



Activation of the left amygdala to a cognitive representation of fear

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We examined the neural substrates involved when subjects encountered an event linked verbally, but not experientially, to an aversive outcome. This instructed fear task models a primary way humans learn about the emotional nature of events. Subjects were told that one stimulus (threat) represents an aversive event (a shock may be given), whereas another (safe) represents safety (no shock will be given). Using functional magnetic resonance imaging (fMRI), activation of the left amygdala was observed in response to threat versus safe conditions, which correlated with the expression of the fear response as measured by skin conductance. Additional activation observed in the insular cortex is proposed to be involved in conveying a cortical representation of fear to the amygdala. These results suggest that the neural substrates that support conditioned fear across species have a similar but somewhat different role in more abstract representations of fear in humans.

A primary adaptive function of emotion is to influence future interactions with stimuli associated with emotional reactions. To discover the neuroscientific principles underlying this emotional learning, fear conditioning has traditionally been used as the behavioral task. The amygdala is critical for learning the aversive properties of events through fear conditioning, across species and stimulus types^{1,2,3}. However, fear conditioning may not be the primary means by which humans learn about the aversive nature of events. Although there are situations in which people learn about the emotional properties of a neutral event by its co-occurrence with an aversive event, we often learn about potentially dangerous situations through language. An analogy to traditional fear conditioning would be learning to fear a neighborhood dog because it once bit you unexpectedly. You develop a fear from direct experience with the dog in conjunction with the aversive experience of a painful bite. However, a similar fear reaction could be elicited not because the dog bit you, but because you heard that it bit someone else. In this case, there is no direct experience with the dog or with an aversive event. Instead, the dog is associated with an imagined and anticipated aversive event, resulting in a cognitive representation of the aversive properties of the dog.

This awareness of the aversive nature of events or stimuli is sufficient to guide our actions. We avoid dangerous neighborhoods or shark-infested waters, not necessarily because we have been crime victims or attacked by sharks in those locations, but rather because we have been told about their aversive properties. Previous research in humans using fear conditioning has found that the amygdala is not necessary for the acquisition of an explicit, cognitive representation of the aversive properties of a stimulus. This cognitive representation depends on the hippocampal memory system, which is important for the acquisition of explicit or declar-

ative memories⁴. However, the amygdala is necessary for the expression of a conditioned fear response to that same stimulus^{4,5}. But what if the cognitive representation of the aversive properties of a stimulus is acquired without direct experience, as in the examples above? Based on previous results, we do not expect the acquisition of this representation to depend on amygdala function⁴. In the present study, we ask if the amygdala is involved in non-experiential fear-evoking situations.

We addressed this question by examining activity in the human amygdala using fMRI with a task called instructed fear. During instructed fear, subjects do not actually receive an aversive stimulus, but they are told that an event might occur in conjunction with a neutral stimulus. Previous research on normal adults has shown that this type of task, which has also been called anticipatory anxiety, results in fear responses similar to those observed in traditional fear conditioning, as measured by startle potentiation and skin conductance response^{6,7,8}. Here we explore whether the same neural system underlies these similar response patterns, focusing on the involvement of the amygdala.

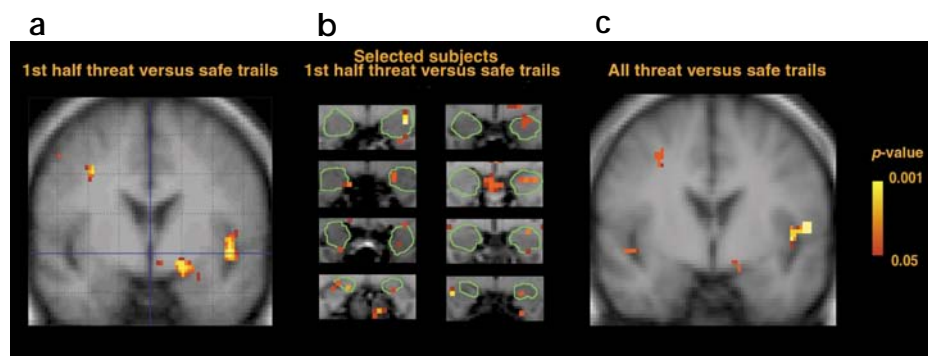
During image acquisition, subjects had an electrode attached to their wrists that they were told would be used to deliver an uncomfortable, but not painful, mild electric shock. They were told to expect no more than three shocks and no less than one shock during the experiment. Three types of stimuli representing the three trial types were presented: a blue square, a yellow square, and the word 'rest.' Subjects were told that they might receive a shock when one of the colored squares were presented (the threat condition), but not when the other colored square was presented (the safe condition) or the word rest (the rest condition) were presented. There were five 18-second trials of each type. Skin conductance responses (SCR) were recorded. No



Fig. 1. Threat versus safe activation.

(a) Contrast composite map of activation for first-half threat versus safe (n = 12). Comparison is averaged BOLD signal across all 5 blocks for the first 6 (of 12) images during threat versus safe trials. Across subjects, significantly active pixels were observed in the left dorsal amygdala (Talairach coordinates, -13, -5, -26), extending into the basal forebrain (-8, -5, -18), the left insula (1.5, -5, -44) and right premotor cortex (37, -5, 32). All coordinates listed are for the largest area of activation within a region,

although additional areas of activation were observed within some regions. The slice represented in this composite is the slice (of three acquired) that covered the largest portion of the amygdala. (b) Representative individual subjects for the first-half threat versus safe comparison. The slice shown for each subject was chosen from among the three slices acquired that covered portions of the amygdala (outlined in green). Individual subjects showed regions of activation consistent with the group composite. However, most subjects also showed regions of activation in the anterior cingulate, the right insula and the striatum. (c) Contrast composite map for the total threat versus safe comparison, including responses in the second-half of the trials. Activation in amygdala is diminished when these later responses are included, suggesting an attenuation of amygdala activation within the 18-s trial. Other regions of activation do not show a similar pattern of attenuation.



shocks were administered in this study. The experimental design was chosen to obtain maximal behavioral effects based on previous research with similar tasks^{7,8} and pilot studies.

RESULTS

To assess if the instructed fear task was successful in eliciting an arousal response consistent with a fear reaction, SCR was compared across the three trial types. There was a significant increase in SCR during the threat trials compared to rest. There was no difference in SCR between safe and rest (mean change in square-root-transformed SCR compared to rest baseline, threat, 0.098 μ S; safe, 0.0069 μ S; $F_{1,11} = 13.94$, $p > 0.01$). In addition, subjective emotional responses were assessed after the imaging session. All subjects indicated that they thought they would receive a shock at some point during the threat stimulus, and all subjects reported feeling more anxious during the threat condition.

We then assessed if the amygdala showed greater activation during the threat trials than the safe trials. Two types of analyses were done. We averaged across all trials for each condition and compared activation patterns across conditions. We also conducted an analysis comparing the activation patterns across conditions for the average of the first half of each trial. This second analysis was done because we expected an attenuation of the amygdala response. Previous studies of both human neuroimaging^{9,10,11} and electrophysiological recording from the amygdala in rats^{12,13,14} have shown that the amygdala response attenuates over time. Two types of attenuation have been observed. Human and animal studies have shown across-trial attenuation in studies with a relatively large number of trials. Specifically, the amygdala response seems to attenuate after four to five presentations of a single conditioned stimulus in fear conditioning^{10,12}, or a few blocks of stimulus presentation in studies that display a series of similar but different stimuli, such as fearful faces⁹. In addition, studies with animals have shown within-trial attenuation of the amygdala response to a longer conditioned stimulus or stimulus presentation, with the maximal amygdala response occurring to the onset of an aversive stimulus^{12,13,14}. In the present study, we had a single-threat stimulus with only five trials of this stimulus. Given the small number of trials, we did not expect significant across-trial attenuation of the amygdala response. However, the duration of the stimulus presentation in each trial was relatively long (18 seconds). Therefore,

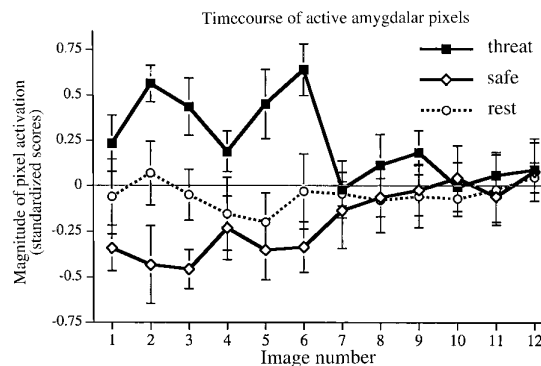
we expected there might be some within-trial attenuation of the amygdala response. For this reason, we chose to examine the early amygdala response across all five of the trials for each condition.

There was significant activity in the left amygdala that extended to the basal forebrain when the first half of the threat trials was compared with the first half of safe trials (Fig. 1). It is sometimes hard to determine precise anatomy on composite activation and anatomical maps because of subject averaging, so we conducted a region-of-interest (ROI) analysis confined to the amygdala. This revealed that 11 of 12 subjects showed significant activity in the left amygdala (Fig. 1b). Only some of these subjects showed additional activation in the basal forebrain, suggesting the activation observed on the composite map may be primarily due to the amygdala. Although the group composite revealed left amygdala activity, the pattern of amygdala response varied somewhat across individuals, and 7 of 12 individuals showed less extensive but significant right amygdala activation. The separate analysis that included the total activation responses, not just those early in each trial, indicated that there was an attenuation of amygdala activation. There was relatively little amygdala/basal forebrain activation on the composite activation map when the analysis was extended to include the entire length of each trial. Additional analyses comparing the other conditions were also conducted. Although most subjects revealed less extensive amygdala activation when comparing first half of threat versus first half of rest trials, this was not significant on the composite analysis. A similar pattern was seen in the first half of threat versus first half of rest comparison for other brain regions in which activation was observed. There was no consistent pattern of activation in the amygdala or other regions when safe was compared to rest.

Although we were primarily interested in the response of the amygdala during instructed fear, and restricted our image acquisition to coronal slices covering the amygdala, there were additional regions of activation. We observed scattered activation in the striatum and prefrontal cortex, primarily premotor cortex, in 10 of 12 subjects. Activation was also observed in the anterior cingulate in 8 of 12 subjects. In all subjects, we observed robust activation of the insular cortex, extensive and strong in the left insula and somewhat more limited in the right insula. The activation in these regions did not show the same pattern of attenuation observed in the amygdala (Fig. 1a and c).



Fig. 2. Time course of amygdala activation. Functional time-course analysis by image number within each trial averaged across all 5 blocks. Average standardized scores of activation for significantly active pixels in early threat versus early safe comparison ($p < 0.05$) within the amygdala ($n = 11$), as defined by ROI analysis. The first image of the time course corresponds to the image acquired 3 s following stimulus onset, to control for the delay in hemodynamic response. Individual subjects' standardized activation levels are calculated relative to their overall average magnitude of BOLD signal throughout image acquisition. These averaged z-scores were submitted to a two-factor (condition \times image number) ANOVA. A significant effect for condition was found ($F_{2,20} = 9.41$, $p < 0.01$) and a significant interaction between condition and trial was found ($F_{22,220} = 2.01$, $p = 0.001$). To investigate the effects of attenuation across image number (over trials), time-course data were collapsed into first half (images 1–6) and second half (images 7–12). Pairwise t -tests between first-half threat and first-half safe trials revealed a significant difference ($t = 10.16$, $p < 0.01$), whereas second-half threat trials were found to be indistinguishable from second-half safe trials ($t = 0.213$, $p > 0.85$). A comparison between first-half safe and first-half rest trials also revealed a significant difference ($t = -4.30$, $p < 0.01$), whereas second half-safe trials were not found to differ from second-half rest trials ($t = 0.183$, $p > 0.85$).



To examine the pattern and attenuation of the amygdala response across each trial type, a time-course analysis was conducted on the significantly active pixels within the amygdala (Fig. 2). The response during threat trials was greater than during safe and rest trials, but only early in the first nine seconds of the block. This result is consistent with other findings showing attenuation of the amygdala response across time, suggesting this response may be primarily linked to signaling the emotional properties of a stimulus^{10,12–14}. In addition, there was a tendency for a reduction in amygdala activation during the safe condition compared to rest early in the block. The mean percent signal change between early rest and early safe conditions was 0.26%, compared to a 1.1% signal change between early threat and early safe. This slight decrease in amygdala activation during the safe trials is consistent with animal studies showing a decrease in amygdala response to stimuli that are thought to represent safety¹⁵.

To evaluate the relationship between the strength of the arousal response as measured by SCR and activation when the aversive event is only anticipated, we correlated the difference in the SCR response to threat trials compared to safe trials across subjects with the magnitude of activation within the amygdala, insular cortex and anterior cingulate, as defined by ROI analysis. There was a strong and significant correlation between the SCR response across the entire block and left amygdala activation ($r = 0.59$, Fig. 3a); the right amygdala showed a non-significant correlation ($r = 0.14$). Unlike amygdala activation that was greater in the first half of each block and attenuated over time, the SCR response was maintained over the entire block. This decoupling of the temporal pattern of the amygdala activation and correlated SCR response has been reported previously¹⁰ and suggests that the amygdala activation is related to this arousal response, but not critical for the response. In addition, there was a significant correlation between the magnitude of activation in the insula and SCR (Fig. 3b). This correlation was stronger than that between amygdala activation and SCR, suggesting that the insula may also be important in the expression of the fear response in this task. Unlike the lateralized response of the amygdala, this correlation was significant for both the right ($r = 0.58$) and left insula ($r = 0.86$), even though the left insula showed greater activation overall, as well as higher correlation with SCR. There was no significant correlation between activation of the anterior cingulate and SCR.

DISCUSSION

In the instructed fear protocol, subjects showed an arousal response, consistent with fear, to a stimulus that they were told might be linked to an aversive event. The presentation of this stimulus led to activation of the amygdala. Across subjects, the magnitude of the amygdala response was correlated with SCR, suggesting that this amygdala activation is related to the expression of the fear response. Unlike previous studies that showed activation of the amygdala in response to stimuli that were linked to aversive events either presented or imagined^{9–11,16}, subjects in the present study had no direct experience with the aversive event. These results suggest that the amygdala is involved in the expression of the fear response in the instructed fear task, which models a common way humans learn about the emotional properties of stimuli.

When we compare the present results using the instructed fear protocol to our previous study with fear conditioning¹⁰, there are several similarities, but also several important differences. In both studies, we found activation of the amygdala that attenuates across time and is correlated with the strength of the fear response. In fear conditioning, this correlation was carried by the right amygdala. In the present study, activation of the left amygdala was predominant and more strongly correlated with the fear response.

What might account for these differences in laterality in conditioned versus instructed fear? In the instructed fear task, subjects are aware of the aversive nature of the stimulus before scanning. A previous study has suggested that the left amygdala responds when subjects are aware of the aversive nature of the stimulus, whereas the right amygdala responds when subjects are unaware of this contingency¹⁷. Although subjects in the fear conditioning study were aware of the conditioned stimulus from the beginning of the study, they were unaware that it predicted an aversive event for the first few trials. However, after a few trials of fear conditioning, subjects acquired an awareness of the aversive properties of the conditioned stimulus. However, in spite of this awareness, the activation did not switch to the left amygdala in the later trials of fear conditioning.

A second possibility for the difference in laterality of the amygdala response across these two protocols is the nature of learned material and the type of representation that is evoked. Studies with brain-injured patients have shown that the right amygdala may modulate the fear response when the aversive properties of the

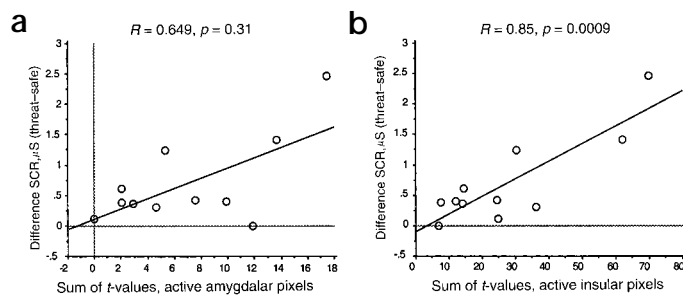


Fig. 3. Correlation between SCR and activation strength for amygdala and insular cortex. Correlation between measures of autonomic arousal (difference SCR) and (a) magnitude of amygdalar functional activation and (b) magnitude of insular functional activation. Difference SCR is expressed as mean threat SCR minus mean safe SCR. Regional magnitude of functional activation was obtained by summing the *t*-values of all significantly active pixels within an ROI for each brain structure for early threat versus early safe trial comparison ($p < 0.05$).

stimulus are visual in nature¹⁸, whereas the left amygdala may modulate the fear response when the aversive nature of the stimulus is learned through verbal communication, as in the instructed fear protocol¹⁹. Visually aversive stimuli elicit an immediate, negative representation that is not dependent on elaboration by the subjects. When the aversive nature of the stimulus is learned verbally, the subjects must generate a mental representation of the aversive event because it does not exist in the immediate environment. The difference in laterality of amygdala activation may reflect the extent to which the representation elicited by the fearful stimulus depends on elaboration and interpretation by the subjects.

The insular cortex is another region that was active in both the fear conditioning and the instructed fear protocols. There are numerous, reciprocal connections between the amygdala and insular cortex²⁰. However, the properties of the insula response across these two protocols differed. In instructed fear, we observed extensive and strong activation of the left insula and additional activation in the right insula. This insular cortex activation was present from the early trials and was strongly correlated with the strength of the arousal response. In fear conditioning, the insula activation was less robust and appeared only after the initial pairings of the neutral and aversive events, when subjects had become aware of the conditioned stimulus–unconditioned stimulus contingency. At that point, the subjects may have been consciously anticipating the aversive shock, consistent with a previous study suggesting the insular cortex is involved in the anticipation of pain²¹.

The present results, when compared to fear conditioning, suggest that the insular cortex may be more integral in the instructed fear task in which the representation of the aversive properties of the event is purely cognitive in nature. It has been suggested that there are two parallel cortical and subcortical pathways for conveying information about the aversive nature of a stimulus to the amygdala²². A previous study²³ suggested that the insula is involved in conveying cortical somatosensory information to the amygdala. In the present study, the aversive stimulus was the imagined discomfort of receiving a shock that was never experienced. We suggest that this imagined and anticipated discomfort results in a cortical representation of fear, which may be relayed to the amygdala via the insula.

Finally, in both fear conditioning and instructed fear, we observed similar patterns of activation in the premotor cortex, striatum and anterior cingulate. The observed activation in premotor cortex and striatum may be related to subtle tensing that occurs when shock is anticipated^{10,11}. Activation in the anterior cingulate has been linked to attentional processes, as well as evaluation of emotional stimuli^{24,25}.

In the present study, we examined the neural substrates involved when subjects encountered an event that was linked to an aversive outcome through verbal communication, in the absence of aversive experience. Fears that are imagined and

anticipated but never experienced can have a profound influence on everyday behavior. In spite of the known involvement of the amygdala in emotional learning, the acquisition of this cognitive representation of the aversive nature of an event does not seem to depend on the amygdala⁴. However, the present study suggests that the left amygdala may be involved in the expression of the fear response when this type of fearful event is encountered. The insular cortex may be involved in conveying this cortical representation of fear to the amygdala.

METHODS

Subjects. Twelve subjects (six female, six male) were submitted to final analysis. A total of 22 subjects were run. Five subjects were excluded because center-of-mass motion during scan exceeded our criterion of 0.33 pixels in any direction. One subject's functional data exhibited severe artifactual activations outside the head, and was also excluded. Two subjects were excluded from analysis as being SCR 'non-responders.' This is characterized in this case by a lack of discernible variability in the subject's SCR output waveform, which can be due to a number of factors. Two additional subjects were also excluded because of SCR recording equipment failure and functional data recording media failure, respectively. All subjects gave informed consent.

Behavioral task. Subjects were asked to lie in an MRI scanner, and electrodes were attached to their left wrist and the second and fourth fingers of the left hand. They were told that the electrodes attached to their fingers would be used to record their SCR during the experiment, but they would not feel any sensation from the electrodes on their fingers. They were told that the electrode attached to their wrist would be used to deliver a mild electric shock during the threat condition and that there would be between one and three shocks delivered throughout the study. The colors representing threat and safe were counterbalanced across subjects. Each trial lasted 18 seconds. The blue and yellow squares had digits presented in the middle of the square that counted down the seconds in the trial from 18 to 1. Each trial type was presented five times. Each block of three trials began with a rest trial. This was followed by a threat and a safe trial, the order of which was counterbalanced across subjects, but did not vary across blocks for an individual subject. After five trials of each type, the experiment ended. The subjects were unaware that the shock electrode on the wrist was not attached to any stimulating device. They were told at the end of the experiment that they were in a non-shock condition. They were asked to indicate whether they thought they would receive a shock in the experiment. All subjects indicated that they believed a shock would be delivered. The subjects were then debriefed.

SCR acquisition and analysis. SCR telemetry was acquired through constant voltage (0.5 V) excitation method with Ag-AgCl electrodes attached to the middle phalanges of the second and fourth digits of the left hand (BIOPAC Systems, Santa Barbara, California). Lafayette Instruments electrode gel was used as an electrolyte (Lafayette, Indiana). Electrode leads were tightly twisted, to minimize electromagnetic interference, and were connected to shielded leads. Lead shield was grounded through an RF filter at an MRI room to control room junction. The SCR signal was



amplified and recorded with a BIOPAC Systems skin conductance module connected to an Apple Powerbook 3400c running AcqKnowledge software (BIOPAC Systems). Data were recorded continuously at a rate of 200 samples per second. Off-line analysis of SCR waveforms was done using AcqKnowledge software. Specifically, waveforms were low-pass filtered (Blackman -61 dB, 7 Hz cutoff) to reduce high frequency noise induced by electromagnetic interference. SCR waveforms were smoothed with a smoothing factor of 30 points. Average, tonic means of SCR levels were then calculated for each 18-s block.

fMRI acquisition. Before image acquisition, the anterior and posterior commissures were localized for slice orientation. Whole-brain sagittal T1-weighted anatomical images were acquired using a spin echo-pulse sequence (5-mm contiguous slices; TE, 12 ms; TR, -600 ms; matrix size, 256 × 192; in-plane resolution, 1.56 × 1.56 mm; FOV, 40 × 40 cm). Five 6-mm coronal slices (slice skip, 2 mm) were then prescribed perpendicular to the AC-PC line, with the middle slice centered on the amygdala. Amygdala localization was accomplished by placing the middle (third) slice 4–5 mm posterior to the anterior commissure in the midsagittal view, and assessing the position of the amygdala in the subsequent coronal sections using anatomical landmarks and a standardized atlas²⁶. During the study, echoplanar functional images were acquired using an asymmetric spin echo pulse sequence (TE, 30 ms; echo offset, 30 ms; TR, 1.5 s; in-plane resolution, 3.125 × 3.125 mm; matrix size, 128 × 64; FOV, 40 × 20 cm). These scanning parameters yielded a total of 192 functional images per slice for each experimental condition.

fMRI analysis. The experimental task was a standard block design consisting of five intermixed trials of each stimulus condition, rest, safe and threat, resulting in 15 trials. During each trial, 12 images were acquired over 18 s (TR, 1.5 s). There were two types of analyses that differed only in the portion of the trial that was included. In the first-half analysis, subjects' functional activation was averaged across the first six images of each trial of each condition (allowing 3 s for the delay in the hemodynamic response). In the total analysis, all 12 images in each trial were included. Resultant *t*-maps were generated by subtraction to reveal differential activation between conditions. Pixels showing significant differential activation ($p < 0.05$) were used in subsequent ROI and time-course analysis. The SPMs and the anatomic images were transformed by in-plane transformation into a proportional three-dimensional grid²⁶. To obtain *p*-values for significantly active pixels across subjects, a contrast composite map was generated using a randomization test to create a distribution of task-related *t*-values²⁷. The *p*-value for each pixel was overlaid upon a mean anatomic image. Only significantly active pixels are displayed.

fMRI ROI and correlation analysis. For each subject, ROI analyses were conducted on the amygdala, insular cortex and anterior cingulate. All ROI analyses were done on data obtained from the first-half threat versus safe trial comparison. The specified regions were first outlined on the three anatomical images acquired per subject that covered portions of the amygdala. The functional maps of threat-safe were then superimposed on the anatomical images to identify active pixels within these regions. To calculate the correlation between arousal response and activation, the two subject variables submitted to the regression, sum of *t*-values and difference SCR were obtained as follows. The magnitude of *t*-values for significantly active pixels (*t*-value; $p < 0.05$; cluster value, 0) occurring within each ROI was summed²⁸. Tonic SCR means of threat and safe for each epoch were obtained from subjects' low-pass-filtered and smoothed SCR waveforms, and averaged across epochs. Subtracting these two values yielded threat-safe SCR. Eleven of twelve subjects were submitted to the regression analysis. One subject was excluded because of a highly uncharacteristic SCR waveform that occurred in the latter half of image acquisition, which may have been the result of equipment malfunction or electrode movement. An additional regression analysis was done identically to the one described above except the strength of amygdala activation was assessed by summing the number of active pixels within a region (as opposed to summing the *t*-values of these pixels). Similar results were obtained with both methods, so only the results of the first analyses are reported.

ACKNOWLEDGEMENTS

The authors acknowledge the inspiration of Charles Oakley. We also thank M. Nordan and K. LaBar for work on a pilot study. This research was supported by McDonnell-Pew Program in Cognitive Neuroscience 97-26 and National Institutes of Health grants MH50812 to E.A.P. and NS33332 to J.C.G.

RECEIVED 2 AUGUST 2000; ACCEPTED 16 JANUARY 2001

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