreases are overlaid on a template MRI image displayed at Talairach coordinates $x = 5, y = -15, z = 2$. The first row of images is the contrast “shock” versus “safe-no shock”. The second row is the contrast “non-delivered shock” (shock expected to be delivered at the CS+ offset) versus “safe-no shock”. The third row is the contrast “shock” versus “non-delivered shock”. Activity changes are displayed at thresholds of $T > \pm 4$.

Figure 3. Sustained and linearly decreasing unconditioned responses

The upper box plot illustrates sustained BOLD signal responses in the anterior insula both to the shock (solid red) and to the non-delivered shock (open red) across the 16 trials.

The lower box plot illustrates diminishing BOLD signal responses in the posterior insula to the shock (solid blue) and absent BOLD signal responses to the non-delivered shock (open blue) across trials. Error bars indicate 90% confidence intervals.

The brain slices (at $x = 8$ and $z = -4$) illustrates medial and insular regions with sustained (red) and decreasing (blue) shock activations, threshold at $T > 4$.

Figure 4. The psychological context modulates inter-regional activity coupling

Psychophysiological interaction (PPI) analysis reveals changes in regional coupling between “non-delivered shock” and “safe-no shock” conditions. Interaction effects are displayed at a threshold of $T > \pm 2.5$. Blue arrows indicate negative interactions, i.e. decreased coupling between regions in the presence of fear. Red arrows indicate positive interactions, i.e. increased coupling in the presence of fear. a) PPIs with the left red nucleus b) right hippocampus. c) Summary of PPI results with decomposed interaction effects for each seed indicating how inter-regional functional coupling changed between safe-no shock (blue boxes) and non-delivered shock (red boxes). Plots display parameter estimates with 90% confidence intervals.

Table 1

Univariate analysis results

Contrast	coordinate peak x,y,z	Z-score	Cluster p		Voxel p [*]	Nearest grey matter region (Brodmann area)
			Size voxels	FWE	FWE	
Shock > Safe-no shock	-40, 14, 5	7.04	28419	<0.0001	<0.0001	L. Insula
	-42, 0, 7	6.91			<0.0001	L. Insula
	-2, -18, -9	6.86			<0.0001	L. Red Nucleus
	-6, 2, 39	6.25	6148	<0.0001	<0.0001	L. Cingulate Gyrus (24)
	-10, 13, 25	5.79			0.0003	L. Cingulate Gyrus (24)
Shock < Safe-no shock	-2, 41, 5	5.78			0.0003	L. Anterior Cingulate (32)
	10, 44, -12	4.09	230	0.0324	0.4725	R. Medial Frontal Gyrus (10)
	-50, -19, 54	6.21	12242	<0.0001	<0.0001	L. Postcentral Gyrus (3)
	-38, -11, 13	6.18			<0.0001	L. Insula
	-40, -19, 56	6.12			0.0001	L. Precentral Gyrus (4)
Shock > Non-delivered shock	38, -3, 15	5.84	5544	<0.0001	0.0002	R. Insula
	51, -13, 14	5.67			0.0006	R. Postcentral Gyrus (43)
	46, -20, 19	5.65			0.0007	R. Insula
	16, -49, -18	4.40	270	0.017	0.1823	R. Culmen
	24, -52, -21	3.87			0.7412	R. Culmen
	4, -53, -11	3.69			0.9104	R. Culmen
	Shock < Non-delivered shock no significant activations					
	-2, -20, -11	6.03	2132	<0.001	0.0001	L. Red Nucleus
	-16, -15, 12	5.28			0.0044	L. Ventral Lateral Thal. Nucleus
	8, -16, -11	4.94			0.020	R. Substantia Nigra
Non-delivered shock > Safe-no shock	40, 20, 5	5.88	1809	<0.001	0.0002	R. Inferior Frontal Gyrus (45)
	36, 21, -6	5.24			0.0053	R. Inferior Frontal Gyrus (47)
	30, 26, 8	4.93			0.021	R. Insula
	-40, 14, 5	5.84	1426	<0.001	0.0002	L. Insula
	-36, 17, -11	5.24			0.0053	L. Inferior Frontal Gyrus (47)
	-40, 25, 1	4.54			0.11	L. Inferior Frontal Gyrus (47)
	-10, 30, 21	4.95	1619	<0.001	0.020	L. Anterior Cingulate (32)

Contrast	Z-score		Size voxels	Cluster p		Voxel p*	Nearest grey matter region (Brodmann area)
	coordinate peak x,y,z	FWE		FWE			
Non-delivered shock < Safe-no shock	-12, 17, 27	4.67				0.066	L. Cingulate Gyrus (32)
	-12, 9, 29	4.47				0.12	L. Cingulate Gyrus (24)
	18, -15, -19	4.90	122	0.225		0.0244	R. Parahippocampal Gyrus (28)
	30, -43, -11	4.67	235	0.030		0.0663	R. Fusiform Gyrus (37)
	48, -63, 23	3.71				0.8964	R. Middle Temporal Gyrus (39)
	8, -22, 58	4.41	512	0.001		0.1737	R. Medial Frontal Gyrus (6)
	10, -27, 51	4.02				0.5518	R. Paracentral Lobule (6)
	32, -25, 53	4.01	507	0.001		0.5727	R. Precentral Gyrus (4)
	32, -10, 65	3.90				0.7078	R. Precentral Gyrus (4)
	40, -25, 51	3.73				0.8801	R. Postcentral Gyrus (3)
	-48, -76, -1	3.77	241	0.027		0.8454	L. Inferior Temporal Gyrus (18)
	-51, -74, 6	3.66				0.9264	L. Middle Occipital Gyrus (19)
-40, -71, 13	3.46				0.9909	L. Middle Temporal Gyrus (39)	

* Uncorrected voxel P values are all < 0.001

Table 2

Trial by trial analysis

Contrast coordinate	Size	Cluster p	Voxel p [*]	Nearest grey matter region
Z-score peak x,y,z	voxels	FWE	FWE	
Sustained activation				
22, -84, -4	7.22	218	<0.001	R. Middle Occipital Gyrus
14, -80, -1	6.26		<0.001	R. Occipital Lingual Gyrus
-32, -78, -6	6.99	198	<0.001	L. Inferior Occipital Gyrus
-24, -82, -6	6.94		<0.001	L. Middle Occipital Gyrus
32, 23, -6	6.12	522	<0.001	R. Inferior Frontal Gyrus
30, 22, 6	6.12		<0.001	R. Anterior Insula
50, 12, 5	4.85		0.016	R. Precentral Gyrus
-32, 27, -3	5.91	471	<0.001	L. Inferior Frontal Gyrus
-32, 25, 4	5.45		0.001	L. Inferior Frontal Gyrus
-38, 16, 5	5.44		0.001	L. Anterior Insula
0, -23, 12	4.58	116	0.002	Brainstem
-6, -1, 48	5.03	19	0.004	L. Medial Frontal Gyrus
-48, -15, 15	4.86	17	0.005	L. Postcentral Gyrus
-51, -20, 21	4.69		0.032	L. Posterior Insula
-46, 10, -4	4.77	10	0.01	L. Superior Temporal Gyrus
Decreasing activation				
48, -26, 22	6.9	968	<0.001	R. Posterior Insula
55, 8, 3	5.8		<0.001	R. Superior Temporal Gyrus
59, -36, 18	5.32		0.002	R. Posterior Insula
-51, -15, 14	6.81	1806	<0.001	L. Postcentral Gyrus
-50, -26, 22	6.79		<0.001	L. Postcentral Gyrus
-38, -17, 16	6.64		<0.001	L. Middle Insula
-6, 0, 39	5.78	298	<0.001	L. Posterior Mid. Cingulate
-50, -21, 54	5.39	132	<0.001	L. Postcentral Gyrus
-42, -11, 50	5.04		0.007	L. Precentral Gyrus
-40, -19, 51	4.98		0.009	L. Postcentral Gyrus
Shock				

Contrast coordinate	Z-score peak x,y,z	Size	Cluster p voxels	FWE	Voxel p [*] FWE	Nearest grey matter region
	14, -72, -1	5.03	46	0.001	0.007	R. Occipital Lingual Gyrus
	14, -68, 9	4.98			0.009	R. Occipital Cuneus
	30, 10, 5	4.83	10	0.01	0.017	R. Claustrum
	4, 24, 23	4.76	12	0.008	0.024	R. Anterior Mid. Cingulate
Shock	Increasing activation no significant regions					
Non-delivered shock	Sustained activation					
	-26, -80, -3	7.82	1089	<0.001	<0.001	L. Middle Occipital Gyrus
	24, -84, -4	6.89		<0.001	<0.001	R. Middle Occipital Gyrus
	6, -80, -3	6.73		<0.001	<0.001	R. Lingual Gyrus
	38, 22, 6	5.97	1237	<0.001	<0.001	R. Inferior Frontal Gyrus
	36, 23, -5	5.28		0.003	<0.001	R. Anterior Insula
	-32, 24, 6	5.14	600	0.004	<0.001	L. Inferior Frontal Gyrus
	-36, 16, 3	4.49		0.039	<0.001	L. Anterior Insula
	-34, 19, -11	4.20		0.100	<0.001	L. Inferior Frontal Gyrus
	0, 14, 44	4.14	201	0.116	<0.001	L. Medial Frontal Gyrus
Non-delivered shock	Decreasing activation no significant regions					
Non-delivered shock	Increasing activation no significant regions					

* Uncorrected voxel P values are all <0.001

Table 3

Psychophysiological interaction analysis results

PPI Seed	coordinate peak x,y,z	Z-score	size	Cluster p		Voxel p		Nearest gray matter region (Brodmann area)
				FWE		FWE *		
L. red nucl. pos.	46, 24, 10	4.19	72	0.5506		0.487		R. Anterior Insula, IFGOp (45)
L. red nucl. neg.	34, -37, -5	5.29	127	0.1756		0.0064		R. Parahippocampal Gyrus (37)
R. hipp pos.	no significant effects							
R. hipp neg.	8, -62, -29	5.59	816	<0.001		0.0015		R. Uvula
	-2, -56, -31	4.28				0.3712		L. Cerebellar Tonsil
	-16, -54, -36	4.11				0.5823		L. Cerebellar Tonsil
	-32, -2, 39	4.02				0.6992		L. Precentral Gyrus (6)
	32, -61, -7	3.65	200	0.0384		0.9807		R. Declive
	28, -55, -4	3.58				0.9919		R. Parahippocampal Gyrus (19)
	38, -78, -15	3.46				0.999		R. Declive
L. hipp pos	no significant effects							
L. hipp neg	-8, -25, 1	5.14	572	<0.001		0.0133		L. Thalamus
	10, -60, -31	4.97	455	<0.001		0.0277		R. Cerebellar Tonsil

* Uncorrected voxel P values are all p<0.001