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Human Amygdala Activation during Conditioned Fear Acquisition and Extinction: a Mixed-Trial fMRI Study

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Summary

Echoplanar functional magnetic resonance imaging (fMRI) was used in normal human subjects to investigate the role of the amygdala in conditioned fear acquisition and extinction. A simple discrimination procedure was employed in which activation to a visual cue predicting shock (CS+) was compared with activation to another cue presented alone (CS−). CS+ and CS− trial types were intermixed in a pseudorandom order. Functional images were acquired with an asymmetric spin echo pulse sequence from three coronal slices centered on the amygdala. Activation of the amygdala/periamygdaloid cortex was observed during conditioned fear acquisition and extinction. The extent of activation during acquisition was significantly correlated with autonomic indices of conditioning in individual subjects. Consistent with a recent electrophysiological recording study in the rat (Quirk et al., 1997), the profile of the amygdala response was temporally graded, although this dynamic was only statistically reliable during extinction. These results provide further evidence for the conservation of amygdala function across species and implicate an amygdalar contribution to both acquisition and extinction processes during associative emotional learning tasks.

Introduction

The amygdala is a brain structure that is hypothesized to play a critical role in emotional memory processes (reviewed by Davis, 1994; LeDoux, 1995; Gallagher and Chiba, 1996; LaBar and LeDoux, 1997; Phelps and Anderson, 1997). This function of the amygdala has been elucidated largely by animal research using classical conditioning (Pavlov, 1927) as a model for understanding how the emotional significance of events is learned and remembered. Across a variety of species, stimuli, and behavioral measures, the amygdala has emerged as an essential component of a neural network mediating conditioned fear associations (reviewed by Kapp et al., 1992; Davis, 1994; LeDoux, 1995; Maren and Fanselow, 1996). However, applications of conditioning paradigms to investigate amygdala function in human populations have produced inconsistent results across techniques. Whereas neuropsychological studies have reported fear conditioning deficits in human patients with amygdala damage (Bechara et al., 1995; LaBar et al., 1995), positron emission tomography (PET) studies have failed to find increased blood flow in the amygdala during fear conditioning in normal human subjects (Fredrikson et al., 1995; Hugdahl et al., 1995; Furmark et al., 1997; Morris et al., 1997). Although failure to report activation in a neuroimaging study does not preclude a role for a given brain region in a particular task (e.g., Cabeza and Nyberg, 1997), the existing PET results are surprising, given the functional anatomy of conditioned fear as revealed in animal studies. Since this model system is important for developing theories of emotional memory mechanisms and their dysfunction in affective disorders (Wolpe and Rowan, 1988; Shalev et al., 1992; Charney et al., 1993), it is critical to establish the role of the amygdala in fear conditioning tasks conducted on human subjects. To help bridge this gap, we applied echoplanar functional magnetic resonance imaging (fMRI) in the present study to examine the role of the amygdala and related structures in the acquisition and extinction of fear conditioning in the normal human brain.

Fear conditioning tasks pose several difficulties for functional neuroimaging, especially in assessing the contribution of the amygdala. We designed the present study to address the difficulties in the following ways. First, the amygdala is a relatively small, subcortical brain structure located near sinus cavities that produce susceptibility artifacts in the echoplanar image. The signal-to-noise ratio was optimized by acquiring a small number of slices centered on the amygdala to maximize the number of images acquired per slice during conditioned stimulus (CS) presentation. Susceptibility artifact was minimized through the use of an asymmetric spin echo pulse sequence that permits imaging in regions containing large scale field gradients and is effective in imaging the amygdala (Breiter et al., 1996; Whalen et al., 1998).

Second, amygdalar responses are relatively transient to discrete cues, with low spontaneous neuronal firing rates (Bordi and LeDoux, 1992; Quirk et al., 1995) and marked habituation over time (Breiter et al., 1996; Quirk et al., 1997). Therefore, neuroimaging procedures that scan across both CS and non-CS epochs over an extended period of time may not be optimal for visualizing signals in this brain region. We took advantage of the increased temporal resolution of fMRI to time-lock the brain activation to the onset of each CS, thereby averaging single, transient responses to the CS and including only those epochs in which the CS was present. In addition, we divided the experimental phases into "early" and "late" periods to examine the coarse temporal dynamics of the elicited responses across trials. The transient fMRI signal changes elicited by a single stimulus were averaged across several presentations in a manner similar to the epoch analyses used to derive event-related potentials in electrophysiology (McCarthy et al., 1997).

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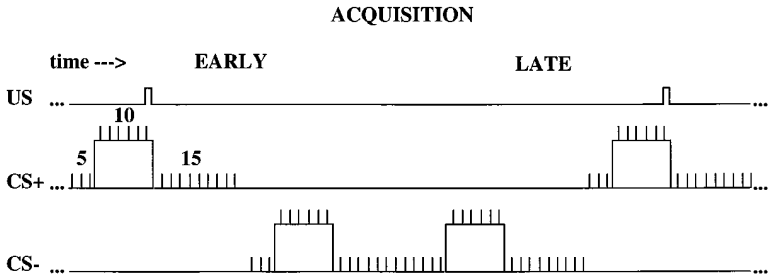

Figure 1. Timeline Illustrating Stimulus Parameters during Conditioned Fear Acquisition

One visual conditioned stimulus (CS+) coterminates with the delivery of a brief electric shock to the wrist (US), while the other visual conditioned stimulus (CS−) is presented alone. Vertical lines depict time points at which scans are acquired. Numbers indicate pre-CS, CS, and post-CS durations in seconds. Only four of the sixteen acquisition trials are depicted. "Early" refers to the first half of the acquisition trials, while "late" refers to the last half of the acquisition trials. Parameters for extinction are identical, except that no US is delivered.

Finally, during fear conditioning, nonassociative factors must be controlled to show that the learning (as reflected in brain activity) is associative in nature. We adopted a simple discrimination paradigm to provide a within-subject control for sensitization effects (Figure 1). In this paradigm, one visual CS (designated the CS+) was paired with an electric shock unconditioned stimulus (US) in acquisition, while the other visual CS (designated the CS−) was presented alone. During habituation and extinction, both CSs were presented alone. Scans were acquired during the acquisition and extinction phases. A statistical index of differential fear conditioning was generated by a double-subtraction statistical logic using within-phase baselines (see Experimental Procedures). This procedure allows one to view conditioning-related activation patterns for acquisition and extinction separately without reference to a preconditioning baseline, which may produce spurious results due to slowly varying signal drift over time. In order to minimize conditioning cues provided by time alone (so-called "temporal conditioning"; Klein, 1987), the CS+ and CS− trial types were intermixed in a pseudorandom order. The mixed-trial design is a relatively new application of fMRI technology (Buckner et al., 1996; Zarahn et al., 1997) that contrasts with the "classic" blocked-trial approach to functional neuroimaging. This combination of methodological advances should overcome several technical limitations that may have obscured the role of the amygdala and other brain regions in previous imaging studies.

Results

fMRI Results

Because the amygdala was our primary region of interest (ROI), we limit our discussion to this brain region, although activations in other brain regions are listed in Table 1. Activation in the amygdala/periamygdaloid cortex was observed during both conditioned fear acquisition and extinction (Figure 2a). During acquisition, the group-averaged results suggest that the amygdala involvement was biased toward the right hemisphere, with a focus in the medial periamygdaloid cortex. The mean percent signal change elicited from this focus was 0.83% in response to the CS+ and 0.06% in response to the CS− (see the Experimental Procedures for details on percent signal change calculations). However, inspection of individual data (Figure 2b) revealed activation of the left amygdala in several subjects (left amygdala, n = 2; left periamygdaloid cortex, n = 4), as well as a more heterogeneous distribution of activation foci within the right hemisphere (right amygdala, n = 1; right periamygdaloid cortex, n = 3). During extinction, the group-averaged results also suggest that the amygdala involvement was biased toward the right hemisphere (Figure 2a). The mean percent signal change elicited from this right amygdala focus was 1.04% in response to the CS+ and 0.26% in response to the CS−. Inspection of individual data (Figure 2b) again revealed a more heterogeneous distribution of activation foci within the right hemisphere (right amygdala, n = 3; right periamygdaloid cortex, n = 3) as well as some left-sided activation (left amygdala, n = 3; left periamygdaloid cortex, n = 1). Interestingly, the right amygdaloid activation seen in the group average habituated across trials during both acquisition and extinction (evident only in the early portion of each phase; Figure 2a). The temporal gradation in the signal intensity, however, was only statistically reliable during extinction, as revealed by Wilcoxon signed-rank tests (acquisition, $z = -1.12$, $p > 0.05$; extinction, $z = -1.96$, $p < 0.05$; see the Experimental Procedures for details).

Behavioral Results

All subjects who participated in the fMRI study were aware of the CS–US contingencies as assessed by a postexperimental interview. In addition, all subjects rated the US as moderately aversive (mean rating [±SD] = 4.09 [±0.63] on a 6-point Likert scale from 1 = "not at all aversive" to 6 = "very aversive"). Skin conductance responses (SCRs) from the follow-up psychophysiological study are depicted in Figure 3. A one way repeated measures ANOVA yielded a significant effect of experimental phase on SCR difference scores ($F[2,8] = 4.67$, $p < 0.05$; Figure 3a). Follow-up–dependent t tests revealed higher differential conditioning during acquisition than during habituation ($t[4] = 2.83$, $p < 0.05$); the difference in conditioning between acquisition and extinction was not significant. Figure 3b replots the SCR data to demonstrate the rate of learning across the acquisition and extinction trial blocks. The rapid rate of extinction may reflect subjects' prior knowledge of the experimental contingencies from the prior conditioning session in the magnet and thus may not truly reflect the extinction rate obtained when the subjects were experimentally naïve. However, even in naïve subjects, extinction of conditioned SCRs tends to be complete by the late extinction period (see LaBar et al.,

Table 1. Brodmann's Areas, Talairach Coordinates, and Proportion of Subjects Showing Statistically Significant ($p < 0.05$) Activations during Conditioned Fear Acquisition and Extinction

Phase	Region	Brodmann's Area	Talairach Coordinate	Subject Prevalence
Early acquisition	Anterior cingulate (rostral)	BA 32′/24′	(−2, 4, 47)	8/10 (80%)*
	Precentral gyrus	BA 6	(53, 4, 4)	8/10 (80%)*
	Periamygdaloid cortex	BA 34	(−14, −4, −19)	7/10 (70%)*
	Striatum		(24, 4, 9)	7/10 (70%)*
	Superior frontal gyrus	BA 6	(−4, −4, 66)	6/10 (60%)*
	Precentral gyrus	BA 4	(41, −4, 27)	5/10 (50%)
	Anterior cingulate (caudal)	BA 24′	(4, −4, 39)	4/10 (40%)
Late acquisition	Middle frontal gyrus	BA 8	(−23, −4, 41)	9/10 (90%)*
	Superior frontal gyrus	BA 6	(−8, −4, 65)	7/10 (70%)*
	Superior temporal gyrus	BA 22	(−43, −4, −7)	7/10 (70%)
	Striatum		(−18, −4, 9)	7/10 (70%)
	Anterior cingulate (caudal)	BA 24′	(3, −4, 40)	6/10 (60%)
Early extinction	Middle frontal gyrus	BA 6	(−27, −4, 59)	8/10 (80%)
	Amygdala		(−17, −4, −11)	6/10 (60%)*
	Precentral gyrus	BA 4	(47, −4, 40)	6/10 (60%)
	Superior frontal gyrus	BA 6	(−12, −4, 61)	6/10 (60%)
	Caudate (head)		(−19, −4, 22)	4/10 (40%)
	Superior temporal gyrus	BA 22	(48, −4, −10)	3/10 (30%)
Late extinction	Superior frontal gyrus	BA 6	(20, −4, 65)	6/10 (60%)

Only areas activated in the group average (Figure 2a) are listed.
*Includes subjects with activation in the contralateral hemisphere.

1995). SCRs to the US (Figure 3c) were robust and did not habituate over the acquisition trials, as revealed by a one way repeated measures ANOVA ($F[3,12] = 0.85$, $p > 0.05$).

To examine whether intersubject variability in amygdala activation during acquisition in the fMRI study related to autonomic indices of conditionability, we computed a correlation between the spatial extent of amygdala activation and conditioned SCRs in the subset of five subjects who participated in both experiments (see Experimental Procedures; Figure 4). Although this correlation is limited in that the fMRI and behavioral measures were not measured concurrently, the correlation was statistically significant and accounted for 77% of the variance in the data set ($r = 0.88$, $p = 0.026$, one tailed). In contrast, significant correlations were not found between conditioned SCRs and activation in rostral and caudal anterior cingulate control regions ($r = 0.17$ and 0.42, respectively; $p > 0.05$).

Discussion

Role of the Amygdala in Conditioned Fear

The amygdala is a key component of a neural network hypothesized to mediate survival functions of the organism by coordinating behavioral plans of action based on the integration of exteroceptive and interoceptive information (Damasio, 1994; LeDoux, 1996). In support of this general role, the amygdala has been linked with the ability of an organism to detect and react to potentially threatening stimuli in the environment through learning based on classical conditioning principles (reviewed by Kapp et al., 1992; Davis, 1994; LeDoux, 1995; Maren and Fanselow, 1996). Although most of this research has been conducted on nonhuman animals, previous neuropsychological studies have found fear conditioning deficits in human patients with amygdala damage (Bechara et al., 1995; LaBar et al., 1995). The present study demonstrates that an amygdalar contribution to conditioned fear learning can be revealed in normal human subjects using fMRI and suggests that the human amygdala actively participates in both conditioned fear acquisition and extinction. Further studies will be required, however, to delineate more carefully the relative contributions of the amygdala and periamygdaloid cortex to task performance.

With the increased temporal resolution of fMRI, we were able to extract coarse temporal dynamics of the brain activations over trials. Across subjects, the response profile within the right amygdaloid region habituated from early to late stages of acquisition and extinction. The follow-up ROI analysis, however, showed that the intensity reduction was only statistically reliable during extinction. These results thus provide partial support for a recent electrophysiological study demonstrating a temporally graded response profile of the amygdala during conditioned fear acquisition and extinction in rats (Quirk et al., 1997). The failure to achieve statistical significance during acquisition in the ROI analysis may be related to the sample size used in the current study or to the overall degree of variability seen in measures of conditioned fear acquisition in human subjects (see LaBar et al., 1995). Our correlational analysis confirmed that the variability in spatial extent of amygdala activation during acquisition accounted for a high proportion of the variance in autonomic indices of conditionability in the same subjects (Figure 4), consistent with a recent PET study (Furmark et al., 1997). Future studies using concurrent physiological indices of conditioning will be able to determine more accurately whether there is a relationship between the time course of amygdala activation and the rate of behavioral acquisition and extinction in individual subjects. At a minimum, the data suggest that the amygdala is most consistently active

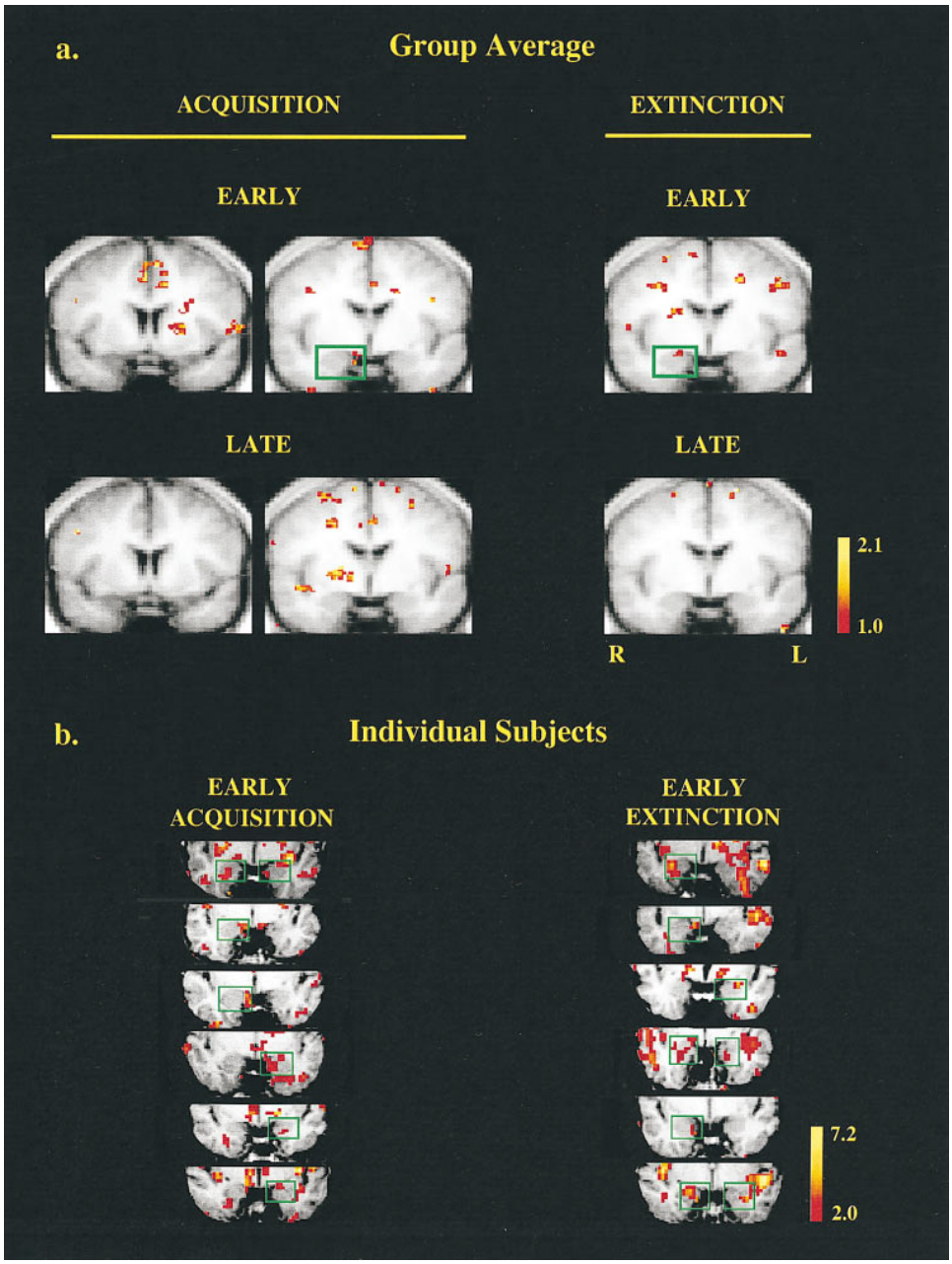

Figure 2. Statistical t Maps of Brain Regions Engaged in Conditioned Fear Acquisition and Extinction

Responses in the amygdaloid region are highlighted in green boxes. All images are depicted in radiological convention (R = right hemisphere, L = left hemisphere).

(a) Group average results (n = 10). Activated pixels reflect the voxel-wise median of t tests computed on individual subjects. Acquisition data are derived from both anterior and middle slices. Extinction data are derived from the middle slice.

(b) Amygdala activation in a sample of individual subjects. Colored bars indicate minimum and maximum t values.

across subjects in the early phases of acquisition and extinction.

Previous studies have found temporally graded amygdala responsivity in both animal and human populations. As mentioned above, Quirk et al. (1997) observed the greatest fear conditioning–induced changes within the lateral amygdala of the rat during the initial acquisition and extinction trials. Moreover, electrophysiological and lesion studies of avoidance conditioning implicate an early amygdalar contribution to aversively motivated learning (e.g., Maren et al., 1991; Parent et al., 1992; Poremba and Gabriel, 1997). In humans, Breiter and colleagues (1996) reported rapid habituation of the amygdala response to facial expressions of emotion using fMRI. The amygdala, therefore, may preferentially signal the detection of affective signals when they are novel or in the initial stages of learning when their emotional meaning is actively encoded.

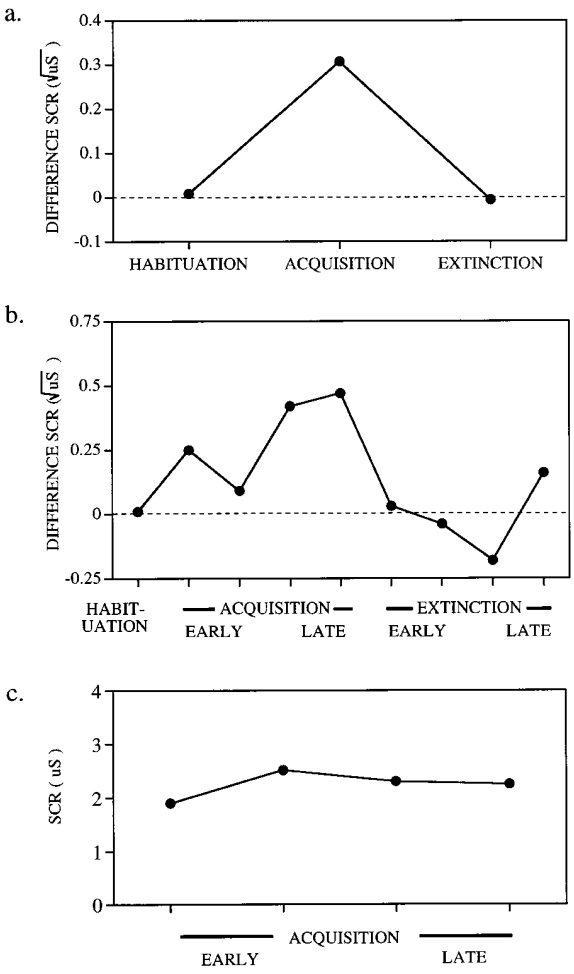

Figure 3. Group-Averaged Psychophysiological Indices of Conditioned Fear in a Subset of Participants in the fMRI Study (n = 5)

(a) Difference SCRs averaged across each phase of the experiment. Positive values indicate relatively greater conditioning to the CS+.
(b) Same data as above, but broken down to illustrate learning rates across early and late periods.
(c) Unconditioned responses to the shock during acquisition. μS, microsiemen.

It is important to emphasize that despite evidence for temporal specificity, emotional learning may still induce long-lasting changes in neural coding within the amygdala. For example, Quirk and colleagues (1995) found that neurons within the lateral amygdala exhibit increased functional coupling during extinction even though the CS no longer elicits neural activity. Such changes would not be observable using current functional neuroimaging techniques. Furthermore, contrary to models of avoidance conditioning (McGaugh et al., 1992), post-training lesion and temporary inactivation studies show that the integrity of the amygdala is required for the expression of previously learned conditioned fear associations (Gentile et al., 1986; Kim and Davis, 1993a; Campeau and Davis, 1995; Muller et al., 1997; but see Kim and Davis, 1993b). However, in spite of the evidence for the involvement of the amygdala in the expression of the conditioned response, the temporal pattern of amygdala activity observed in the present study suggests that this activity may only partially underlie expression. It is likely that the observed activation may also be related to encoding the emotional meaning of the conditioned stimulus. This is consistent with the resurgence of amygdala activity during early extinction, when the emotional meaning of the stimulus has changed (see also Falls et al., 1992). Further clarification of the amygdala's temporal involvement in fear conditioning and other associative emotional tasks requires exploration with converging evidence across species and research techniques.

In the group-averaged results of the present study, the amygdala's contribution to fear conditioning appears to be right hemisphere dominant (Figure 2a). However, the hemispheric distribution of the activation at the level of individual subjects was considerably scattered during acquisition, with some subjects showing bilateral activation or selective activation in the left hemisphere (see Figure 2b; Results). Previous studies have found that damage to either the left or the right amygdala is sufficient to impair conditioned fear acquisition in both humans (LaBar et al., 1995; Peper et al., 1997, Soc. Neurosci., abstract) and rats (LaBar and LeDoux, 1996), although the deficits observed are not as severe as those following bilateral damage (Bechara et al., 1995; LaBar and LeDoux, 1996). A more robust right-hemispheric amygdaloid response occured during extinction, although again left-sided activation was seen in some subjects (see Figure 2b; Results). To our knowledge, it is unknown whether a right hemisphere specialization for extinction has been reported with other techniques. It is therefore best to interpret the hemispheric asymmetries with caution.

Comparison to Prior Neuroimaging Efforts

Previous functional neuroimaging studies using PET have not found activation in the amygdala during fear conditioning tasks using subtractive methodology (Fredrikson et al., 1995; Hugdahl et al., 1995; Furmark et al., 1997; Morris et al., 1997). Morris and colleagues (1997) did report a correlation between amygdala and pulvinar activation in their study, but the implications of this pattern are unclear, given that the pulvinar is not a key thalamic structure through which conditioned associations are formed (LeDoux, 1990) and the amygdala has only weak projections to the pulvinar (Amaral et al., 1992). The PET methodology employed in the human fear conditioning studies may be limited in several regards. First, Fredrikson et al. (1995) state that they did not have adequate data sampling from the ventral aspect of the brain. Second, PET studies typically rely on a postconditioning-minus-preconditioning subtraction to infer conditioning effects. Although postconditioning measures are interpreted as reflecting residual learning influences, they overlook acquisition-specific effects and confound processing unique to extinction (especially when averaged over large blocks of extinction trials). Third, the temporal constraints of PET require that scans be averaged over both CS− and non-CS epochs, which is not optimal for viewing the kind of transient activity (both within and across trials) that characterizes the response profiles of amygdala neurons to

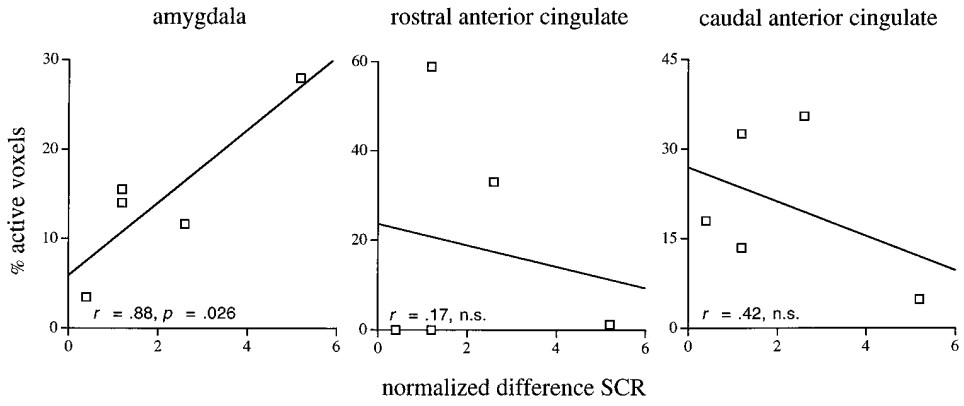

Figure 4. Linear Regression Analysis between Measures of Autonomic Conditionability and Spatial Extent of Activation in the Amygdala and Cingulate Control Regions

Data are derived from subjects who participated in both the fMRI and psychophysiological studies (n = 5). The number of voxels activated during acquisition is averaged bilaterally and expressed as a percentage of regional volume of the amygdala, rostral anterior cingulate, and caudal anterior cingulate in the same slice. Difference SCRs are averaged across all acquisition trials and normalized to each subject's mean difference SCR during habituation. n.s., not significant.

discrete conditioned fear cues (Pascoe and Kapp, 1985; Quirk et al., 1995, 1997). One way to circumvent this issue in PET studies is to utilize a contextual CS (as in Fredrikson et al., 1995). While lesions of the amygdala impair both contextual and cued fear (e.g., Phillips and LeDoux, 1992; LaBar and LeDoux, 1996), the neuronal correlates of contextual fear have not been examined systematically in the amygdala, and contextual fear engages additional neural structures, such as the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). Finally, conditioning paradigms in humans show some degree of between-subject variability in behavioral measures and in localization of foci within the amygdala (see above) that introduce further difficulties when individual subject data are not examined.

In conclusion, the fMRI methods used in the present study confer several advantages for assessing the functional anatomy of conditioned fear in normal human subjects. The application of single trial analyses permitted us to employ a mixed-trial design to control for nonassociative effects within subjects and provided a means to examine event-related activation optimal for viewing transient amygdala responses. Together with studies on fearful facial expression (Adolphs et al., 1994, 1995; Breiter et al., 1996; Calder et al., 1996; Morris et al., 1996; Whalen et al., 1998), the data reported here implicate the conservation of amygdala function across species. Our results extend neuropsychological findings of impaired fear conditioning in patients with amygdala damage (Bechara et al., 1995; LaBar et al., 1995) to offer insight into the neural bases of associative emotional learning processes in the normal human brain.

Experimental Procedures

Subjects

Eighteen healthy college students without a history of neurological impairment were paid to participate in the study. Subjects were prescreened for suitability to be scanned in the MRI facility and provided informed consent. fMRI data from seven of the subjects were excluded from the final analysis due to motion artifacts involving in-plane motion greater than 1/3 of a pixel (~1 mm) in either the x or y direction during the course of the experiment. One additional subject was excluded because he was left handed. The mean age [±SD] of the remaining 10 subjects was 22.50 [±4.25] years. Of these subjects, five were male and five were female. The experimental procedure was approved for use on human subjects by the Institutional Review Board at Yale University.

Apparatus

The visual CSs were displayed on a Macintosh 8100 PowerPC and backprojected into the scanner by a Sharp QA-1150 LCD panel (Sharp Electronics, Mahwah, NJ). In the scanner, subjects used an adjustable mirror located directly above their eyes to view the backprojected images on a screen placed at the foot of the scanner bed. The US was an electric shock delivered transcutaneously over the subject's left median nerve by a stimulating bar electrode (30 mm electrode spacing, Nicolet Instruments model 019–722400, Madison, WI). The electrode leads were secured by a velcro strap placed near the subject's wrist and attached to a Grass Instruments SD-9 stimulator (Quincy, MA) via coaxial cable leads that were magnetically shielded and grounded through an RF filter. Lafayette Instruments electrode gel (model 76621, Lafayette, IN) served as an electrolyte. SuperLab software (Cedrus Corporation, Phoenix, AZ) controlled the stimulus presentation and triggered the shock generator via a National Instruments DIO-24 data acquisition card (Austin, TX). MRI scans were acquired on a GE Signa 1.5 T scanner (General Electric, Waukesha, WI) equipped with whole-body gradients (Advanced NMR, Wilmington, MA) and a quadrature head coil.

Experimental Design and Procedure

Figure 1 summarizes the conditioning parameters used in the present study. The CSs consisted of blue and yellow squares presented in a pseudorandom sequence (CS duration, 10 s; intertrial interval, 30 s) that was initiated concurrently with the MRI console. The sequence was constructed so that subjects received no more than two trials of the same type (CS+/CS−) in a row within each experimental phase. Color assignment for the CS+ and CS− was counterbalanced across subjects. After subjects were placed into the magnet but before scans were acquired, subjects were attached to the shock electrodes, and two to four shocks (200 ms duration, 50 pulses/s) were delivered to adjust the voltage level for each subject's tolerance and to reduce startle-related motion artifact during scanning. Subjects were told that only a few stimulation pulses would be delivered at the same intensity during the course of the experiment and that the shocks should feel mildly aversive but not painful. Voltage levels were initially set at 30 V and were adjusted in ±5 V increments until the subject indicated that his or her tolerance level was reached. The mean US intensity level [±SD] set by the subjects

included in the final analysis was 33 [±8.41] V. Subjects were given four habituation trials (two CS+ and two CS−) in a pseudorandom order (30 s intertrial interval) to adjust the mirror for viewing the visual stimuli and to habituate orienting responses to the CSs. Anatomical MR images were then acquired for ~30 min, followed by functional echoplanar imaging during the task itself.

Subjects were instructed that the task was passive but that they should try to notice a pattern between the presentation of the visual stimuli and the delivery of the wrist stimulation during the experiment. They were also told that the experiment would be paused halfway to download the echoplanar images for subsequent image reconstruction. During this brief download time (~30 s), a crosshair appeared on the screen which, unbeknownst to the subjects, separated the acquisition from the extinction phases of the experiment (sixteen trials/phase, eight CS+ and eight CS− trials intermixed). During acquisition, the CS+ and US coterminated. Subject awareness of the reinforcement contingencies was assessed immediately following the study in a postexperiment interview conducted in the control room outside the magnet. Subjects were also asked to rate the shock intensity on a Likert-type scale from 1 (not at all aversive) to 6 (very aversive).

Scanning Parameters

To localize the anterior (AC) and posterior (PC) commisures 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 mm × 1.56 mm, and FOV = 40 × 40 cm). Three 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 most anterior slice on the anterior pituitary in the midsagittal view and assessing the position of the amygdala in the subsequent coronal sections using anatomical landmarks (Bronen and Cheung, 1991) and a standardized atlas (Talairach and Tournoux, 1988). Echoplanar functional images were acquired using an asymmetric spin echo pulse sequence (TE = 30 ms, echo offset = 30 ms, TR = 1.67 s, in-plane resolution = 3.125 × 3.125 mm, matrix size = 128 × 64, and FOV = 40 × 20 cm). These scanning parameters yielded a total of 288 functional images per slice for each experimental phase.

Statistical Analysis

Functional scans were acquired continuously throughout acquisition and extinction (see Figure 1). For purposes of analysis, only those scans that occured during the CS onset periods and pre-CS resting baselines were used, with one scan skipped per time series to accommodate the delay in the hemodynamic response. Note that scans acquired during or immediately following US presentation are not included in the analysis. Functional scans were segmented into four time epochs: early acquisition, late acquisition, early extinction, and late extinction. "Early" refers to the first half of each phase (i.e., the first eight trials of acquisition/extinction), while "late" refers to the last half of each phase (i.e., the last eight trials of acquisition/extinction). Within each of these four time epochs, statistical t maps were generated by a double subtraction method: CS+ and CS− onset periods were first subtracted from their respective pre-CS resting baselines and then were subtracted from each other (i.e., [CS+ minus rest] minus [CS− minus rest]). Positive activations thus provide a statistical index of differential fear conditioning within each time epoch. Although negative activations are not reported, the double-subtracted group average images did not show negative activations in the amygdala. All scans were subject to motion correction using SPM96. The average maximum motion correction estimates for the subjects included in the final analysis were 0.20 mm (acquisition) and 0.19 mm (extinction). All voxels correlated with the motion estimates were removed from the analysis. Amygdala activations were compared against the mean echoplanar functional image for each subject to confirm that they did not overlap areas of susceptibility artifact (defined as dropoff in signal intensity to <10% of the mean echoplanar signal).

The double-subtracted t maps from individual subjects were Gaussian smoothed (FWHM = 6.3 mm), warped to 3-D Talairach space, and group averaged by taking the voxel-wise median of the individual t maps (see Pugh et al., 1996). The group results were thresholded at a voxel-wise t cutoff of 10.0 (cluster size = 5) and superimposed onto a group mean anatomical image to visualize regions of consistent activation across subjects (Figure 2a). Data from individual subjects (Figure 2b) was then examined at a voxel-wise t cutoff of 1.96 (nominal $p < 0.05$; cluster size = 3) to compute the prevalence of statistically significant activations within these regions. To derive the mean percent signal change from the group-averaged amygdaloid activations in Figure 2a, an ROI was drawn around the pixels activated in the "early acquisition" and "early extinction" phases separately on the group-averaged double-subtracted image using a mouse-driven computer program. The resultant ROIs were then applied to the group-averaged single subtraction maps to estimate the mean percent signal change elicited within early acquisition and early extinction phases in response to the CS+ and CS− separately, relative to the pre-CS resting baseline periods. The values for the mean percent signal change (see Results) thus represent the mean signal change for voxels constituting the activated amygdaloid regions in Figure 2a averaged over all CS+ and CS− trials within the early acquisition and early extinction phases (i.e., an average of four CS+ and four CS− trials, each containing four pre-CS resting baseline and six CS-related scans). All pixels with a minimum of 0.01% signal change were included in the calculations.

A statistical ROI analysis was performed to examine the effects of time on the activation pattern in the amygdala during acquisition and extinction. For this analysis, individual amygdalae from each subject's anatomical scan were outlined using a mouse-driven computer program. The outlines were derived from the middle slice and contained both the amygdala proper and subjacent periamygdaloid cortex, as the border between these regions was not always distinctive. The following calculations were then derived from this circumscribed region in each subject: the total number of voxels, the total number of activated voxels within each experimental phase (e.g., early acquisition, late acquisition, early extinction, and late extinction), and an aggregate t value for each phase. A statistical voxel-wise threshold of $p < 0.05$ was used for the ROI analysis, although similar results were obtained at a more stringent threshold ($p < 0.01$). Because the group average data showed an intensity decrease from early to late phases in both acquisition and extinction in the right amygdala, the ROI analysis was targeted on the aggregate t values in the right hemisphere. Tests for normality of the activation distributions showed that the data were positively skewed and kurtotic, so nonparametric tests were employed. Specifically, Wilcoxon signed rank tests were computed to evaluate the change in signal intensity from early to late phases of acquisition and extinction separately.

Behavioral Data

Because we were not equipped to record concurrent physiological measures of conditioning in the MRI facility, those subjects included in the final analysis were asked to participate in a follow-up psychophysiological study 1–3 months after the initial fMRI session. The purpose of the follow-up experiment was to ensure that the experimental parameters used in the scanner would produce reliable conditioning as assessed by SCR, a standard psychophysiological measure of conditioning (LaBar et al., 1995). Five of the ten subjects participated in the follow-up session and were included in the behavioral data analysis. One additional subject of the ten comprising the fMRI group participated but was dropped from the behavioral portion of the study because no detectable SCRs were elicited when the subject was tested in the follow-up session (i.e., a "nonresponder"). It is not clear why this one subject did not show any SCRs during the follow-up session, but psychophysiological studies of conditioning typically report a subset of subjects who are dropped from the SCR analysis for similar reasons (e.g., Björkstrand, 1990; Schell et al., 1991). It may be that in these cases, the electrode contact is not sufficiently coupled to the skin surface to detect small changes in conductance, or these subjects may possess behavioral habits (e.g., smoking) or personality traits that influence conditioned electrodermal activity (Levey and Martin, 1981). Stimulus parameters were identical to those used in the fMRI study, except that the CS colors were changed to red and green to reduce the influence of preexisting associations from the prior testing session on subjects' conditionability.

SCRs were measured by Ag–AgCl electrodes attached to the middle phalanges of the third and fourth digits of the nondominant hand by velcro straps (model TSD 103, BIOPAC Systems, Santa Barbara, CA). Lafayette Instruments electrode gel was used as an electrolyte (model 76621, Lafayette, IN). A BIOPAC Systems skin conductance module (GSR 100A) was triggered to begin recording at the start of each trial by a National Instruments DIO-24 card (Austin, TX) controlled by SuperLab software (Cedrus Corporation, Phoenix, AZ). Skin conductance was sampled at 250 Hz during the course of each trial, amplified, and stored on a Macintosh Quadra 700 computer for offline analysis using AcqKnowledge software (BIOPAC Systems, Santa Barbara, CA). The recorded waveforms were low pass filtered using a Blackman window (cutoff frequency = 31 Hz) and smoothed over three successive data points prior to scoring.

First interval SCR amplitudes were scored according to conventional criteria (1–4 s after CS onset; Lockhart, 1966) and were square root–transformed prior to statistical analysis to reduce skewness. SCRs to the US were also scored as a measure of unconditioned responding. A minimal deflection criterion of 0.02 siemen (S) was established for inclusion in the analysis. The scorer was blind to the trial type (CS+/CS−) during the raw data analysis. A difference score was derived as a measure of differential conditioning by subtracting CS− responses from CS+ responses (LaBar et al., 1995). According to this measure, difference scores greater than zero reflect greater relative conditioning to the CS+, difference scores equal to zero reflect no differential conditioning, and difference scores less than zero reflect greater relative conditioning to the CS−.

To assess the relationship between amygdala activation during acquisition and measures of autonomic conditionability, a correlational analysis was performed on the subset of subjects who participated in both experiments. Amygdala ROIs were drawn as described above for the statistical ROI analysis. In addition, rostral and caudal portions of the cingulate gyrus (BA 32′/24′) were outlined from the anterior and middle slices, respectively, as control regions. These regions were chosen because they were the only other limbic regions with readily identifiable anatomical borders whose activations were relatively robust, and we had no a priori expectation that their activations would relate to measures of conditioned SCRs. Each subject's mean difference SCR during acquisition was normalized to the mean difference SCR during habituation to account for differences in relative levels of habituation across subjects. The spatial extent of activation in the amygdala and cingulate control regions (in terms of voxel counts) was averaged bilaterally across all acquisition trials and expressed as a percentage of region volume estimated within the same slice as the functional data. The statistical t maps were thresholded at a voxel-wise $p < 0.05$. The results from the simple linear regression analysis are summarized in Figure 4.

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