

Impaired Fear Conditioning Following Unilateral Temporal Lobectomy in Humans

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Classical fear conditioning was used in the present study as a model for investigating emotional learning and memory in human subjects with lesions to the medial temporal lobe. Animal studies have revealed a critical role for medial temporal lobe structures, particularly the amygdala, in simple and complex associative emotional responding. Whether these structures perform similar functions in humans is unknown. On both simple and conditional discrimination tasks, unilateral temporal lobectomy subjects showed impaired conditioned response acquisition relative to control subjects. This impairment could not be accounted for by deficits in nonassociative sensory or autonomic performance factors, or by differences in declarative memory for the experimental parameters. These results show that medial temporal lobe structures in humans, as in other mammals, are important components in an emotional memory network.

[Key words: amygdala, classical conditioning, discrimination learning, emotion, fear, skin conductance response]

The neural substrate of emotional learning and memory in animals has been extensively studied using classical fear conditioning paradigms as a model approach (for reviews, see Kapp et al., 1990; Davis, 1992; Fanselow, 1994; LeDoux, 1995). The emerging view from this research suggests that the amygdala is an essential component in a distributed neural network through which emotional reactions are coupled to novel stimuli. Although the role of other temporal lobe structures is less clear, recent lesion studies indicate that the hippocampus is not essential for simple conditioned fear associations but is important for more complex aspects of fear conditioning, such as contextual conditioning (Selden et al., 1991; Kim and Fanselow, 1992; Phillips and LeDoux, 1992, 1994). This model of emotional memory formation, however, has not been applied to investigate analogous brain mechanisms in humans, where little is known about the neural basis of emotional information processing. The goal of the present study is to assess the role of medial temporal lobe

structures in emotional learning and memory in humans, as measured by classical fear conditioning.

Fear conditioning is a generic term used to describe an aversive classical conditioning procedure in which a neutral conditioned stimulus (CS), such as a tone, is presented in association with a noxious unconditioned stimulus (US), such as an electric shock. The US elicits an unconditioned response (UR), and over several trials of CS-US pairings, the CS itself comes to elicit a conditioned fear response (CR; Estes and Skinner, 1941; McAllister and McAllister, 1971). Normal human subjects exhibit reliable autonomic and behavioral URs to unconditioned stimuli (shocks and loud noises), as well as CRs to conditioned fear stimuli (e.g., Öhman, 1979; Hodes et al., 1985; Grillon et al., 1991).

Fear conditioning in human clinical populations, however, has not been well-characterized. Existing reports have primarily involved anxious or schizophrenic patients, where the criteria for study inclusion were based on psychiatric evaluation (e.g., Howe, 1958; Ax et al., 1970; Gorham et al., 1978; Pitman and Orr, 1986; Ashcroft et al., 1991). Since information regarding the underlying brain etiology was not available in the patient samples, these studies have not been informative in advancing knowledge of the neural structures involved in conditioned fear. Fear conditioning was once attempted in H.M., a well-studied amnesic subject with extensive bilateral medial temporal lobe damage, but H.M. did not show skin conductance responses (SCRs) to electric shock, so evaluation of conditioned responding in H.M. was suspended (Milner et al., 1968). Human patients with restricted bilateral damage to the amygdala are extremely rare (Tranel and Hyman, 1990). Given these factors, brain-behavior relations in fear conditioning phenomena in humans have been difficult to assess.

In the present study, we examined fear conditioning in human subjects with unilateral anteromedial temporal lobe resection to control medically intractable epilepsy. These patients have sustained damage to the amygdala and hippocampus associated with complex partial seizures of medial temporal lobe origin, but they do not show the severe declarative memory impairment typical of amnesics with bilateral hippocampal lesions (Novelly et al., 1984). Because the etiology of this population sample is well-defined, brain-behavior relations can be assessed more carefully than in previous studies. Both simple and conditional discrimination tasks were conducted to examine the role of the medial temporal lobe in simple and complex aspects of associative emotional responding. A finding of impaired performance in this group would demonstrate that the relationship between

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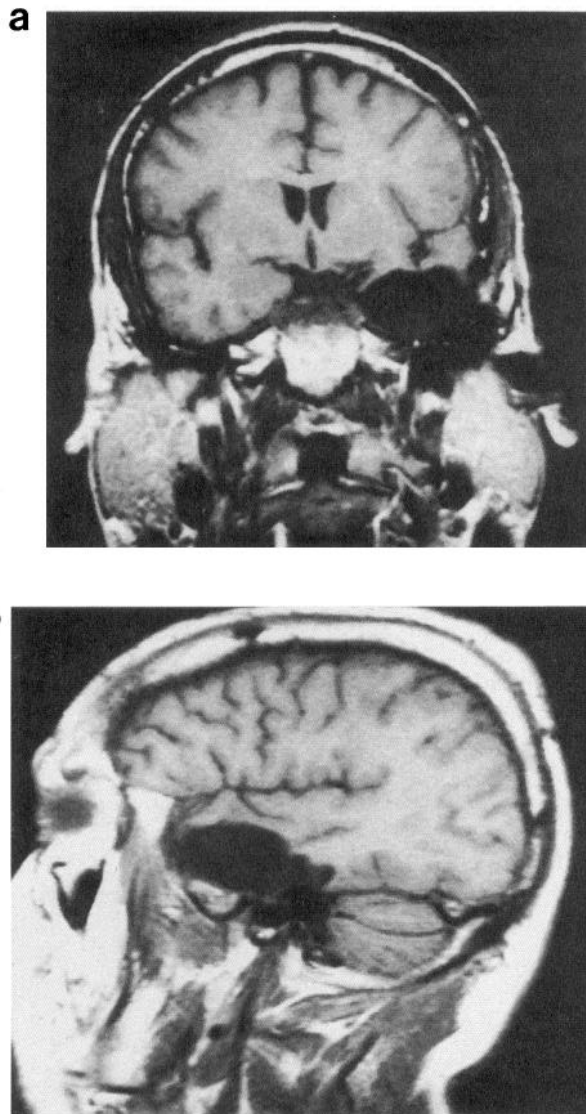


Figure 1. Representative postoperative T1-weighted MRI scan demonstrating the extent of the surgical procedure. *a*, Coronal section at the level of the amygdala. *b*, Parasagittal section.

medial temporal lobe structures and learned emotional responses, which has been established in animal models of fear conditioning, extends to the human brain.

Materials and Methods

Subjects

Twenty-six patients with medically refractory complex partial seizures of medial temporal lobe origin were studied 2–6 years following unilateral anteromedial *en bloc* temporal lobe resection. The surgical procedure involves an approximate 3.5 cm resection of the anterior middle and inferior temporal gyri allowing access to the temporal horn. This is followed by dissection of the occipito-temporal fasciculus and subsequent removal of 70–80% of the amygdala and all of the hippocampus, parahippocampus, and projection fibers to their posterior extent at the atrium of the lateral ventricle (Spencer et al., 1984; Spencer and Spencer, 1985). All patients received postoperative anatomical MRI scans confirming the extent of the surgical procedure. A representative scan in coronal and parasagittal sections is provided in Figure 1. This procedure is highly successful in controlling seizure activity in these patients, with most patients experiencing few, if any, seizures postoperatively. These patients typically show some selective cognitive improvement following surgery due to reductions in seizure activity (Nov-

elly et al., 1984), and most patients are gradually withdrawn from anticonvulsant medication in accordance with postoperative neurological assessment.

Twenty-two patients participated in the simple discrimination task (11 left hemisphere, 11 right hemisphere; 9 male, 13 female), and 23 patients participated in the conditional discrimination task (12 left hemisphere, 11 right hemisphere; 8 male, 15 female). Most subjects participated in both experiments in a counterbalanced presentation order separated by a 1 week interval. All but four subjects were taking anticonvulsant medication at the time of testing.

Neuropsychological testing results, including the Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler, 1981) and the Russell adaptation of the Wechsler Memory Scale (WMS; Russell, 1975), are presented in Table 1 along with data on other clinical variables. Neuropsychological data was available for a subset of patients tested both 6 months preoperatively and 12 months postoperatively. Consistent with previous studies (e.g., Novelly et al., 1984), patients with left (speech-dominant) medial temporal lobe epilepsy showed more impaired verbal memory in comparison to patients with right (nondominant) medial temporal lobe epilepsy on WMS subtest scores [preoperative: WMS delayed verbal $t(17) = -2.80, p = 0.012$; WMS immediate verbal $t(17) = -2.45, p = 0.026$; postoperative: WMS delayed verbal $t(12) = -2.82, p = 0.016$; WMS immediate verbal $t(13) = -2.42, p = 0.031$]. Right- and left-medial temporal lobe epilepsy patients were not significantly different on any of the WAIS-R IQ scores or other subject characteristics.

The control group consisted of nonepileptic adult subjects matched for age and gender without a history of epilepsy or other neurological impairment. Twenty-two subjects participated in the simple discrimination task (10 male, 12 female), and 23 subjects participated in the conditional discrimination task (10 male, 13 female). As in the temporal lobectomy group, most of the control subjects participated in both tasks in a counterbalanced presentation order separated by a 1 week interval. Control subjects were recruited through local newspaper advertisements.

An additional epileptic control group ($N = 3$; 1 male, 2 female) was run on the simple discrimination task to address issues of the specificity of the results. These patients have a similar clinical profile as the temporal lobectomy group, including a history of epileptic seizure activity uncontrolled by anticonvulsant medication followed by surgical treatment. However, in these patients, the focus of the seizure activity was not localized to the medial temporal lobe: 2 of the subjects received a partial left occipital lobectomy, while the remaining subject received section of the anterior 2/3 of the corpus callosum. Data from this control group will be presented separately due to the small sample size. This group was not run on the conditional discrimination task, which included a visual component, as the occipital lobectomy subjects in this group had mild visual deficits in the contralateral visual field.

All subjects provided informed consent and were paid for their participation. All subjects showed normal hearing thresholds based on an audiometric screening (Belton Instruments). None of the subjects run on the conditional discrimination task had color vision deficits. The protocol received Human Subjects Committee approval at New York University and Yale University.

Materials

Auditory stimuli were delivered by Coulbourn Instruments tone and white noise generators (models S81-06 and S81-02, Lehigh Valley, PA) and were presented binaurally to the subjects through Koss headphones (model TD/60). A Realistic sound level meter was used to maintain sound levels to within ± 2 dB. Visual stimuli for the conditional discrimination task were presented centrally on an IBM-compatible VGA monitor, which was adjusted to full brightness and contrast under ambient room luminance.

SCRs were recorded bilaterally from Thought Technology skin conductance units (Lafayette Instruments, Lafayette, IN), consisting of Ag-AgCl electrodes attached by velcro straps to the middle phalanges of the subjects' third and fourth digits. Lafayette Instruments electrode gel was used as an electrolyte. The incoming analog signal was amplified and digitized by a LabMaster A/D converter controlled by ASYST software on an IBM AT computer. Skin conductance was sampled at 100 Hz throughout a trial and was stored for off-line amplitude analysis. The minimum SCR resolution was 0.0144 μ Siemen (μ S).

Table 1. Demographic and neuropsychological profile of patient population

Group	Age	Gender	Education (yrs)	Seizure onset (age)	WAIS-R		WMS	
					(6 mo. pre-op)	(12 mo. post-op)	(6 mo. pre-op)	(12 mo. post-op)
LTL (N = 13)	36 ± 10	7M, 6F	13 ± 2	9 ± 10	94 ± 10 F	99 ± 14 F	11 ± 1 IP	11 ± 2 IP
					96 ± 16 P	102 ± 13 P	8 ± 3 DP	8 ± 3 DP
					94 ± 16 V	104 ± 14 V	15 ± 6 IV*	14 ± 5 IV*
							9 ± 5 DV*	7 ± 6 DV*
RTL (N = 13)	38 ± 9	3M, 10F	14 ± 2	7 ± 7	97 ± 10 F	107 ± 12 F	10 ± 4 IP	12 ± 3 IP
					98 ± 11 P	110 ± 11 P	7 ± 4 DP	10 ± 5 DP
					96 ± 13 V	104 ± 14 V	21 ± 5 IV*	20 ± 5 IV*
							16 ± 7 DV*	16 ± 6 DV*

WAIS-R: F, full scale; P, performance scale; V, verbal scale. WMS: IP, immediate pictorial; DP, delayed pictorial; IV, immediate verbal; DV, delayed verbal. All values represent means (±SD) unless otherwise indicated.

*Statistically significant [left temporal lobectomy (LTL) vs right temporal lobectomy (RTL), $p < 0.05$].

Task instructions

Before being attached to the skin conductance units, subjects washed their hands and were given a description of the recording instruments. Subjects were instructed that their SCRs were being recorded in response to tones heard through the headphones. They were asked to attend to the sounds (and lights in the conditional discrimination task) and to try to detect a pattern to the stimuli, which the experimenter would ask them to report periodically throughout the session. Subjects were asked to keep their hands still during the 25 min session. A 1 min period of relaxation was given prior to the start of the experiment.

Experimental paradigm

Simple discrimination. Two tones served as CSs in this task (950 Hz and 3000 Hz; 70 dB; 5 msec rise/fall time; 8 sec duration). The US was a white noise burst (100 dB; 10 Hz to 20 kHz; 4 cycles/sec; 1 sec duration). The conditioning paradigm consisted of three phases: habituation, acquisition, and extinction. One of the two CSs was paired with the US in the acquisition phase (CS+ trial), while the other CS was not (CS- trial). Choice of auditory stimulus for CS+/CS- was counterbalanced across subjects. Trial type (CS+/CS-) was presented to subjects in a pseudorandom order, subject to the limitation that no more than two of the same trial type be presented successively. Two trial type orderings were composed and were counterbalanced across subjects.

In the habituation phase, subjects received four trials each of the CS- and CS+ alone. In the acquisition phase, subjects received eight trials of the CS- presented alone, and eight trials of the CS+ terminated with US onset (nonoverlapping). In extinction, eight trials each of the CS- and CS+ alone were presented. The intertrial interval used throughout the experiment was 30 ± 6 sec.

At the end of each experimental condition, subjects were asked to describe what they heard during the preceding phase. If subjects did not verbally report the correct stimulus parameters, their knowledge was probed by a series of questions (e.g., How many sounds did you hear? Did you notice any pattern to the stimuli? Did the noise come after one of the tones you heard?). This verbal assessment provided a measure of the subjects' declarative knowledge of the experimental parameters throughout the session.

Conditional discrimination. The CS used in this task was a 2000 Hz tone (70 dB; 5 msec rise/fall time; 8 sec duration). All other aspects of auditory stimulus presentation, including US characteristics, were the same as in the simple discrimination task. Visual stimuli consisted of 1-sec duration red and green blocks subtending 2.15° of visual angle. This task also incorporated three conditioning phases: habituation, acquisition, and extinction. In the acquisition phase, if the CS was preceded by a colored light (e.g., green), the CS was reinforced immediately by the US (CS+ trial). If the CS was preceded by the color-opponent light (e.g., red), the trial was unreinforced (CS- trial). A 2 sec interval separated the onset of the visual cue and onset of the CS. Color choice for CS+/CS- trials was counterbalanced across subjects. As in the simple discrimination task, two pseudorandom trial schedules were constructed and were counterbalanced across subjects.

During habituation, subjects received four trials of the tone CS alone.

In the acquisition phase, subjects received eight CS+ trials (tone preceded by a colored light and terminated with US onset) and eight CS- trials (tone preceded by the color-opponent light without reinforcement). During extinction, eight CS- trials and eight CS+ trials, now unreinforced, were given. A 30 ± 6 sec intertrial interval was used throughout the session. Subjects were again asked to verbally report their knowledge of the experimental parameters at the end of each phase.

Response measurement

SCRs to the conditioned stimuli were used as the dependent measure of conditioning. Any SCR deflection with a peak latency less than 0.5 sec or greater than 5 sec was rejected as artifact. Both first interval and second interval SCRs were recorded, as defined by the largest SCR occurring within 0.5–4 sec or 4–8 sec after tone onset, respectively. Consistent with previous studies (Öhman et al., 1976; Hugdahl, 1978; Lovibond, 1992; Fredrikson et al., 1993), the first interval response was more reliable and was therefore used as the primary measure of conditioning. SCRs to the US were scored as a measure of unconditioned responding in the subjects. All SCRs were square-root transformed to attain statistical normality (Levey, 1980).

During SCR amplitude measurement, left- and right-hand responses for each subject were scored independently, and the scorer was blind to the trial type (CS+/CS-). For the purpose of analysis, trials were averaged into two-trial blocks separately for each trial type. A difference score was obtained as a measure of differential conditioning by subtracting CS- block responses from CS+ block responses (Ax et al., 1970). According to this measure, difference scores greater than 0 reflect greater relative conditioning on CS+ trials; difference scores equal to 0 reflect no differential conditioning as a function of trial type; and difference scores less than 0 reflect greater relative conditioning on CS- trials. An α level of 0.05 was used in all analyses.

Results

Four general observations were noted across both simple and conditional discrimination tasks: (1) no significant hand differences (left hand vs right hand) were found for any of the experimental groups in any experimental phase, so SCRs were averaged across both hands in all analyses; (2) no differences emerged between subjects who performed the simple discrimination task first and those who performed the conditional discrimination task first, so subject data was collapsed across task presentation order; (3) although the small sample size precluded direct statistical comparison, there were no apparent differences in responding between temporal lobectomy patients free of anticonvulsant medication ($N = 4$) and medicated patients, so data from all temporal lobectomy subjects was used; (4) finally, no significant differences in differential responding were found between the left and right temporal lobectomy groups in any ex-

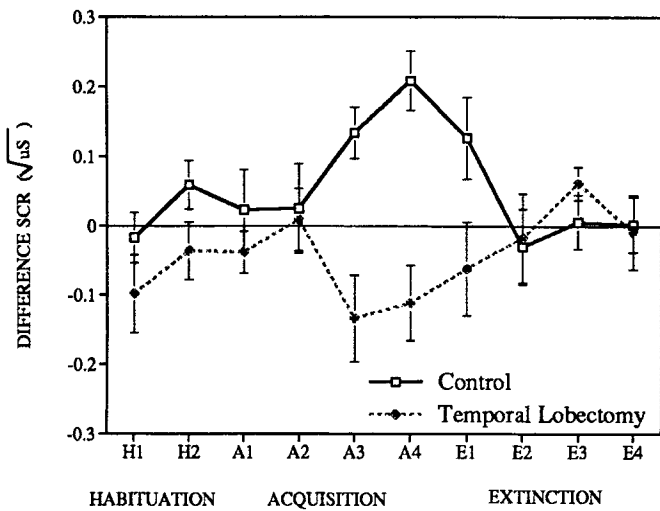


Figure 2. Differential conditioning during simple discrimination. Trials were averaged into two-trial blocks, and CS- blocks were subtracted from CS+ blocks to yield a mean SCR difference score (\pm SEM) for each subject. A difference score of 0 indicates no differential conditioning. Temporal lobectomy subjects demonstrated impaired differential conditioning in acquisition relative to nonepileptic control subjects.

perimental phase, so data from these subjects were combined to form one temporal lobectomy group.

Simple discrimination

CR acquisition. Figure 2 presents the differential conditioning data for simple discrimination expressed as a function of experimental group. To investigate CR acquisition, a two-way mixed ANOVA was performed on this data using experimental group (temporal lobectomy vs nonepileptic control) as the between-subjects variable and experimental block as the within-subjects variable. The only trial blocks included in the analysis were blocks 2–4 of acquisition and blocks 1–2 of extinction, as these were the blocks where discriminative learning was hypothesized to be observed. All subsequent analyses of CR acquisition uti-

lized the same set of trial blocks. The ANOVA revealed a significant main effect of group, $F(1,42) = 17.13$, $p < 0.001$, and a significant group \times block interaction, $F(4,168) = 3.66$, $p = 0.007$. Follow-up ANOVAs on the group interaction with a significance level adjusted by the number of follow-up tests (Tabachnick and Fidell, 1983) showed a significant block effect only in the control data, $F(4,84) = 3.58$, $p = 0.010$, which, in the trend analysis using orthogonal polynomial decomposition, emerged as a significant quadratic trend, $F(1,21) = 8.65$, $p = 0.008$.

These data suggest that control subjects responded more to CS+ trials during CR acquisition, with a peak on the last acquisition trial and subsequent decline in discrimination performance on early extinction blocks. Although it appears from Figure 2 that temporal lobectomy subjects responded more to CS- trials in the acquisition phase, in the trend analysis using orthogonal polynomial decomposition, this trend was not statistically significant. These results provide evidence for significant differences in group performance during discriminative learning, with control subjects demonstrating normal differential conditioning ability and temporal lobectomy subjects showing impaired CR acquisition.

To further explore these CR acquisition effects, separate two-way mixed ANOVAs (group \times block) were computed for CS+ trials and CS- trials separately. SCR data averaged by experimental group as a function of trial type is given in Figure 3. The ANOVA computed on CS+ trials revealed a main effect of group, $F(1,42) = 5.37$, $p = 0.025$, indicating significantly higher SCRs elicited by the CS+ in the control group. A marginally significant group \times block interaction was also found, $F(4,168) = 2.10$, $p = 0.083$, showing a trend of response habituation in the control group late in acquisition. Both groups did show an enhanced response on the first block of acquisition (block A1 in Fig. 3a). However, this trend was not statistically significant—it was also apparent on the CS- trials (Fig. 3b) and probably reflects a nonassociative arousal effect due to the introduction of the noxious US at the beginning of this phase of the experiment.

A two-way mixed ANOVA (group \times block) computed on

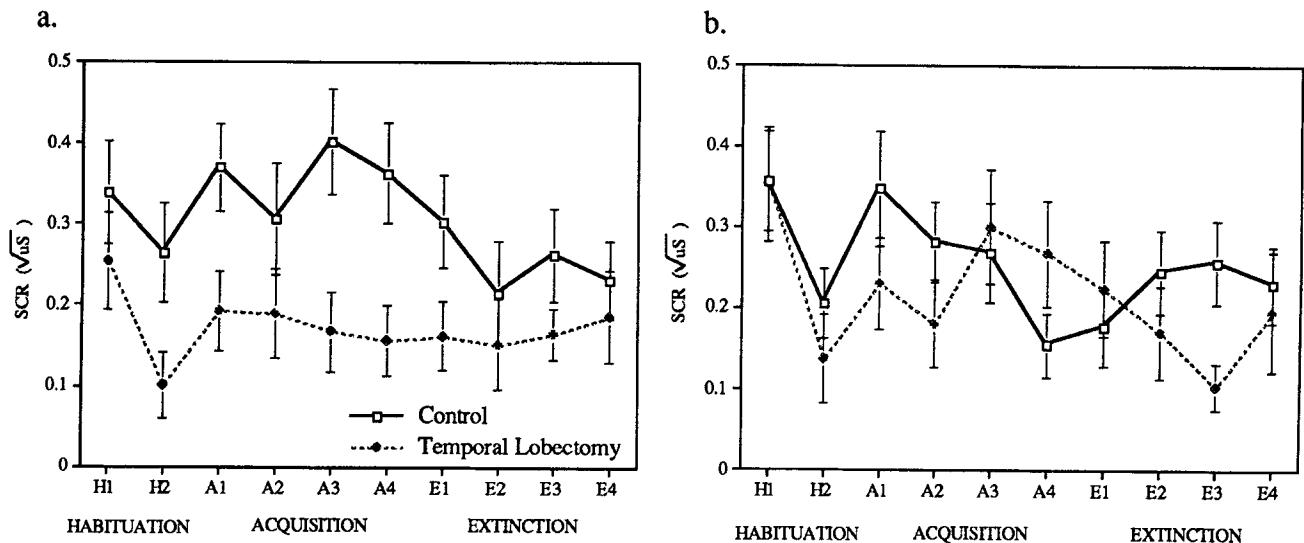


Figure 3. Simple discrimination by trial type. SCRs were averaged into two-trial blocks by experimental group. *a*, Mean SCRs (\pm SEM) elicited on CS+ trials. *b*, Mean SCRs (\pm SEM) elicited on CS- trials. Temporal lobectomy subjects showed deficits on CS+ trials (*a*) relative to nonepileptic controls, while no significant differences emerged on CS- trials (*b*).

Table 2. CS+/CS- Difference scores for the last acquisition block expressed in raw units (microsiemens) and in standard deviation of habituation units (SD_{hab})

Group	Raw score (mean ± SD)	Proportion of subjects with scores > 2 SD _{hab}	Proportion of subjects with scores < -2 SD _{hab}
Temporal lobectomy	-0.11 ± 0.05	0/22 (0%)	2/22 (9%)
Nonepileptic controls	0.21 ± 0.04	11/22 (50%)	0/22 (0%)
Epileptic controls	0.15 ± 0.10	2/3 (66%)	0/3 (0%)

CS- trials yielded no significant main effects, although a marginally significant group × block interaction was found, $F(4,168) = 2.40$, $p = 0.052$. This interaction suggests a marginal trend for temporal lobectomy subjects to increase responding to the CS- while controls decrease responding to the CS- at the end of acquisition. Analysis of the discrimination index discussed above (Figure 2), however, revealed that the trend in temporal lobectomy subjects was not statistically reliable, and Table 2 (see "Individual Variability" below) shows that this pattern is carried primarily by only two subjects. These data show that the deficit in differential conditioning during acquisition in the temporal lobectomy group (Fig. 2) is mainly attributable to relatively low responses on CS+ trials, with no significant group differences evident on CS- trials.

Habituation, extinction, and UR analysis. Differential responding during habituation was analyzed by a two-way mixed ANOVA with experimental group as the between-subjects factor and trial block as the within-subjects factor. Only a marginally significant group effect, $F(1,42) = 3.19$, $p = 0.081$, and a marginally significant block effect, $F(1,42) = 3.77$, $p = 0.059$, were found. These results signify a trend for temporal lobectomy subjects to respond more to the CS- on the first habituation block and for control subjects to respond more to the CS+ on the second habituation block (see Fig. 2). The group × block interaction, however, was not significant. Despite some marginal trends, there is no significant statistical evidence for response differences in the experimental groups during the habituation phase.

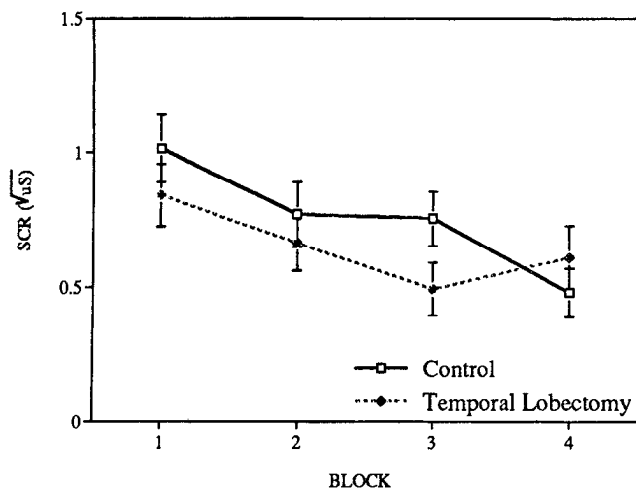


Figure 4. Mean unconditioned response performance (\pm SEM) during simple discrimination. SCRs to the US were averaged into two-trial blocks by experimental group. Temporal lobectomy and nonepileptic control subjects showed comparable levels of unconditioned responding on this task.

Differential responding during extinction was analyzed by a two-way mixed ANOVA (group × block) performed on the last two extinction trial blocks. No significant main effects or interaction terms emerged from this analysis, suggesting equivalent performance in the experimental groups during extinction (see Fig. 2). In addition, a two-way (group × block) ANOVA performed on the UR data yielded no significant group differences in levels of unconditioned responding (see Fig. 4). However, the UR analysis did reveal a significant block effect, $F(3,108) = 19.03$, $p < 0.0001$, showing a trend for URs to decrease over time across groups, and a significant group × block interaction, $F(3,108) = 4.83$, $p = 0.003$. Follow-up dependent t tests indicated that the group interaction was due to marginally significant differences between the groups on block 3. These data show that SCRs during habituation and extinction were at comparable levels in the experimental groups, and that the response deficit seen in the temporal lobectomy group during acquisition cannot be attributed to a reduction in US sensitivity in these subjects.

Role of awareness. All but four temporal lobectomy subjects were able to successfully report the correct experimental parameters at the end of each experimental phase. Because so few subjects were unaware of the CS-US relationships in this task, a direct statistical comparison of conditioning in aware and unaware subjects was not possible. However, observation of the data from these patients revealed no qualitative differences in comparison to the results obtained from aware patients. This verbal assessment provides evidence that declarative knowledge of the rules operating during the conditioning procedure was spared in most of the temporal lobectomy subjects studied. The impairments seen during CR acquisition in the temporal lobectomy group therefore cannot be explained by impaired declarative memory for the reinforcement contingencies.

Specificity of the results. An epileptic control group ($N = 3$) was also run on the simple discrimination task to address issues regarding the specificity of the findings. These patients received surgical treatment of medically refractory seizure activity, but the epileptic focus was not localized to the temporal lobe (see Materials and Methods). Consequently, medial temporal lobe structures in these subjects are intact. The differential conditioning data from this group is given in Figure 5, along with the data from the other subjects (taken from Fig. 2) collapsed across trials for comparison purposes. The data show that, as in the nonepileptic control group, the epileptic controls demonstrated simple discrimination ability, with relatively higher SCRs elicited on CS+ trials during acquisition. Formal statistics were not computed because of the small sample size. Although preliminary, the results from the epileptic control group suggest that the impairment seen in the temporal lobectomy subjects is not due to history of anticonvulsant medication, epilepsy in general, or other factors related to brain surgery. This finding supports

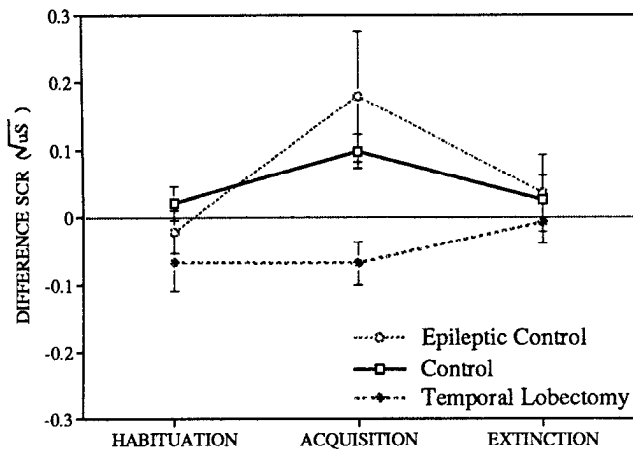


Figure 5. Comparison of differential conditioning during simple discrimination with epileptic controls. Data from the temporal lobectomy and nonepileptic control groups are collapsed across trials from Figure 2 for comparison with data from epileptic controls with lesions to other brain areas (see Materials and Methods). CS- blocks were subtracted from CS+ blocks to yield a mean SCR difference score (\pm SEM) for each subject. A difference score of 0 indicates no differential conditioning. Epileptic control subjects showed intact differential conditioning, suggesting that the impairment in the temporal lobectomy group may be specific to this epileptic population.

the interpretation that damage specific to medial temporal lobe structures is related to the deficits observed in this group. Because of mild visual impairment in some of the epileptic control subjects (see Materials and Methods), this group was not run on the conditional discrimination task, which contained a visual component.

Individual variability. The differential conditioning data from the last acquisition block is given in Table 2 to provide a measure of individual variability within each group. For each group, baseline mean and SDs were derived from habituation trials, and the raw CS+/CS- difference scores from the last block of acquisition were converted into standard deviation units of habituation (SD_{hab}). We used a 95% confidence interval criterion to describe the proportion of subjects in each group who showed greater relative conditioning on CS+ trials in this acquisition block ($>2 SD_{hab}$), and the proportion of subjects in each group who showed greater relative conditioning on CS- trials in this acquisition block ($<2 SD_{hab}$). These data show that, according to this criterion, none of the temporal lobectomy subjects showed greater relative conditioning on CS+ trials in this block, and only two temporal lobectomy subjects showed greater relative conditioning on CS- trials in this block. In addition, the data show that there was some intersubject variability in control subjects in terms of conditionability, a finding which is consistent with other conditioning studies (e.g., Woodruff-Pak and Thompson, 1988).

Conditional discrimination

CR acquisition. The differential conditioning data for the conditional discrimination task is given in Figure 6. A two-way mixed ANOVA (group \times block) was computed to examine CR acquisition in the experimental groups. As in the simple discrimination task, trial blocks 2–4 of acquisition and blocks 1–2 of extinction were included in this and all subsequent CR acquisition analysis. The ANOVA yielded a significant main effect of group, $F(1,44) = 7.73$, $p = 0.008$, indicating discriminant

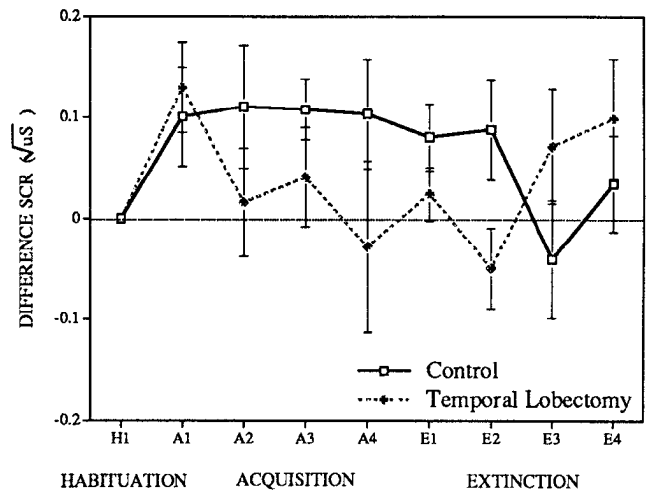


Figure 6. Differential conditioning during conditional discrimination. Trials were averaged into two- 1 blocks, and CS- blocks were subtracted from CS+ blocks to yield a mean SCR difference score (\pm SEM) for each subject. Habituation data is extrapolated to 0, as there was no distinction between CS+ and CS- trials during this phase. A difference score of 0 indicates no differential conditioning. Temporal lobectomy subjects showed impaired differential conditioning in acquisition relative to nonepileptic control subjects.

learning impairment in the temporal lobectomy group relative to nonepileptic controls.

To assess the relative contributions of CS+ and CS- trials to this response deficit, two-way mixed group \times block ANOVAs were performed on CS+ and CS- trial data separately (see Fig. 7). The ANOVA computed on CS+ trials revealed a significant main effect of group, $F(1,44) = 17.73$, $p < 0.001$, with higher CRs elicited in the control group. There was also a significant block effect, $F(4,176) = 4.46$, $p = 0.002$, showing response habituation in both groups across acquisition trials. Although both groups did show an enhanced response to the CS+ on the first acquisition block (see block A1 in Fig. 7), as discussed in the simple discrimination task (see “CR Acquisition”), this response may be artificially elevated by nonassociative factors due to the introduction of the noxious US. This arousal effect would mask the appearance of a more gradual learning curve in controls (Fig. 6) and should not be interpreted as evidence of learning in the temporal lobectomy subjects—it is the only trial block in which an enhanced response occurs, is only an average of two trials, and is not a statistically significant increase in this group.

A group \times block ANOVA on CS- trials yielded a significant main effect of block, $F(4,176) = 3.28$, $p = 0.013$, indicating response habituation in both groups late in acquisition. Although there is a trend for group differences in acquisition (see Fig. 7), this trend was only marginally significant, $F(1,44) = 3.37$, $p = 0.073$. In addition, no significant group \times block interaction was evident. Thus, comparable response patterns between the experimental groups were seen during acquisition on CS- trials. The impairment in differential conditioning for the temporal lobectomy subjects (see Fig. 6) can therefore be primarily attributed to low CRs during CS+ acquisition. These results parallel the findings obtained in the simple discrimination task.

Habituation, extinction, and UR analysis. An independent *t* test computed on responses elicited by the CS during habituation revealed no significant effects, indicating equivalent response patterns in the experimental groups during the habituation phase

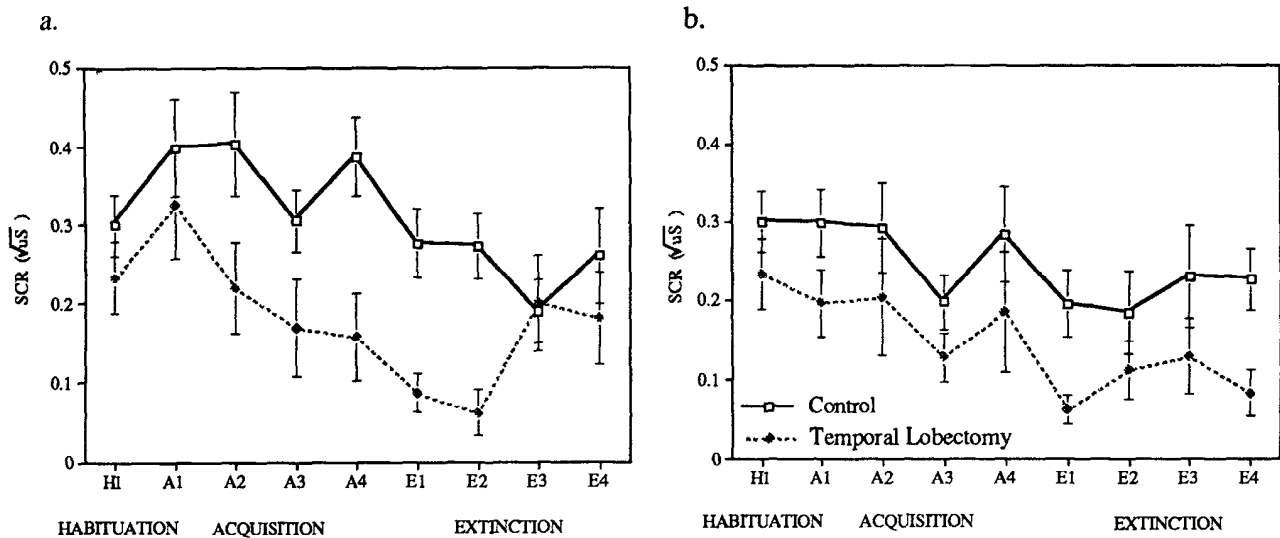


Figure 7. Conditional discrimination by trial type. SCRs were averaged into two-trial blocks by experimental group. *a*, Mean SCRs (\pm SEM) elicited on CS+ trials. *b*, Mean SCRs (\pm SEM) elicited on CS- trials. Habituation data is averaged across all trials, as there was no distinction between CS+ and CS- trials during this phase. Temporal lobectomy subjects showed deficits on CS+ trials (*a*) relative to nonepileptic controls, while no significant differences emerged on CS- trials (*b*).

(see Fig. 7). A group \times block ANOVA computed on differential extinction performance utilizing the last two extinction blocks also yielded no significant main effects or interactions (see Fig. 6). Finally, an analysis of unconditioned responding showed no significant group differences in SCRs to the US (see Fig. 8), although a significant block effect was found, $F(3,117) = 37.05$, $p < 0.0001$, showing an overall decline in UR amplitudes in both groups over time. Habituation, extinction, and UR performance was therefore comparable in the experimental groups, as previously seen in the simple discrimination task.

Role of awareness. With the exception of two temporal lobectomy patients and one control subject, all subjects correctly reported the CS-US relationships in this paradigm at the end of each experimental phase. As before, this verbal assessment provides evidence that declarative knowledge of the conditioning

procedure was spared in most of the temporal lobectomy patients studied. This finding again reflects a dissociation between intact declarative memory for the experimental parameters and impaired conditioned responding in these subjects, even in this more complex conditioning paradigm.

Discussion

In the present set of experiments, fear conditioning was investigated as a model for studying the neural basis of emotional learning and memory in humans. Results from the simple and conditional discrimination procedures demonstrated impaired conditioning in patients with unilateral anteromedial temporal lobe resection to control medically intractable epilepsy. The impairment was found to be primarily attributable to deficits in responding on paired CS-US acquisition trials. Since unconditioned responses and responding during habituation, CS- acquisition, and extinction appeared to be preserved, the discriminative learning impairment could not be accounted for by deficits in autonomic performance factors. The individual subject analysis, which accounted for group performance levels, also revealed an acquisition impairment in the temporal lobectomy subjects irrespective of baseline SCRs in habituation. Moreover, the impairment was not attributable to nonassociative sensory factors (as measured by audiometric screening), or to differences in declarative memory for the experimental parameters. These findings suggest that the role of medial temporal lobe structures governing simple and complex conditioned fear associations, as postulated in animal models of fear conditioning, extends to the human brain.

It is important to emphasize that the impairment reported here is related to *conditioned* fear acquisition, that is, the ability of a neutral stimulus to come to elicit an autonomic response through repeated pairings with an aversive unconditioned stimulus. In the present study, subjects did not necessarily report feeling "afraid" during the task, but they did report that the noxious US was annoying or unpleasant—this anticipation of an aversive event, signaled by the CS and measured physiologically, is de-

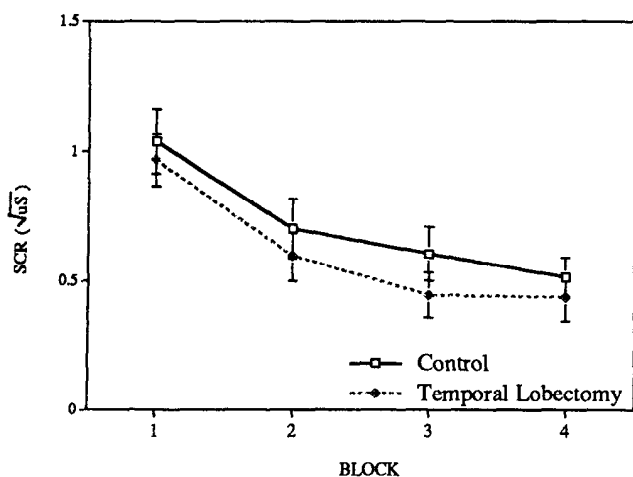


Figure 8. Mean unconditioned response performance (\pm SEM) during conditional discrimination. SCRs to the US were averaged into two-trial blocks by experimental group. Temporal lobectomy and nonepileptic control subjects showed comparable levels of unconditioned responding on this task.

defined as conditioned fear. It is also important to note that the patients did not show a complete lack of conditioned responding, but rather, their SCRs on CS-US acquisition trials were significantly attenuated in comparison to controls. In humans, SCRs have been shown to provide a reliable index of conditioning (e.g., Fredrikson et al., 1993), and fear conditioning paradigms have been proposed as a model for studying human affective disorders (Öhman, 1979; Charney et al., 1993). Although autonomic nervous system activity was used as the dependent measure of conditioning in the present study, we believe that other measures of conditioned fear acquisition (e.g., fear-potentiated startle) would also be impaired in these patients. This hypothesis, however, remains to be tested. In contrast, the patients' *unconditioned* response to an eliciting stimulus (a loud white noise) appeared to be intact, and we have not examined other aspects of fear-related behavior in these patients, such as recognition of facial expressions of fear (Adolphs et al., 1994).

We did not observe any hemispheric differences during conditioning on either the simple or conditional discrimination tasks. Left and right temporal lobectomy patients responded similarly, and no SCR asymmetries in the left versus right hands were seen in these subjects or in controls. Studies in human subjects which have been specifically designed to assess hemispheric asymmetries have found some hemispheric differences in electrodermal orienting and conditioning, although other results have been inconclusive (for review, see Hugdahl, 1984, 1995). Asymmetric differences may have been masked in our tasks by bilateral stimulus presentation and the response measure chosen (SCR). However, it is possible that hemispheric specialization in conditioning is mediated by other brain regions, or may be more apparent in behavioral and neuroimaging studies in normal subjects.

Daum et al. (1992) reported preserved simple discrimination ability on an eyelid conditioning task in unilateral temporal lobectomy patients similar to those studied in the present set of experiments. Their finding directly contrasts the outcome of the simple discrimination experiment reported here. This dissociation is most likely due to differences in the integrity of the neural circuitry essential for mediating these conditioning paradigms. In animal models of eyelid conditioning, learning ability is critically dependent on cerebellar and brainstem structures (for review, see Lavond et al., 1993), while in animal models of fear conditioning, the amygdala is crucial for performance in this paradigm (for review, see LeDoux, 1995). The temporal lobectomy subjects tested in the human studies have sustained lesions to the amygdala, with cerebellar and brain stem circuitry left relatively intact (with the possible exception of some cerebellar deterioration due to long-term Dilantin therapy; see Woodruff-Pak, 1993). Thus, a dissociation between impaired fear conditioning and intact eyelid conditioning on simple learning paradigms in this subject population is consistent with the known role of these structures from animal studies. However, alternative explanations related to qualitative task differences between the human conditioning studies cannot be ruled out at the present time. These include procedural differences (experimental parameters, such as reinforcement schedules, CS-US intervals, etc.), differences in US modality (white noise vs airpuff), and differences in response measurement (SCR vs eyeblink; nonadaptive vs adaptive responses).

Furthermore, Daum et al. (1991) also investigated conditional discrimination in temporal lobectomy subjects, and, consistent with the main result from our study, they reported deficits in

learning ability on this more complex procedure. This parallel result may be related to similar contributions of the hippocampus on more complex versions of both fear conditioning and eyelid conditioning tasks (e.g., Rickert et al., 1978; Berger and Orr, 1986; Solomon et al., 1986; Kaye and Pearce, 1987). However, the response pattern underlying the conditioning impairment was different in the two experiments: while Daum and colleagues reported a deficit due to excess responding on CS- trials, the current study found a deficit due to inadequate responding on CS+ trials. High responses on unreinforced trials is a characteristic of hippocampal dysfunction in animal models of discriminative somatomotor learning (Gray and McNaughton, 1983; Ross et al., 1984), while insufficient CR development is a characteristic of amygdala damage in animal models of fear conditioning (e.g., LeDoux, 1995). This contrasting pattern of results suggests the possibility that different medial temporal lobe structures may have been critical for the emergent response patterns across the human fear and eyelid conditioning studies.

Because unilateral temporal lobectomy patients have resection of most structures in the medial temporal lobe area, including the amygdala, hippocampus, and surrounding cortices, direct assessment of the relative contributions of these structures to task performance was not possible. Based on animal studies, it is likely that the amygdala damage in these patients was primarily responsible for the learning impairment for the following reasons: (1) the patients exhibited similar response patterns across both simple and complex tasks, suggesting a common neurobiological substrate; and (2) animal studies have shown that selective amygdala lesions disrupt both simple and complex aspects of fear conditioning (for review, see Kapp et al., 1990; Davis, 1992; LeDoux, 1995), while selective hippocampal lesions impair higher-order but not simple CS-US associations (e.g., Rickert et al., 1978; Kaye and Pearce, 1987; Phillips and LeDoux, 1992). One issue raised in extrapolating from the animal literature to the present report is that bilateral lesions are typically made in animal models of fear conditioning. The subjects used in the present study had predominantly unilateral damage, although it is possible that dysfunction in some structures contralateral to the site of the epileptic focus may have contributed to the response impairment (see also Daum et al., 1991).

Given the present results in humans, we recently examined the effects of unilateral amygdala lesions in rats, using a simple delay paradigm with a loud white noise as the US (LaBar and LeDoux, 1995). Unilateral lesions attenuated but did not eliminate conditioned fear responses, a finding consistent with the results reported here. Bilateral lesions, however, completely eliminated conditioned responding. We expect that bilateral lesions of the amygdala would have a similar effect in humans. Although human patients with selective bilateral amygdala lesions are extremely rare (Tranel and Hyman, 1990), future research investigating human subjects with more restricted damage and functional imaging studies in normal adults will be useful to further characterize the relative contributions of medial temporal lobe structures to performance on conditioning tasks.

In both the fear conditioning studies reported here and in eyelid conditioning tasks (Weiskrantz and Warrington, 1979; Daum et al., 1989; Daum et al., 1991, 1992; Woodruff-Pak, 1993), declarative knowledge of the conditioning parameters was dissociated from (or unrelated to) learning performance, even in more complex paradigms. Awareness of stimulus relationships has generally been found to enhance conditionability in normal human subjects (for discussion, see Grant, 1973; Furedy et al.,

1982; Frcka et al., 1983; Dawson and Schell, 1987). Current theories propose that relatively simple conditioning procedures, such as a simple delay paradigm, are governed by nondeclarative learning processes, while more complex conditioning procedures, such as a conditional discrimination paradigm, are regulated by declarative learning processes (Squire and Cohen, 1984; Squire, 1987). The main conclusion to be drawn from conditioning studies in brain-damaged populations conducted to date is that intact declarative memory may not be sufficient for successful performance, even in more complex conditioning paradigms. In order for conditioning to occur, conditioned stimuli representations must enter a functional neural network which mediates the CS-US association and directs an efferent signal to other brain regions controlling the conditioned response.

To conclude, we have demonstrated impaired response acquisition during simple and complex fear conditioning tasks following unilateral temporal lobectomy in humans. As a model for how fear and other aversive emotional events are learned and remembered, fear conditioning paradigms have been instrumental to uncovering the neural mechanisms of emotional processing in animals. In humans, however, the brain structures involved in emotional information processing are still poorly understood, although recently there has been an interest in applying animal models of emotional memory to human populations (Charney et al., 1993; Adolphs et al., 1994; Cahill et al., 1994). These results extend what is currently known about the neural substrate of emotional learning and memory from animal models, suggesting that medial temporal lobe structures are integral components of an emotional memory network in the human brain.

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