Indelibility of Subcortical Emotional Memories

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Abstract

Acquisition and extinction of fear responses conditioned to a visual stimulus were examined in rats with ablations of visual cortex. Visual cortex lesions did not interfere with acquisition, indicating that visual fear conditioning, like auditory fear conditioning, is mediated by subcortical, probably thalamo-amygdala, sensory pathways. In contrast to acquisition, extinction was greatly prolonged, if not prevented, by cortical ablation. This resistance to extinction of subcortical emotional memories may explain certain aspects of emotional memory in man.

Introduction

Stimuli associated with a highly charged emotional situation take on the affective qualities of that situation and can subsequently have a profound impact on mental life and behavior. This is easily demonstrated in the laboratory using classical conditioning procedures. If an animal is exposed to an emotionally neutral conditioned stimulus (CS), such as a light or a tone, at the same time as an aversive unconditioned stimulus (US), the CS will later, in the absence of the US, evoke a conditioned emotional (fear) reaction. Classical conditioning, therefore, can be used to examine how the brain learns and remembers the affective associations of the stimuli we encounter in daily life.

Studies over the past several years have demonstrated that the classical conditioning of fear reactions to acoustic stimuli is mediated by projections from auditory processing areas of the thalamus to the amygdala (LeDoux et al. 1984, 1985, 1986, 1987, 1988; Iwata et al. 1986, 1988). This circuit bypasses the auditory cortex and thus constitutes a subcortical mechanism of emotional learning.

Most contemporary theories of emotion suggest that affect occurs after cognition in a sequential processing chain (see Zajonc 1980). However, the existence of subcortical emotional processing circuits argues that at least some aspects of emotional processing are organized in parallel to cortical functions and that affect can be processed independent of cortically-dependent higher cognitive processes, such as pattern recognition and categorization.

It is important to determine whether subcortical emotional learning is the rule or is instead an exception restricted to the auditory modality. In the present studies we therefore examined whether visual fear conditioning, like auditory fear conditioning, is mediated subcortically. As expected, we found that visual conditioning does not depend upon the integrity of the visual cortex. Unexpectedly, we also found that emotional memories established in the absence of the visual cortex persisted (resisted extinction) for unusually long periods of time. This indelibility of subcortical emotional learning has important implications for understanding how emotional memories are established and maintained.

Results

Four groups of animals were studied. Rats were given either paired (P) or random (R) presentations of the CS (flashing light) and US (footshock) 20 days following visual cortex (VC) lesions (VC-P, n = 10; VC-R, n = 5) or sham (SH) operation (SH-P, n = 11; SH-R, n = 6). The next day, the suppression of drinking by the CS was used as a measure of fear conditioning (Bouton and Bolles 1980; Leaf and Muller 1965).

The basal lick rate was evaluated by examining the

Table 1

VALUES REPRESENT MEAN ± SEM

	SH-P (11)	VC-P (10)	SH-R (6)	VC-R (5)
Latency: 1st 5 licks:	65 ± 16	68 ± 15	78 ± 17	71 ± 31
Next 100 licks:	17 ± 1	23 ± 6	19 ± 1	16 ± 1

The four groups did not differ in the time taken to complete the first 5 licks (latency to start drinking) or in their latency to complete the next 100 licks. Abbreviations: SH-P: sham operated, paired training; VC-P: visual cortex lesioned, paired training; SH-R: sham operated, random training; VC-R: visual cortex lesioned, random training. Numbers of subjects per group is shown in parentheses.

amount of time taken to complete the first 5 licks once placed in the box and the amount of time taken to complete the next 100 licks before CS presentation (Table 1). A two-way analysis of variance was performed on each of these measures. The factors were surgical treatment (lesion vs. sham) and training procedure (paired vs. random). Insignificant F values were obtained for the effects of training and surgery and for their interaction in both analyses. The basal lick rate was thus similar in the four groups.

With the onset of the CS, the sham and lesioned animals given paired training (SH-P and VC-P) stopped drinking while the sham and lesioned animals given random training (SH-R and VC-R) continued drinking. Licking during the CS was standardized for each rat by computing the difference between the latency to complete 100 licks before and after the onset of the CS. A log (base 10) transform of these difference scores was then performed to allow for parametric data analysis. The higher the difference score the greater the suppression of drinking and thus the larger the magnitude of the conditioned fear response. As shown in Figure 1a, the sham and lesioned animals given paired training both had relatively high log difference scores and the sham and lesioned animals given random training had relatively low difference scores. A two-way analysis of variance of the log difference scores produced a significant effect of training ($F(1, 28) = 288.3$, $p < .01$) but insignificant F values for surgical treatment and for the interaction effect. Thus, the sham and lesioned groups given paired training did not differ relative to each other but each differed from their respective control group given random training. Visual cortex lesions, therefore, do not prevent the associative conditioning of fear responses to visual stimuli.

Figure 1b illustrates the average number of times animals in each group licked the drinking tube during each minute of the CS. A three-way analysis of variance of these data was performed. The main effect of surgery was not significant. Significant effects were found for training procedure (paired vs. random, $F(1, 28) = 7.3$, $p < .01$) and for the repeated measure (time during the CS, $F(9, 252) = 2.4$, $p < .05$). The interactions between surgery and training ($F(1, 28) = 5.6$, $p < .05$), surgery and the repeated measure ($F(9, 252) = 2.4$, $p < .05$) and training and the repeated measure ($F(9, 252) = 9.0$, $p < .01$) were also significant, as was the three-way interaction ($F(9, 252) = 2.8$, $p < .01$). Post hoc analysis indicated that the sham and lesioned groups given paired training each differed from their respective random control groups throughout the CS ($p < .01$). Post hoc tests also showed that the sham and lesioned animals given paired training did not differ from each other during the first 7 minutes of the test but did differ during minutes 8–10 ($p < .01$).

Thus, both sham operated and visual cortex lesioned animals acquired the conditioned association (as indicated by the differences between the paired and random groups for each surgical treatment). However, the conditioned response was somewhat weaker towards the end of the CS in the lesioned than in the sham group given paired training. This suggests that the lesioned group might have started to extinguish and thus that visual cortex lesions might enhance extinction.

The CS test was readministered 5 times (once every 5–7 d) over the subsequent month to determine the rate of extinction of the conditioned fear response in the visual cortex and sham operated animals given paired training. The animals were deprived of water at 1700 hr the night before the test and the test administered between 0900 and 1300 hr the following day. Figure 1c shows that with each administration of the test the CS suppressed licking less in the sham operated group. In marked contrast, the performance of the lesioned group, illustrated in Figure 1d, did not significantly change over the course of 5 tests. A three-way analysis of variance was performed, with surgery (sham vs. lesion), minutes 1–10 of the CS, and extinction test days 1–5 as factors. A significant F value was obtained for minutes of the CS ($F(9, 171) = 71.4$, $p < .01$) and for days of the extinction test ($F(4, 76) = 16.3$, $p < .01$), but not for surgery. The interaction between surgery and extinction test days ($F(4, 76) = 4.7$, $p < .01$) and between extinction test days and CS minutes ($F(36, 684) = 6.9$, $p < .01$) was significant, but the interaction between surgery and CS minutes was not. The three-way interaction was also significant ($F(36, 684) = 5.4$, $p < .01$). Separate two-way analyses of variance performed on the data from the sham and lesioned groups determined that the performance of the sham group changed significantly over the 5 tests ($F(4, 40) = 18.4$, $p < .01$),

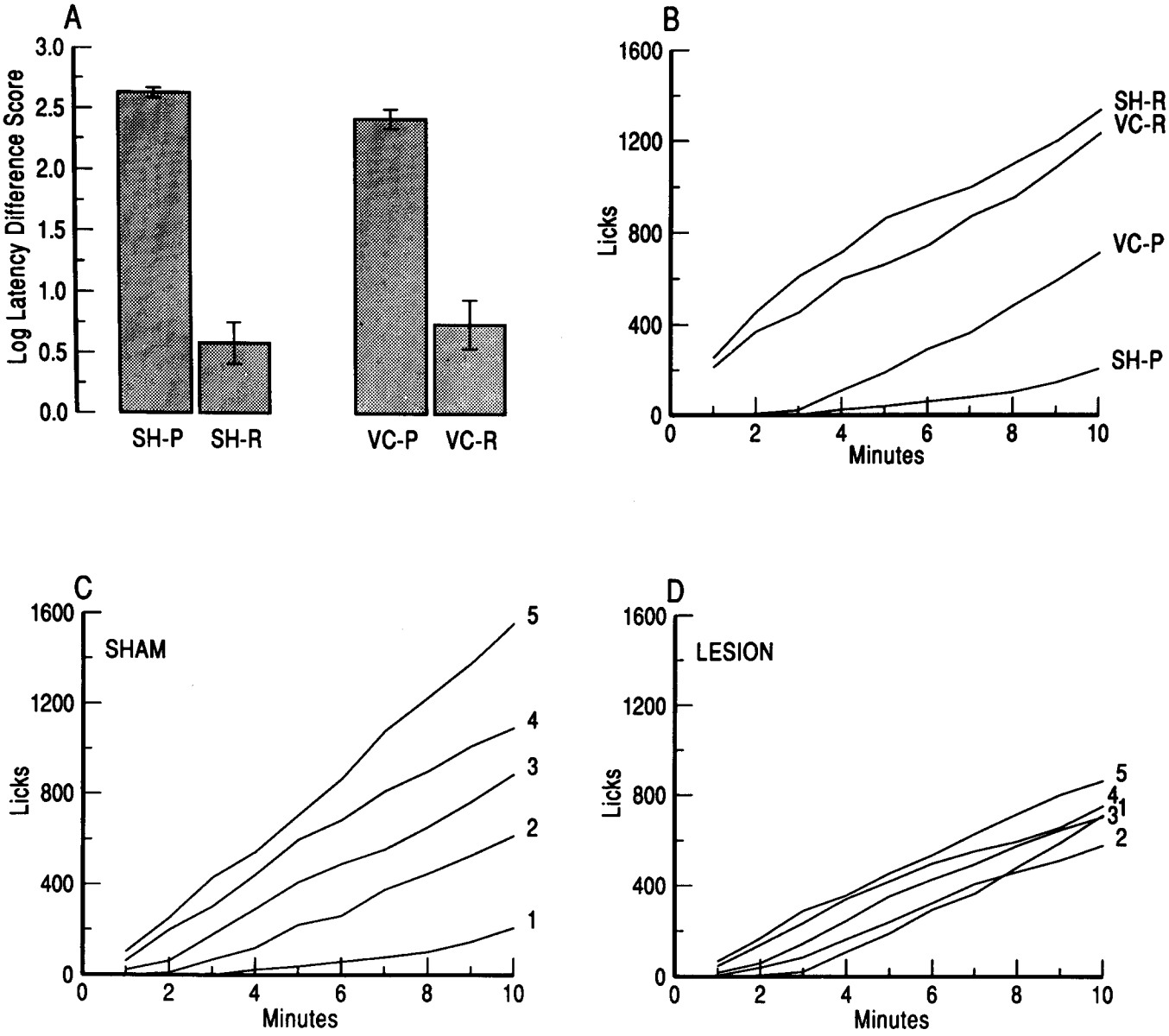

Figure 1. Lesions of the visual cortex did not prevent the acquisition of conditioned fear responses to a visual stimulus but greatly prolonged extinction. (A) The difference in time (transformed to log base 10) to complete 100 licks before and after the onset of the CS is illustrated for the four groups tested. Both sham operated (SH) and lesioned (VC) animals given paired training (P) differed from their respective control group given random training (R). However, the sham and lesioned animals given paired training did not differ, nor did the sham and lesioned animals given random training. (B) Number of licks completed during each minute of the 10-min CS for each group. Sham and lesioned animals given paired training each differed from their respective control groups given random training throughout the CS. The lesioned group given paired training did not differ statistically from the sham group given paired training during the first 7 minutes, but did differ during minutes 8–10. (C and D) Repetition of the 10-minute extinction test 5 times over 30 days for the sham operated (C) and visual cortex lesioned (D) animals given paired training. Sham operated animals showed gradual extinction of the response with repeated non-reinforced presentations of the CS. In contrast, the performance of the lesioned group did not change over the 5 tests.

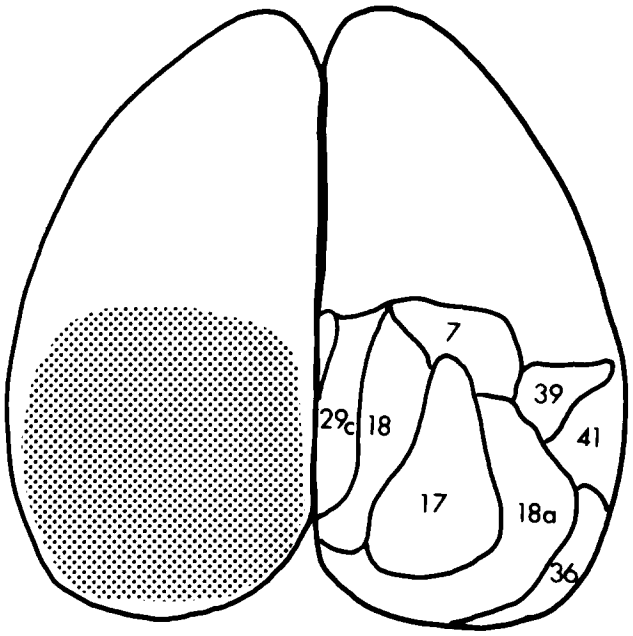

Figure 2. A typical lesion of visual cortex is illustrated on the left, with the cytoarchitecture of the posterior neocortex illustrated on the right. Visual cortex includes areas 17, 18, and 18a. The cytoarchitectural map is based on Hughes (1974).

whereas the lesion group did not.

All animals in the lesioned groups (VC-P and VC-R) had extensive damage to the visual cortex, as defined on the basis of published criteria (Thompson 1969; Hughes 1974). In some cases the lesions were complete. However, even the incomplete lesions damaged all of striate and most (at least 80% and often more) of extrastriate visual cortex. Since the performance of the animals with complete and partial lesions did not differ statistically, these were treated together. Little or no brain damage was present in the sham operated groups (SH-P and SH-R). A typical lesion is illustrated in Figure 2.

Discussion

The present results demonstrate that in the absence of visual cortex, fear responses were readily established to visual stimuli. Visual fear conditioning, like auditory fear conditioning, does not depend upon the integrity of the sensory receptive cortex and involves subcortical sensory pathways. However, in the absence of the visual cortex, fear responses to visual stimuli persisted in the face of unreinforced presentations of the CS long after the responses began to extinguish in sham operated animals. Although the visual cortex is not necessary for the establishment of conditioned associations between visual and aversive stimuli, it does appear to be required to efficiently extinguish conditioned fear responses once the CS is no longer systematically related to the US.

The subcortical auditory pathways involved in emotional learning are known to involve projections from the acoustic thalamus to the amygdala (LeDoux et al. 1984, 1985, 1986, 1987, 1988; Iwata et al. 1986, 1988). Visual fear conditioning is disrupted by lesions of the amygdala (Hitchcock and Davis 1986; LeDoux, unpublished) and the amygdala receives afferents from thalamic areas that may function as secondary visual structures (LeDoux et al. in preparation). Thalamo-amygdala projections may be involved in visual, as well as auditory, fear conditioning.

Since visual cortex lesions interfere with extinction, it is possible that extinction is mediated by visual cortex. However, a more likely possibility is that the visual cortex provides access to other cortical structures that perform this function. For example, it has been known for some time that damage to the frontal lobes produces perseveration of unreinforced conditioned responses (Butter 1969; Fuster 1980). Hippocampal lesions have similar effects (Gray 1982). Visual inputs reach these areas by way of cortico-cortical connections arising in visual cortex (Nauta 1972; Turner et al. 1980; van Hoesen et al. 1972). Lesions of visual cortex may prolong extinction by preventing the relay of visual inputs to areas such as the frontal cortex and/or hippocampus.

Emotional memories established in the absence of sensory cortex, probably by way of thalamo-amygdala projections, are, therefore, relatively indelible. If emotional memories are also established through these pathways when the cortex is intact and functioning, it might explain why experimental extinction appears to operate more on performance than on memory variables. In laboratory animals, extinguished responses often recover spontaneously (Pavlov 1927) and re-exposure to the US alone (without the CS) can reinstate the emotional potency of the CS (Rescorla and Heth 1975). Similarly, in humans, infantile emotional memories and adult fears and phobias can lay dormant (unconscious and unexpressed) for years until reinstated by stress (Jacobs and Nadel 1985). Emotional behavior may extinguish, but the underlying association is not lost. Behavioral extinction of emotional responses may represent a temporary suppression, by cortex, of subcortical (thalamo-amygdala) emotional circuits that maintain the learned association over long, perhaps indefinite, periods of time, in spite of repeated exposure to the CS without the US. This subcortical maintenance of underlying emotional associations in the face of behavioral extinction may also help explain why the treatment of affective disorders in humans is so difficult. Treatments that emphasize cognitive (cortical) control of emotion may prove to be more effective over the long run than those that attempt to eliminate indelible and cognitively impenetrable emotional associations.

Methods

Male, Sprague-Dawley rats (275–325 g) were anesthetized with pentobarbital (40 mg/kg, ip) and placed

in a stereotaxic frame. Using aseptic procedures, the cranium was exposed and the skull removed over the visual cortex. An incision was made in the dura, which was folded back, and the underlying visual cortex was removed through subpial aspiration. Hemorrhaging was controlled by application of Gelfoam. The surgical field was cleaned, the dura sutured together (when possible), and the wound closed. Sham operated controls were anesthetized, placed in the stereotaxic frame, and the skull was removed, but the dura was left intact. Body weight and food and water consumption were routinely checked in order to detect any postoperative complications that might arise.

After at least 20 d recovery from surgery, the rats were removed from their home cages, placed individually in a rodent conditioning chamber (E10–10, Coulbourn Instruments, Lehigh Valley, PA) enclosed by a sound attenuating cubicle (E10–20, Coulbourn Instruments), and subjected to aversive classical conditioning trials. The conditioned stimulus (CS) consisted of the illumination of a 25W incandescent lamp mounted behind a translucent panel located on the front wall of the conditioning chamber. The lamp flashed on and off for 10 sec at a rate of 2.5 Hz. The unconditioned stimulus was a brief (500 ms) presentation of regulated direct current (2.2 mA) produced by a grid floor shocker (E13-08, Coulbourn Instruments) and delivered through the grid floor of the conditioning chamber.

Stimulus presentation was controlled by a microprocessor (IBM-XT) equipped with a digital output board (Opto 22). For the first 10 trials, the CS was presented alone. Over 30 conditioning trials, the US appeared during the final 500 ms of the CS for animals given associative training (paired presentations of the CS and US). For animals given non-associative (random) training, the CS and US were each delivered 30 times, but the onset of the US with respect to the CS was randomized.

The effects of conditioning were assessed by measuring the extent to which presentation of the CS suppressed drinking behavior. In order to ensure that the animals would drink during the test period, they were deprived of water at 1700 hr the night before conditioning. Approximately 40 h after deprivation and 18–22 hours after conditioning, the rats were placed in a chamber similar to the conditioning chamber except that a drinking tube filled with 10% sucrose protruded through the front wall. The metal tip of the drinking tube and the metal floor grids immediately in front of the drinking tube formed the open ends of an electric circuit. Each time the rat's tongue made contact with the drinking tube the circuit was closed. One lick was counted electronically when the tongue broke contact. After 100 licks were counted, the CS was presented for 10 minutes. During the conditioned response test, the following measures were taken: latency to lick the tube 5 times after being placed in box; latency to lick the tube 100 times after the initial 5 licks; latency to complete 100 licks after the onset of the CS; number of licks during each minute of the CS.

After completion of the behavioral studies, the animals were given free access to food and water. The next day an overdose of pentobarbital (120 mg/kg) was administered and the animals were perfused through the left ventricle of the heart with normal saline and 10% buffered formalin. The brains were removed, frozen and sectioned (at 40 um). The sections were stained with thionin and the lesion location evaluated.

Note Added in Proof

The finding that visual cortex lesions prolong extinction may generalize to other sensory modalities. A recent study shows that auditory cortex lesions prolong extinction of heart rate responses conditioned to acoustic stimuli in rabbits (Teich, A.H., McCabe, P.M., Gentile, C.C., Schneiderman, L.S., Winters, R.W., Liskowsky, D.R., and Schneiderman, N. (1989) *Brain Research, 480*, 210–218).

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