Behavioral Neuroscience
2006, Vol. 120, No. 5, 1187–1195

0735-7044/06/$12.00 DOI: 10.1037/0735-7044.120.5.1187

Human Amygdala Activity During the Expression of Fear Responses

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The initial learning and subsequent behavioral expression of fear are often viewed as independent processes with potentially unique neural substrates. Laboratory animal studies of Pavlovian fear conditioning suggest that the amygdala is important for both forming stimulus associations and for subsequently expressing learned behavioral responses. In the present article, human amygdala activity was studied during the autonomic expression of conditional fear in two differential conditioning experiments with event-related functional magnetic resonance imaging and concurrent recording of skin conductance responses (SCRs). Trials were classified on the basis of individual participants' SCRs. Significant amygdala responding was detected only during trials on which a signal both predicted shock and elicited significant conditional SCR. Conditional stimulus presentation or autonomic activity alone was not sufficient. These results indicate that amygdala activity may specifically reflect the expression of learned fear responses and support the position that this region plays a central role in the expression of emotional reactions.

Keywords: amygdala, fear conditioning, memory, emotion, fMRI

In Pavlovian fear conditioning, a conditional stimulus (CS) is paired with an aversive unconditional stimulus (UCS) such as electric shock. As the CS and UCS are repeatedly and consistently paired, the CS alone begins to elicit behavioral responses in anticipation of a UCS presentation. Learning the predictive relationship between the CS and UCS and then expressing an appropriate response to the CS after learning has taken place are often viewed as two dissociable processes that may rely on distinct neural circuits.

The brain circuits underlying Pavlovian fear conditioning support both the acquisition and expression of fear responses (Davis, 2000; LeDoux, 2000; Maren, 2001). The amygdala, a critical component within this circuit, is made up of several distinct nuclei that appear to be differentially involved in either learning stimulus associations or expressing fear responses (Bellgowan & Helmstetter, 1996; Campeau & Davis, 1995; Fanselow & LeDoux, 1999; Helmstetter, 1992; Maren, 1999). Although distinctions between memory acquisition and behavioral performance have received considerable experimental attention in laboratory animal studies (Campeau & Davis, 1995; Helmstetter, 1992), it remains unclear whether all properties of this system extend to intact humans. Functional neuroimaging studies of human Pavlovian fear conditioning (Armony & Dolan, 2002; Büchel, Dolan, Armony, & Friston, 1999; Büchel, Morris, Dolan, & Friston, 1998; Knight, Smith, Cheng, Stein, & Helmstetter, 2004b; Morris, Büchel, & Dolan, 2001) have largely focused on the importance of the amygdala for learning stimulus associations. However, laboratory animal data (Campeau & Davis, 1995; Helmstetter, 1992) and human lesion studies (Bechara et al., 1995; LaBar, LeDoux, Spencer, & Phelps, 1995) have reported that this region may also be critically involved in the expression of fear responses.

Early efforts to characterize the functional role of the amygdala in human fear learning include studies of patients with damage to this region and surrounding tissue. Medial temporal lobe patients were unable to demonstrate learned autonomic responses to both simple and conditional discrimination paradigms (LaBar et al., 1995). Similarly, patients with damage to the amygdala were unable to demonstrate autonomic discrimination in a fear conditioning experiment but were able to acquire declarative knowledge of the stimulus relationships (Bechara et al., 1995). These studies suggest that the human amygdala may be involved in the production of learned autonomic fear responses.

Previous behavioral (Kimmel, 1959; Stewart, Stern, Winokur, & Fredman, 1961) and functional magnetic resonance imaging (fMRI) studies (Büchel et al., 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Phelps et al., 2001) of human fear conditioning have shown that both skin conductance responses (SCR) and amygdala activity are transient and habituate over time. Given that SCR topography is remarkably similar to the pattern of responding observed in the amygdala during fear conditioning, it is not surprising that several different laboratories (Büchel et al., 1998; Cheng, Knight, Smith, Stein, & Helmstetter, 2003; LaBar et al., 1998; Phelps et al., 2001) have demonstrated a correlation between these two measures. These findings support the possibility

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This research was supported by the McDonnell-Pew Foundation, National Institute of Mental Health Grant MH060668 awarded to Fred J. Helmstetter, and a Bill and Melinda Gates Foundation Scholarship awarded to Dominic T. Cheng.

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that human amygdala activity may not exclusively reflect the learning of stimulus associations but may also be involved in the expression of autonomic conditional fear.

Consistent with this view, one study from our laboratory (Cheng at al., 2003), conducted with an analytical approach emphasizing sensitivity to the response-generating functions of the amygdala, found significant amygdala activity, whereas another study (Knight, Cheng, Smith, Stein, & Helmstetter, 2004a) involving a more traditional analysis technique and focusing on stimulus-processing properties of the amygdala, failed to detect differential amygdala activity. The current article revisited this data set that failed to show significant amygdala activity (Knight et al., 2004a) in order to determine whether activity within this region can be better predicted by an analytical approach emphasizing response expression. Additionally, new data were collected with a modified experimental design to test the generality and reliability of these findings.

In order to test the idea that amygdala activity could be better predicted by learned autonomic fear responses, we correlated the SCR waveform of individual participants with their amygdala activity (Cheng et al., 2003). This cross-correlation technique showed that the topography of early amygdala activity resembled SCR waveforms that contained conditioned responses. Such interpretations are inherently limited on account of the correlational design of the approach, thus necessitating a closer examination of the link between amygdala activity and the autonomic expression of conditional fear.

In order to more closely investigate this relationship, we believed that isolating amygdala activity to individual conditioning trials that elicit autonomic fear responses would be informative. For example, event-related fMRI has been used previously to observe neural activity related to specific behavioral responses (Wagner et al., 1998; Williams et al., 2001). Differential neural activity in prefrontal cortex has been demonstrated as a function of remembered and forgotten items (Wagner et al., 1998) and amygdala activity has been observed during trials that elicit SCRs to images of fearful facial expressions but not during trials that fail to elicit similar autonomic responding (Williams et al., 2001). Similar to these previous analytic approaches, event-related fMRI and concurrent SCR measurements were used to investigate the human amygdala response during the autonomic expression of conditional fear in two separate experiments. Both experiments exposed participants to a general differential fear conditioning procedure but differed on several parameters, allowing us to test the reliability of the findings.

In Experiment 1, we reanalyzed data from a previously published study that failed to detect amygdala activity (Knight et al., 2004a). By reexamining this same data set with an analysis that focuses on fear responses, we sought to determine whether the lack of effects seen in the amygdala could have been due to an insensitive analysis technique emphasizing stimulus-processing properties of the amygdala. In this experiment, one stimulus coterminated with shock (CS+), one stimulus was presented alone (CS−), and another stimulus signaled shock following a 10-s trace interval (CS_{10}). Because CS+ presentations evoked larger and more reliable SCRs than did CS− and CS_{10} presentations early in training (see Knight et al., 2004a, for details) and because SCR habituation is common, data analyses focused on amygdala activity related to CS+ and CS− presentations during the first two blocks of training trials. To assess the generality and reliability of the findings from Experiment 1, we applied the same analysis procedure to data collected with a slightly different design and methodology. In Experiment 2, participants were exposed to a habituation phase prior to acquisition and only needed to discriminate between two conditional stimuli (CS + and CS−). Similar to Experiment 1, data analysis in Experiment 2 only focused on early acquisition trials.

Prior to imaging analysis in both experiments, CS+ and CS− trials were classified into response and nonresponse categories (Figure 1) on the basis of SCR patterns, which resulted in four distinct trial types: CS+ response, CS+ nonresponse, CS− response, and CS− nonresponse. If amygdala activity is related to the autonomic expression of conditional fear, then differential amygdala activity should be detected between trials that signal shock and produce a conditional SCR (CS+ response) and trials that signal shock but fail to elicit a conditional SCR (CS+ nonresponse).

Method

Experiment 1

Participants

Seventeen (8 male, 9 female) healthy, right-handed subjects ranging from 18 to 43 years of age ($M = 23.35$, $SE = 6.15$ years) volunteered for this study. All procedures were approved by the Institutional Review Boards for human subject research at both the Medical College of Wisconsin and the University of Wisconsin—Milwaukee.

Apparatus

MRI. Whole brain functional imaging was performed on a 3 Tesla/60 Bruker Biospec scanner (Ettlingen, Germany) with a multislice gradient-echo echoplanar pulse sequence. Contiguous sagittal slices (3.75 × 3.75 × 8.00 mm) were collected (repetition time [TR] = 2,000 ms, echo time [TE] = 27.2 ms, field of view [FOV] = 24 cm, flip angle = 90°) in a series of 340 sequential images (for a total of 680 s) during four blocks of stimulus presentations. High-resolution anatomical images were obtained with a three-dimensional multi-planar inversion recovery gradient-echo imaging sequence to serve as an anatomical map over which functional images were superimposed.

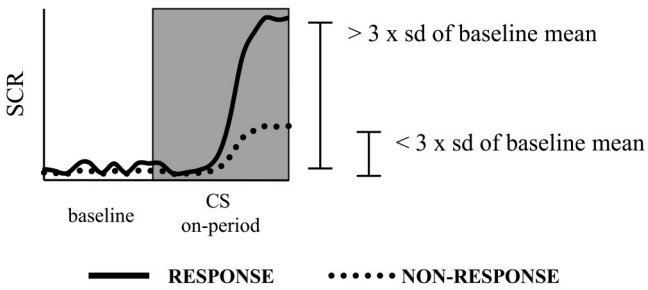

Figure 1. Definition of a response and a nonresponse trial. All conditional stimuli (CS+ and CS−) trials were classified into one category or the other on the basis of individual participants' skin conductance response (SCR) patterns (see Method section) and were subsequently used in both behavioral and imaging analysis.

Electrical stimulus. The unconditional stimulus (UCS) was electrical stimulation (0.5 s) presented with a custom-made alternating current (60-Hz) source through two aluminum surface electrodes (2-cm diameter) positioned over the right tibial nerve above the right medial malleolus. The maximum possible current used for the UCS was 7.35 mA. Each participant determined the UCS intensity that he or she used for the experiment.

Visual stimuli. Five trials of three conditional stimuli (CS+, CS−, CS_{10}) varying in shape, number, and color were presented (10-s duration, 40- or 50-s intertrial interval) during each stimulus block. Conditioned stimuli were counterbalanced and presented in a pseudorandom order such that no more than two trials with the same CS were consecutively presented. The CS+ and UCS presentations co-terminated on all stimulus blocks. The CS_{10} was followed by a 10-s trace interval prior to UCS presentation, and the CS− was presented alone. Participants were presented with four blocks of 15 trials per block of the three conditioned stimuli.

Skin conductance response. Two surface cup electrodes (silver/silver chloride, 1-cm diameter; Beckman Instruments, Fullerton, CA) filled with electrolyte gel (Teca Corporation, Pleasantville, NY) were attached 2 cm apart to the sole of the 'participant's left foot. Skin conductance data were digitized and stored continuously at 250 Hz with Asyst software (Version 3.10, Rochester, NY).

UCS expectancy. A custom-made rotary dial was used to monitor the participant's expectancy of receiving electrical stimulation. The dial controlled a rating bar presented at the bottom of the visual display. Realtime feedback of the participant's dial position was displayed throughout the experiment. The dial was attached to the right thigh with a Velcro strap, and participants were instructed to manipulate the dial with their right hand. Whole brain analyses of CS-evoked activity and additional procedural details for Experiment 1 can be found in Knight et al. (2004a).

Procedure

Prior to the start of the experiment, participants were instructed in the use of the dial and rating bar. Ratings were made on a continuous scale ranging from 0 to 100, where 0 reflected the expectation that shock definitely would not be presented and 100 reflected the expectation that shock definitely would be presented. A rating of 50 indicated that participants were uncertain as to whether the shock would be presented. Participants were instructed to continuously update their rating to accurately reflect their current shock expectancy.

Participants were asked to individually set the intensity of the electrical stimulus. Prior to training, practice trials were given in which participants rated the level of electrical stimulation on a scale ranging from 0 to 5 (0 = *no sensation,* 5 = *painful, but tolerable*). Gradual increases in intensity, interspersed with two declinations, were given until the stimulus intensity that elicited a subjective report of 5 was achieved.

Anatomical scans were conducted in the absence of stimuli. Following anatomical scans, visual and electrical stimuli were presented while functional images were obtained. Participants were debriefed at the end of the experiment.

Data Analysis

Trial classification. Prior to imaging analysis, all CS+ and CS− trials were classified into response or nonresponse trial types on the basis of the individual participant's SCR. We focused on activity during the second interval response (last 5 s of the CS) because it is generally considered to be an emotional anticipatory response to the predicted UCS and should accurately reflect learning the relationship between the CS and the UCS (Prokasy & Kumpfer, 1973; Wolter & Lachnit, 1993). If the difference between the maximum and minimum responses during the second interval response was greater than three times the standard deviation of the mean of the baseline period (10 s prior to CS), the trial was classified as a response. CS+ and CS− trials that did not meet these criteria were categorized as nonresponses (Figure 1). Thus, four trial types were identified and used in both the behavioral and imaging analysis: CS+ response, CS+ nonresponse, CS− response, and CS− nonresponse.

This classification scheme prevented the analysis of some participants on the basis of their behavioral performance. For example, if all of a participant's Block 1 CS+ trials were labeled a response, there would be no Block 1 CS+ nonresponse trials to which a comparison could be made. The number of participants that showed at least one response and one nonresponse trial for Block 1 CS+, Block 1 CS−, Block 2 CS+, and Block 2 CS− were 11, 13, 15, and 9 respectively. Because of the need for equal sample sizes across groups, 9 common participants with activation in each of the four categories were selected for group analysis.

Behavioral data (SCR). Repeated measures analysis of variance (ANOVA) was performed, revealing significant differences in SCR (Knight et al., 2004a). CS+ presentations evoked significantly larger SCRs than did CS− and CS_{10} early in training. Because of this early differential responding and the early, transient nature of amygdala activity, the present analysis focused on responding during CS+ and CS− trials in Blocks 1 and 2. Accordingly, mean SCR values during the second interval response for all four trial types during Blocks 1 and 2 were calculated, and paired *t* test comparisons were performed between response and nonresponse trial types.

Imaging data. Functional MRI data were processed and analyzed with Analysis of Functional NeuroImages software (AFNI; Cox, 1996). Three-dimensional motion correction algorithms were applied to the functional data set to correct for minor movement artifacts. Participants whose fMRI time series had perceptible, residual head movements based on cinematic viewing were excluded from further analysis.

Functional MRI time series data were analyzed with 3D Deconvolution software[1] utilizing the measured blood-oxygenated level dependent (BOLD) response and input reference functions representing the temporal location of the four trial types (CS+/CS− response and CS+/CS− nonresponse) to estimate the impulse response functions evoked by each trial type. The percentage of area under the curve (%AUC) of the impulse response function was used as a measure of response strength.

Functional and anatomical images for each participant were transformed into stereotaxic coordinate space relative to the line between the anterior and posterior commissures (Talairach & Tournoux, 1988). To compensate for anatomical variability between subjects, the procedures included application of a 4-mm full width at half maximum Gaussian blur to each participant's functional data set following transformation to common coordinate space. Although whole brain imaging was performed during both experiments, our hypothesis specifically predicted activity within an a priori region of interest (right amygdala) on the basis of results from our laboratory and others (Büchel et al., 1998; Cheng et al., 2003; LaBar et al., 1998). A two-factor (CS Type × Response Type) ANOVA was performed on the %AUC within this region of interest, and thus a significance level of $p \leq .05$ and a minimum cluster size of 150 mm^3 was used to threshold the data. Volumes of active tissue passing these criteria were used in a functional region of interest analysis.

Experiment 2

Participants

Thirteen (4 male, 9 female) healthy, right-handed subjects ranging from 18 to 31 years of age ($M = 22.38$, $SE = 3.80$ years) participated in this experiment. All procedures were approved by the Institutional Review Boards for human subject research at both the Medical College of Wisconsin and the University of Wisconsin—Milwaukee.

[1] Information about the Deconvolution software is available from http://afni.nimh.nih.gov/pub/dist/doc/manual/Deconvolvem.pdf

Apparatus

MRI. Imaging parameters were identical to those used in Experiment 1 with the exception of the scan length. Experiment 2 consisted of one run, which lasted 1,140 s (570 sequential images).

Electrical stimulus, skin conductance response, and UCS expectancy. Materials used to present the electrical stimulus and to measure SCR and UCS Expectancy were the same as in Experiment 1.

Visual stimulus. Forty trials of two conditioned stimuli (20 CS+ and 20 CS−) varying in shape, number, and color were presented (8-s duration, 28-s intertrial interval). Participants were given eight habituation trials (four CS+ and four CS−) and 32 acquisition trials (sixteen CS+ and sixteen CS−) in one seamless phase. During habituation, no UCS presentations were delivered, whereas during acquisition the CS+ co-terminated with the UCS, and the CS− was presented alone. One of two colors (blue or green) served as the CS+. Conditioned stimuli were counterbalanced and presented in a pseudorandom order such that no more than two trials of the same CS were presented consecutively.

Procedure

The sequence of experimental procedures in Experiment 2 was the same as in Experiment 1.

Data Analysis

A repeated measures ANOVA was performed on SCR data for the first eight CS+ and CS− trials during acquisition. Differential responding during CS presentations was observed by a significant main effect for CS, $F(1, 8) = 12.53$, $p \leq .05$. CS+ presentations (25.07 ± 0.09 μmhos) evoked larger SCR amplitude than did CS− presentations (24.73 ± 0.03 μmhos). Because differential SCR was evident early during acquisition and amygdala responding has also been detected early (Büchel et al., 1998; Cheng et al., 2003; LaBar et al., 1998; Phelps et al., 2001), the current analysis focused on the first eight CS+ and CS− trials. Similar to Experiment 1, trial classification reduced our number of participants included in the imaging analysis to nine. All analysis procedures (trial classification, imaging analysis, etc.) were performed exactly as in Experiment 1.

Results

Experiment 1

Behavioral Results (SCR and UCS Expectancy)

During Block 1, increased SCR during CS+ response trials relative to CS+ nonresponse trials was observed, $t(8) = 3.56$, $p \leq .05$. However, CS− response and CS− nonresponse trials did not differ, $t(8) = 1.06$, $p > .05$. Similarly, SCR amplitudes during Block 2 CS+ response trials were significantly greater than Block 2 CS+ nonresponse trials, $t(8) = 2.31$, $p \leq .05$, whereas Block 2 CS− response and CS− nonresponse trials were not different, $t(8) = 0.99$, $p > .05$ (Figure 2a).

Corresponding UCS expectancy ratings showed a different pattern. Unlike SCR during Block 1, no differences were seen be-

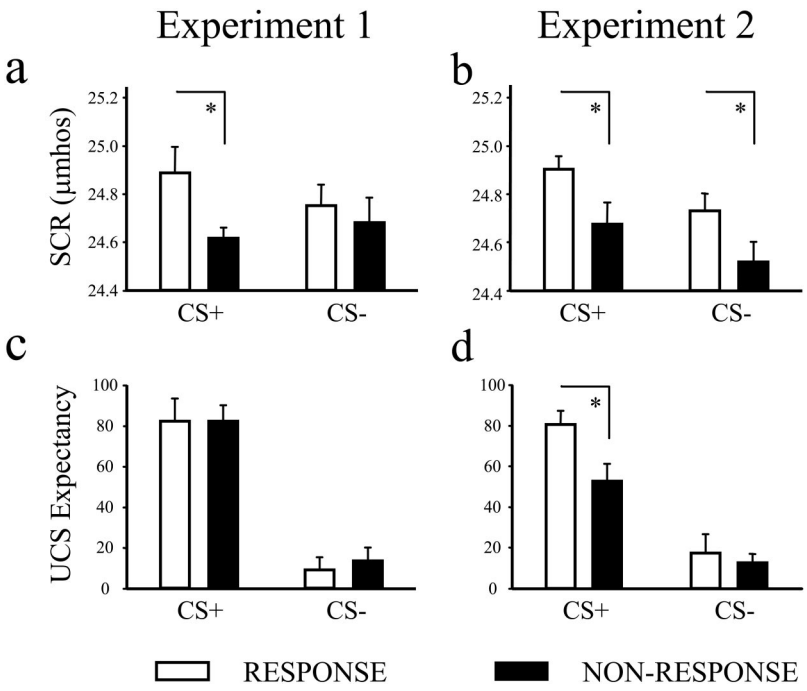

Figure 2. Mean skin conductance response (SCR) values (μmhos) of all four trial types during Experiments 1 and 2 and corresponding unconditional stimulus (UCS) expectancy ratings. Significantly greater SCRs were observed during CS+ response trials relative to CS+ nonresponse trials during Block 2 in both Experiment 1 (a) and Experiment 2 (b). Similar differences were seen between CS− response and CS− nonresponse trials in Experiment 2 but not during CS− trials in Experiment 1. Participants did not explicitly report (UCS expectancy) differences between trials on which they demonstrated an SCR and on trials on which they did not during Experiment 1 (c) but did so in Experiment 2 (d). These behavioral data suggest that the changes observed in the amygdala were closely related to differences observed in participants' SCR patterns during a visual cue that predicts shock. Error bars represent standard errors of the mean. *$p \leq .05$ (paired t test).

tween response and nonresponse trials for CS+, $t(8) = 0.15$, $p > .05$ and CS−, $t(8) = -1.00$, $p > .05$. Similarly, Block 2 also showed no significant differences between response and nonresponse trials for CS+, $t(8) = 0.01$, $p > .05$ and CS−, $t(8) = -1.11$. $p > .05$ (Figure 2c).

In summary, only in the presence of a CS+ did response trials elicit greater SCR amplitude than nonresponse trials. Similar SCR differentiation between response and nonresponse trial types during CS− presentations was not observed. Corresponding UCS expectancy ratings indicated that participants did not explicitly differentiate between trials on which they showed an SCR and trials on which they did not. This pattern of responding was consistent for CS+ and CS− trials during both Blocks 1 and 2.

Imaging Results

We performed a two-factor ANOVA (CS Type × Response Type) on %AUC within our a priori region of interest. No significant main effect or interaction in the amygdala was found during Block 1. However, a significant CS Type × Response Type interaction in Block 2 revealed a cluster of activation in the right amygdala (Talairach coordinates: 19.2, –0.7, –9.7; 200 μl), $F(1, 8) = 7.37$, $p \leq .05$ (top portion of Figure 3a). Using this cluster as a functional region of interest, we detected increased responding to CS+ response trials ($M \pm SE = 1.61 \pm 0.45$) relative to CS+ nonresponse trials (-0.18 ± 0.30). In contrast, CS− response trials (-0.00 ± 0.72) did not differ from CS− nonresponse trials (0.30 ± 0.24; bottom portion of Figure 3a).

Experiment 2

Behavioral Results (SCR and UCS Expectancy)

Similar to Experiment 1, increased SCR during CS+ response trials relative to CS+ nonresponse trials was observed early in acquisition (first eight CS+ trials), $t(8) = 2.35$, $p \leq .05$. Furthermore, CS− response trials also showed increased magnitude when compared with CS− nonresponse trials, $t(8) = 3.54$, $p \leq .05$ (Figure 2b).

Corresponding UCS expectancy ratings showed differential responding between CS+ response and CS+ nonresponse trials,

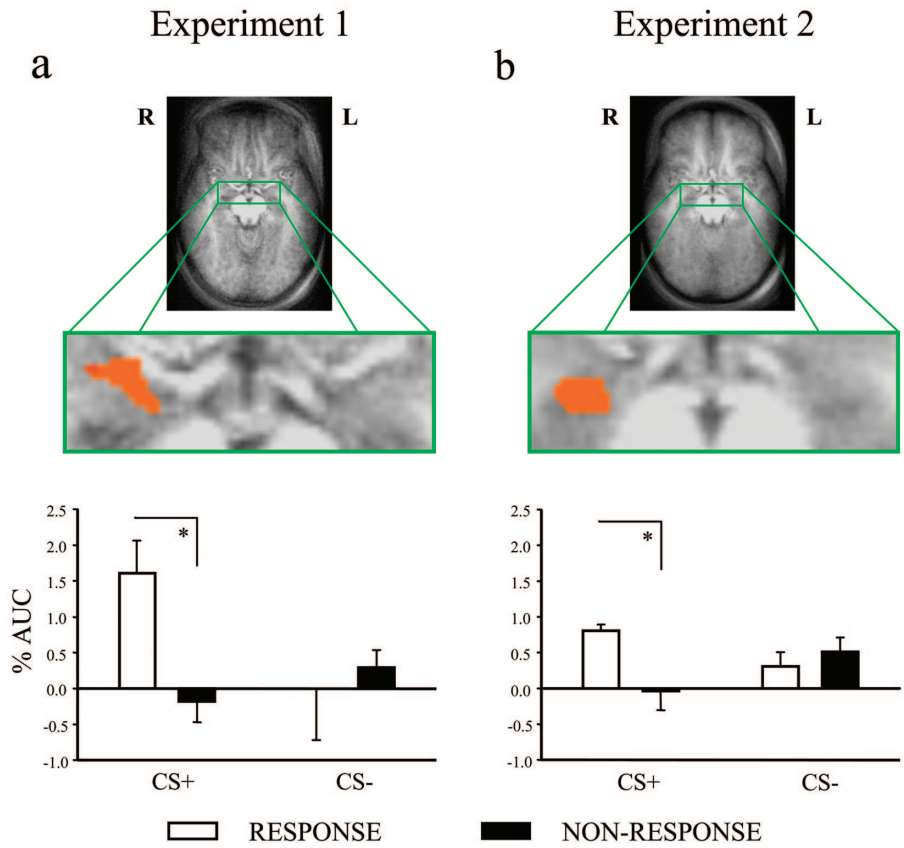

Figure 3. Amygdala activation as a function of autonomic fear responses. (a) Analysis of variance results revealed a Conditional Stimulus (CS) Type × Response Type interaction ($p \leq .05$) in the right amygdala during Experiment 1 (Talairach coordinates: 19.2, −0.7, −9.7; 200 μl). Functional region-of-interest (ROI) analysis showed differential amygdala responding only between CS+ trials that elicited a conditional response and CS+ trials that did not. (b) Results from Experiment 2 show a similar CS Type × Response Type interaction ($p \leq .05$) in the right amygdala (Talairach coordinates: 19.1, –8.8, –8.7; 296 μl). These data suggest that responding in this structure may not exclusively reflect learning stimulus associations but also the expression of conditional fear responses. AUC = area under the curve.

$t(8) = 5.99$, $p \leq .05$, but no differences between CS− response and CS− nonresponse trials were observed, $t(8) = 0.78$, $p > .05$ (Figure 2d).

In summary, response trials evoked larger SCRs than nonresponse trials for both CS+ and CS− trial types. Corresponding UCS expectancy ratings indicated that, during CS+ but not CS− trial types, participants explicitly differentiated between trials on which they showed an SCR and trials on which they did not.

Imaging Results

In order to test the reliability of the amygdala effect found in Experiment 1, we applied the same imaging analysis technique in Experiment 2. One procedural difference between the two experiments is that functional images were acquired in separate runs (Blocks 1 and 2) in Experiment 1, whereas images were collected in one long, seamless run in Experiment 2. Another two-factor ANOVA (CS Type × Response Type) was performed on %AUC for the first eight CS+ and CS− trials. Similar to Experiment 1, a significant CS Type × Response Type interaction revealed a cluster of activation in the right amygdala (Talairach coordinates: 19.1 −8.8 −8.7; 296 μl), $F(1, 8) = 5.33$, $p \leq .05$ (top portion of Figure 3b). Using this cluster as a functional region of interest, we detected increased responding to CS+ response trials ($M \pm SE = 0.80 \pm 0.09$) relative to CS+ nonresponse trials (-0.04 ± 0.27). In contrast, CS− response trials (0.31 ± 0.20) did not differ from CS− nonresponse trials (0.51 ± 0.20; bottom portion of Figure 3b). These activation patterns resemble the results found in Experiment 1. Differential right amygdala activity was observed only on CS+ trials, as indicated by increased amygdala responding on trials that produced a SCR (Figures 3a and 3b).

Discussion

Laboratory animal models suggest that forming CS–UCS associations and expressing autonomic and behavioral reactions during Pavlovian fear conditioning are two dissociable processes that rely upon distinct neural structures. Although numerous studies (Armony & Dolan, 2002; Büchel et al., 1999; Büchel et al., 1998; Knight et al., 2004b; Morris et al., 2001) have focused on the human amygdala's role in learning stimulus associations, there has not been a direct investigation of its role during response expression in Pavlovian fear conditioning. In the present article, event-related fMRI and concurrent SCR measurements were used to explore the functional role of the amygdala during the autonomic expression of human conditional fear in two different experiments. Unlike previous investigations (Armony & Dolan, 2002; Büchel et al., 1999; Büchel et al., 1998; Cheng et al., 2003; Knight et al., 2004b; LaBar et al., 1998; Morris et al., 2001; Phelps et al., 2001) of the amygdala during fear conditioning, the present analyses isolated amygdala responding to a particular trial type to more directly address the relationship between human amygdala activity and the expression of autonomic fear responses in two separate experiments. Results from both experiments showed differential amygdala activity between trials that signaled shock and produced a conditional SCR (CS+ response) and trials that signaled shock but failed to elicit a conditional SCR (CS+ nonresponse), suggesting that metabolic activity in this structure may reflect the expression of conditional fear responses.

Several procedural differences between the two experiments are important to note. First, functional images were acquired in separate runs (Blocks 1 and 2) in Experiment 1, whereas images were collected in one long, seamless run in Experiment 2. Although both experiments involved differential conditioning paradigms, Experiment 1 involved three different CSs, whereas Experiment 2 included only two. Experiment 2 also included a habituation phase before acquisition, whereas Experiment 1 did not. Despite these procedural differences, reliable significant amygdala responses across two different experiments were observed only under specific conditions and, consequently, lend strong support to our hypothesis.

Analyses of SCR data from both experiments showed learning-related changes. Participants showed increased SCR amplitude to the CS+ relative to the CS−, indicating that participants were able to implicitly discriminate between the two conditional stimuli. Although the expression of conditional responses is important during fear learning, it is just one component of learning. Specifically, showing discriminative responses to the CS+ and CS− suggests successful differential conditioning. Nonspecific fear expression to all stimuli, including those that serve as a safety signal would not qualify as fear learning. This is important to note because our amygdala activity was closely associated with only conditional responses occurring in the presence of a visual cue that predicts shock, whereas responses during CS− trials do not appear to be related to amygdala activity.

Differences between the two experiments might have yielded relatively minor discrepancies in the behavioral data. Following classification of all trials into response and nonresponse trial types, differential SCR was observed only during CS+ trials in Experiment 1 (Figure 2a), whereas differential responding was seen for both CS+ and CS− in Experiment 2 (Figure 2b). Given that differential responding was not seen during CS− trials in Experiment 1, one possible interpretation of the pattern of amygdala activity is that differential BOLD responding occurs only when there is a significant SCR difference regardless of the conditional stimulus. However, significant behavioral differences between CS− response and CS− nonresponse trials in Experiment 2 did not yield similar amygdala differentiation, suggesting that amygdala activity is related to increased SCR in the presence of a visual cue that predicts shock.

In Experiment 2, participants explicitly reported differences between CS+ trials on which they demonstrated an SCR and trials on which they did not. Taken alone, these results make it difficult to suggest that the amygdala effect is due solely to conditional SCRs. However in Experiment 1, significant UCS expectancy differences between these same trials were not detected, yet differential responding in the amygdala was. This observation rules out the possibility that differences in the amygdala response might have been confounded by differential contingency awareness during response and nonresponse trial types. These behavioral data suggest that the changes observed in the amygdala were closely related to differences observed in participants' SCR patterns and were not due to differences in other learning-related parameters. One likely explanation for the UCS expectancy differences between Experiments 1 and 2 is that participants in Experiment 2 had just undergone a habituation phase and were entering the acquisition phase, whereas participants in Experiment 1 have already had

five acquisition trials and were already at asymptotic behavioral performance.

With the use of a similar analytic approach to investigate the role of the amygdala while participants viewed fearful facial expressions, researchers have reported (Williams et al., 2001) that amygdala activity was detected only in the presence of SCR arousal, whereas hippocampal activity was observed only in the absence of SCR arousal, suggesting that the amygdala may be uniquely involved in SCR production related to fearful experiences. The present results extend these findings by demonstrating that amygdala activity may be related to autonomic conditional fear responses elicited by a stimulus that predicts shock.

Changes in amygdala activity did not simply correlate with normal SCR fluctuations. First, differential amygdala activity was not seen between CS− trials that elicited a significant positive SCR and CS− trials not able to elicit a SCR (Experiment 2). Additionally, the amygdala is not believed to be a necessary neural component underlying normal SCR fluctuations, as lesion data have shown that amygdala patients show intact SCR patterns (Tranel & Damasio, 1993, 1989). Furthermore, functional neuroimaging studies (Critchley, Elliot, Mathias, & Dolan, 2000; Patterson, Ungerleider, & Bandettini, 2002) investigating brain regions important for the general production and maintenance of SCR do not observe amygdala activity.

Although the current studies were not optimally designed to address laterality effects, right amygdala activity was observed in both experiments. The unilateral findings in both experiments were largely consistent with previous neuroimaging studies of fear conditioning (Büchel et al., 1998; Cheng et al., 2003; LaBar et al., 1998). However, left amygdala activity during fear learning has also been reported (Morris, Ohman, & Dolan, 1998; Phelps et al., 2001). One possibility for this discrepancy may be that conditions contributing to activation of the left amygdala are associated with higher order cognitive processes, such as awareness (Morris et al., 1998) and verbal instructions of CS–UCS contingencies (Phelps et al., 2001).

Surprisingly, changes in activity within the amygdala were not observed during Block 1 in Experiment 1, even though SCR differences were evident. This would appear to be inconsistent with our working hypothesis and results from a previous study (Cheng et al., 2003) that detected early amygdala activity. However, on closer examination, the levels of cognitive demand between the current and former study (Cheng et al., 2003) were not equal. First, participants in our prior imaging study were not required to discriminate between different stimuli, as that study used single-cue conditioning, whereas participants in the present study were asked to differentiate between three distinct stimuli. Discrimination paradigms are more likely to retard acquisition rates relative to that typically seen in single-cue conditioning. Second, participants in the present study were engaged in an active task that required the manipulation of a dial in order to report their cognitive UCS expectancy, whereas no such task was required of the participants in the previous study. Other fMRI studies of fear conditioning that detected early amygdala activity were also passive tasks and relatively simple to learn (Büchel et al., 1998; LaBar et al., 1998; Phelps et al., 2001; Cheng et al., 2003). Taken together, it is likely that the increased cognitive demands and the addition of an active task in the current study may have influenced the rate at which amygdala responses developed.

Experiment 2 also addressed the timing of amygdala responses and levels of cognitive demand. Recall that Experiment 2 required participants to discriminate between two conditional stimuli, whereas Experiment 1 required participants to discriminate between three conditional stimuli. In the more simple design (Experiment 2), amygdala activity was observed early during acquisition, whereas in a more complicated paradigm (Experiment 1), functional activity in the amygdala was observed following several acquisition trials (Block 2). These results appear to be consistent with the view that the levels of cognitive demand may affect when amygdala responses occur. Specifically, the more complex designs may result in a delay in the development of amygdala responses.

This idea of complex cognitive demands influencing amygdala activity has been previously noted. On the basis of previous functional imaging (Beauregard, Levesque, & Bourgouin, 2001; Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003) and patient lesion studies (Bechara et al., 1999) of the neural mechanisms underlying cognitive demands, regulation of emotional experiences, and their interactions with amygdala functioning, one might conjecture that the lack of amygdala responding during Block 1 could have resulted from cognitive appraisal of stimuli. It has been proposed (Hariri et al., 2003) that increased prefrontal cortical activity, as a result of conscious evaluation of fearful stimuli, may inhibit amygdala responses. At the end of Block 1, asymptotic behavioral performance on subject's UCS expectancy was seen (Knight et al., 2004a), suggesting that participants were aware of the CS–UCS relationships by the beginning of Block 2. It is possible that changes in the amygdala were detected in Block 2 of Experiment 1 and early acquisition of Experiment 2 might have occurred in part because participants were no longer actively evaluating stimulus relationships and, thus, no longer inhibiting amygdala activity.

The pattern of amygdala activity between Experiments 1 and 2 were qualitatively similar but the magnitude of amygdala responding during CS+ response trials in Experiment 2 appeared weaker. This effect could be interpreted as a result of the habituation phase implemented in Experiment 2. Habituation often leads to latent inhibition, which refers to the retardation of the acquisition of conditional responses as a result of pre-exposure to nonreinforced CSs. Conditioning studies have shown that the amygdala may play a role during latent inhibition (Coutureau, Blundell, & Killcross, 2001; Sotty, Sandner, & Gosselin, 1996). Specifically, Sotty et al. (1996) used c-*fos* immunolabeling to report decreased neuronal density in the amygdala of rats receiving CS pre-exposure trials compared with rats receiving no pre-exposure period during fear conditioning. These results are consistent with the decreased magnitude of amygdala responses observed for participants receiving habituation (Experiment 2). Although quantitative differences between the two experiments were observed, the overall qualitative pattern of activity was similar in both experiments, as indicated by the significant increase in amygdala responding to CS+ trials that produce an SCR relative to those CS+ trials failing to elicit a SCR.

Although this article has focused on implicit responses during Pavlovian fear conditioning, this form of learning involves both implicit and explicit components. Explicit responses are often used to define awareness of stimulus contingencies and can also be used, in a similar manner to the way we used the participant's implicit responses, to explore brain regions important for declarative knowledge. Unfortunately, in many simple experimental

designs, the development of explicit knowledge of CS–UCS relationships often occurs in a small number of trials. This relatively short acquisition period makes it difficult to observe learning-related changes in the brain related to explicit responding. Current efforts in our lab have concentrated on retarding acquisition by introducing more complex conditioning procedures in order to consider brain regions important for the development of explicit knowledge during fear conditioning (Richards, Cheng, Smith, & Helmstetter, 2006). Slowing acquisition may also address some questions about the temporal properties of amygdala activation. This paper focused only on amygdala activity and conditional SCRs during early acquisition trials. This time window was selected because responses in the amygdala have been shown to rapidly attenuate in human neuroimaging studies of fear conditioning (Büchel et al., 1998; LaBar et al., 1998; Phelps et al., 2001); moreover, electrodermal responses often habituate (Kimmel, 1959; Stewart, Stern, Winokur, & Fredman, 1961), making it difficult to classify trial types on the basis of SCR characteristics late in acquisition. More complex conditioning procedures designed to retard acquisition may be able to address response-related amygdala activity during later training trials.

Cheng et al. (2003) was an initial attempt at using autonomic fear responses to guide functional imaging analyses. The current analysis addressed specific concerns of this study, which include limitations that accompany a block design, correlational findings, and a between-groups analysis. Consistent results from two different experiments extended our previous findings and the general approach of using autonomic fear responses to map neural activity. Despite the use of slightly different procedures in the two experiments, differential amygdala responding was consistently observed under specific conditions. Differential amygdala activity was detected between trials that signaled shock and produced a conditional SCR (CS+ response) and trials that signal shock but failed to elicit a conditional SCR (CS+ nonresponse). These results further implicate the involvement of the amygdala in the autonomic expression of conditional fear and suggest that studies that view the human amygdala as a functionally homogenous structure may be restricted in their conceptualization of the data. Future studies should give more consideration to the interpretation that amygdala activation may be related to the performance of autonomic conditional fear as well as acquiring stimulus associations.

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Received September 29, 2005
Revision received September 6, 2006
Accepted September 14, 2006 ■