The Amygdala, Fear, and Memory

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ABSTRACT: Lesions of the frontotemporal region of the amygdala, which includes lateral and basal nuclei, cause a loss of conditional fear responses, such as freezing, even when the lesions are made over a year and a half from the original training. These amygdala-damaged animals are not hyperactive and show normal reactivity to strong stimuli such as bright lights. After receiving tone-mild shock pairings rats normally display an appropriately weak response when exposed to the tone. Rats' fear of the tone can be inflated by giving them exposure to strong shocks in the absence of the tone between training and testing. This inflation of fear memory is abolished if the frontotemporal amygdala is inactivated by muscimol only during the inflation treatment with strong shocks. Based on such findings we suggest that the frontotemporal amygdala permanently encodes a memory for the hedonic value of the aversive stimulus used to condition fear.

KEYWORDS: fear; pavlovian conditioning; freezing; memory; amygdala; basolateral amygdala; frontotemporal amygdala; hedonic value; affect; hippocampus; retrograde amnesia

INTRODUCTION

There is near universal agreement that the amygdala contributes to fear-motivated learning. Many amygdala manipulations profoundly affect behavioral indices of fear conditioning.[1] Changes in the firing properties of amygdala neurons after fear conditioning have also been observed. However, it is incorrect to think of fear as something localized to a single brain region. Fear represents a complex functional behavior system, and widely distributed neural circuitry contributes to the perception and recognition of danger, the learning and remembering about dangerous experiences, and the coordination of defensive behaviors to environmental threat.[2] The circuit involves many brain regions and distinct patterns of communication between those regions.[3] Our laboratory is interested in determining the unique and special contributions each of these components of the circuit makes to the effective operation of this functional behavior system we call fear. This chapter focuses on one such component, the frontotemporal amygdala.[4]

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The frontotemporal amygdala (FTA), often referred to as the basolateral complex, receives extensive information from the neocortex, thalamus, and hippocampus. Thus, it is in an excellent position for sensory integration. It has extensive projections to the amygdala's central nucleus and thereby has access to many of the structures that generate and coordinate fear-related behavior.[4] Lesions and pharmacologic manipulations that selectively target this region have profound effects on both the acquisition and the expression of fear.[1,5] Although these findings clearly establish a critical role for the FTA, the precise nature of its contribution to fear processes remains an open question. We describe a few experiments that lead us to a rather bold and specific conclusion: the frontotemporal amygdala permanently encodes the emotional significance (hedonic value) of the aversive reinforcer used in pavlovian fear conditioning. The experiments are conducted so that this role cannot be attributed to factors such as alterations in general activity or interference with behavioral indices of fear.

PERMANENT INVOLVEMENT IN FEAR MEMORY

A number of findings suggest that the FTA plays a relatively permanent role in fear conditioning.[6,7] These studies employed a posttraining lesion strategy in which the amount of time between training and lesion is manipulated in order to assess how long a structure contributes to the learned behavior. This strategy has been successful at characterizing temporally limited contributions of the hippocampus to fear. Posttraining lesions of the hippocampus are effective in blocking expression of some forms of fear when made shortly after training but ineffective when made at longer intervals between training and testing.[8] To investigate how long the FTA is involved in mediating a fear memory, we made posttraining lesions of this structure.[9] Previous studies showed that amygdala lesions made up to 1 month after fear conditioning severely attenuated fear, as assessed by both potentiation of startle and freezing.[6,7] This pattern of results appears consistent with a "permanent" role for the FTA in fear conditioning. However, in some instances the hippocampus showed a time-dependent involvement in fear conditioning for periods longer than 1 month.[10] To test conclusively the duration of the contribution of the FTA to fear conditioning, we interposed an interval of 1.5 years, which encompasses most of a rat's life span.

To maximize the power of our test procedure we used the within-subjects design developed by Anagnostaras *et al.*[11] to explore the time-dependent involvement of the hippocampus in fear conditioning (FIG. 1). Adult rats were placed in one distinctive context and received pairings of tone and shock. After this, they were maintained in their home cages for 1.5 years (the Anagnostaras *et al.*[11] hippocampus study only waited 50 days). At that time, rats were placed in a second distinctive context that differed in terms of location, shape, lighting, odor, and background noise. There, they received pairings of a different tone with shock. The day after this second training the rats received bilateral lesions of the FTA made by injecting NMDA into that region. Although these lesions cause extensive cell loss in the lateral and basolateral nuclei, nearby regions such as the central nucleus were completely spared. Rats were given 10 days to recover from surgery prior to a series of tests designed to independently test fear of the contexts and tones. We tested fear of both contexts in the absence of tone and shock. Fear to both tones was tested in a novel third context.

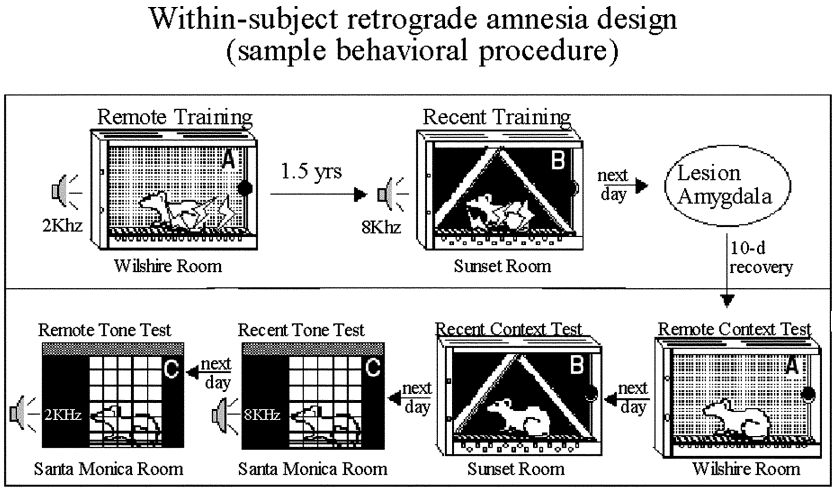

FIGURE 1. The design of an experiment to investigate the duration of the frontotemporal amygdala's (FTA) mediation of a fear memory. Rats received fear conditioning in either the Wilshire Room or the Sunset Room of the laboratory. Fear conditioning consisted of 10 tone-shock pairings, and a different tone was used in each context. The two experiences were separated by about a year and a half. What tones were used in what context, what combination of tone and context was trained first and second, and the order of testing were all counterbalanced across animals. The design allows comparisons of different types of training to be made in a statistically powerful within-subjects manner.

Freezing served as our behavioral index of conditioning. The procedure shown in FIGURE 1 is a sample procedure; all aspects of the experiment (which tone was recently or remotely trained, which context was used first and second, and the order of tests) were counterbalanced across animals.

Data for tone testing (FIG. 2) and context testing (FIG. 3) are remarkably similar. One particularly striking outcome is apparent from comparing the freezing scores of the sham surgery animals for the remote and recent memory test. This reveals that absolutely no forgetting of fear occurs over a period of 1.5 years, most of the life span of the rat. This is the longest retention of fear memory tested in this species and, we believe, the longest retention of fear memory experimentally documented for any species. The lack of forgetting is equally apparent for tone and context memory.

The effects of the FTA lesion were very consistent. Freezing was drastically reduced regardless of whether the test stimulus was tone or context and whether the memory was old or new. These results can be contrasted with the Anagnostaras *et al.*[11] experiment that used the same apparatus and parameters except that only 50 days transpired between initial training and hippocampal lesions. In that study, there was no forgetting in the sham surgery animals either. However, there was no effect of the lesion on tone fear, and only recent, not remote, context fear was affected.

In conclusion, fear memories are permanent, and the FTA is critically involved in mediating these memories. The FTA plays a general role in that it is critical for both tone and context fear. It also plays a permanent role, as evidenced by the profound

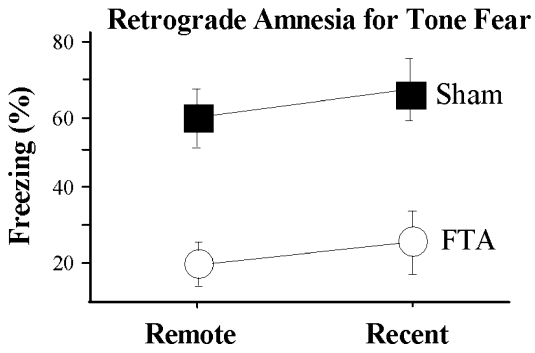

FIGURE 2. The results for conditioning to the tone. In comparison to "sham" lesioned controls, excitotoxic lesions of the frontotemporal amygdala (FTA) reduced fear to a memory formed 1 day before the lesion (Recent) and 1.5 years (Remote) before the lesion, equivalently.

deficits in freezing to stimuli conditioned a very long time ago. Clearly, the FTA's involvement in this behavior is not related to consolidation of a memory in some other brain region. Perhaps it could be argued that amygdala-dependent consolidation occurs, but 1.5 years is not long enough for it to exert a detectable effect. Of course, this would mean that such consolidation is so slow as to be functionally meaningless given the life span of the rat.

PERFORMANCE FACTORS

Although the data in FIGURES 2 and 3 clearly demonstrate a permanent role for the FTA in this behavior, they do not necessarily mean that this role is mnemonic. Because the amygdala is not functioning during testing regardless of the interval between training and testing, the lesions may have affected performance in some other way. Perhaps the lesioned animals are simply unreactive to significant environmental stimuli or are hyperactive. Alternatively, the behavioral deficits may reflect an inability of these rats to perform the freezing response. We conducted a set of follow-up tests on these animals after completion of the four freezing tests just described to address these possibilities.

To determine if these rats were hyperactive, we placed them in a rectangular open field divided into eight square sections. Our measure of activity was the number of crossovers between the sections. They were first observed for 4 minutes, while the open field was dark (FIG. 4, left side). Crossovers start out high during the first minute as a result of exploration and then decline over 4 minutes. Lesioned animals show the exact same pattern and level of activity as the controls. Clearly, these animals are not hyperactive and display normal habituation of exploratory activity.

Following the 4-minute dark phase, bright lights situated at both ends of the open field were turned on for an additional 4 minutes of observation. Normally in this test, rats react with a sudden increase in movement that is revealed as a small elevation in crossovers during the fifth minute, but then activity is markedly suppressed during

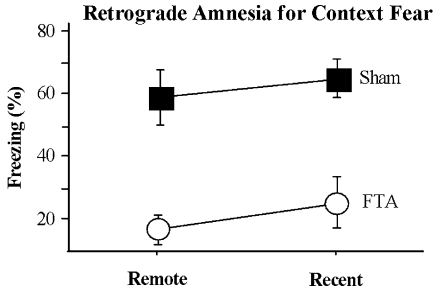

FIGURE 3. Results for conditioning to the context are displayed. Frontotemporal amygdala (FTA) lesions reduced fear to a memory formed 1 day before the lesion (Recent) and a year and a half before the lesion (Remote) to a similar degree.

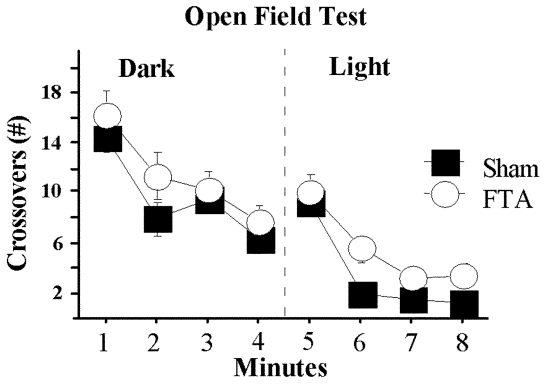

FIGURE 4. Number of crossovers between sections of an open field for each minute of an 8-minute session. The first 4 minutes were in the dark and the last 4 were in bright light. Frontotemporal amygdala (FTA) lesions did not affect this measure of general activity.

the remainder of the session. Our lesioned rats and their sham controls showed exactly this pattern (FIG. 4, right side). The fluctuations in activity precipitated by exposure to bright light indicate that the lesioned rats are reactive, in terms of increases and decreases, of activity to a significant environmental stimulus. The profound suppression of activity in the lesioned animals during the seventh and eighth minutes of the test clearly demonstrates that the lesions do not produce hyperactivity.

Freezing is a far more specific response than not moving between sections of an open field. It is the complete absence of all movements, including sniffing and head movement; only movement of the animal's flanks that accompanies respiration is tolerated in the criteria we employ. Although these rats can suppress their movement enough not to cross between sections of an open field, the lesions may have rendered them unable to generate a freezing response. Recent findings do not support this characterization. Maren[12] demonstrated that with very extended overtraining, amygdala-lesioned rats could freeze at normal levels, and he has forcefully argued

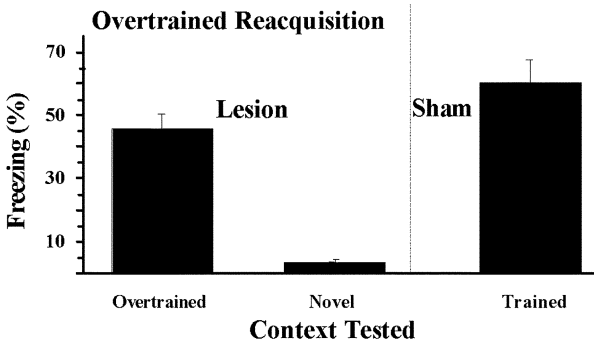

FIGURE 5. Rats with frontotemporal amygdala (FTA) lesions shown in the previous three figures were capable of freezing when they were given substantial overtraining. Lesioned rats were overtrained and tested in both the overtrained context and a novel context. Data on sham rats were obtained from the context tests of FIGURE 1.

that the deficits in freezing shown by amygdala-damaged rats do not stem from an inability to respond. Therefore, after the open field test, we gave the animals an overtraining session, similar to that used by Maren,[12] containing 75 unsignaled shocks. As can be seen in FIGURE 5, overtraining resulted in high levels of freezing in lesioned animals when they were later tested in the overtrained context. The freezing was associative, as these rats did not freeze when placed in a novel chamber. The level of freezing obtained with this overtraining was comparable to that attained by the unlesioned controls, whose data are presented in FIGURE 5 for comparison purposes.

We do not know what brain structures subsume the role of the amygdala with this overtraining protocol; those have yet to be determined. Here, we merely wish to point out that these rats are capable of executing a freezing response. It is important to note that Maren[12] has shown that if intact rats are first overtrained and then given a lesion, all freezing behavior is lost, and there is no savings when a second course of overtraining is begun. Therefore, whatever neural structures compensate for loss of the amygdala, they are only engaged when substantial overtraining is given to an amygdala-damaged subject. Thus, the amygdala is usually responsible for fear learning whether normal or overtraining procedures are used.

This set of findings makes it unlikely that the deficit in freezing after conditioning can be explained by an inability to react to significant stimuli, hyperactivity, or an inability to freeze. Rather, we believe the total pattern of results points to a mnemonic function for the amygdala.

WHAT DOES THE AMYGDALA ENCODE?

Fear memories are complex; they contain many components. It is incorrect to think that any one regions encodes the "fear memory." Different components of the fear circuit extract and encode different aspects of the fear experience, and the memory is as much in the interconnections between these regions as it is in any particular

region. That being said, it is important to determine just what component of the fear memory the FTA encodes. Given its general and permanent involvement in fear memory, that component must be essential. But what is the content of that memory?

The question of the content of memory has long been a focus of learning theorists, particularly with respect to pavlovian conditioning. One early and rather intuitive hypothesis, formally proposed by Tolman,[13] is that the conditional stimulus (CS) calls up a memory of the unconditional stimulus (US). For the memory of the US to generate behavior, one component of that US memory would have to be its affective or hedonic value. According to this view, conditioning requires the encoding of an association between the CS and US and also a hedonic representation of the US. The association determines how well the CS can arouse the US representation, and behavior is a joint function of the associative strength and hedonic value of the US representation.

The most satisfying empirical attack on this view was accomplished in a series of experiments conducted by Rescorla.[14,15] He reasoned that we should be able to alter conditional behavior not only by changing the associative strength between the CS and US but also by independently manipulating the hedonic value of the US. In one experiment, he first gave rats several pairing of a tone and a mild shock.[15] The tone acquired the ability to produce a low level of fear, presumably because the representation of the shock reflected the low hedonic impact of the mild US. On another day, Rescorla gave the rats a series of strong shocks in the absence of the CS. Because there was no CS during this treatment, such manipulation should not enhance associative strength. However, receiving strong shocks should change how the subject remembers shock, inflating the hedonic value of the memory. Later, Rescorla tested the tone in the absence of any shock. Rats that received the inflation shocks showed enhanced fear, behaving as if they were remembering the strong inflation shock rather than the mild shock that was actually paired with the tone. The inflation effect depended on the tone being paired with the weak shock; rats that received tone and weak shock unpaired and the inflation shocks did not show the effect. Therefore, a CS-US association was critical to performance. Because no US was present during the test, the effect of the CS had to occur via a memory of the US. Inasmuch as the strong shock was never directly paired with the CS, the inflation manipulation was not having its effect on the CS-US association. Thus, Rescorla demonstrated that the hedonic value of the memory of the US could independently be changed with the inflation procedure.

Weiskrantz[16] suggested that the amygdala allows animals to recognize that stimuli are reinforcing, and since then, many variants of this idea have been put forth.[17–19] How do you recognize that a stimulus is reinforcing? Perhaps, it can be done by recalling its hedonic value. By combing these ideas with those of Rescorla, we propose that what the FTA encodes is the hedonic value of the US. A loss of this component of memory would go a long way in explaining why FTA lesions have such a devastating effect on pavlovian fear conditioning. Rescorla's inflation procedure offers a way to empirically test this conjecture. Studies examining both rodents[20] and primates[21] with amygdala lesions performing instrumental behavior (e.g., lever pressing for food) are consistent with this idea. Amygdala lesions do not disrupt instrumental performance *per se,* but they do prevent reenforcer devaluation from changing performance. However, the fact that amygdala lesions produce such subtle effects on positively reinforced instrumental behaviors compared to the devastating

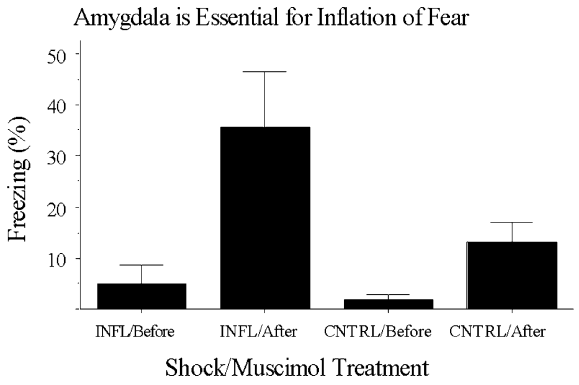

FIGURE 6. Data from an inflation experiment are summarized in which rats first received pairing of tone and an 0.5-mA shock. On the next day, rats were presented with a series of 3-mA shocks (INFL/–) or simply placed in the conditioning chamber (CNTRL/–). The amygdala was inactivated with muscimol either immediately before (–/Before) or after (–/After) this shock inflation treatment. On the day after inflation, tone fear was tested in a novel chamber. There was no infusion and no shock on this test day.

effects the same lesions have on pavlovian fear conditioning suggests that the structure plays a different role in these two types of behavior. Indeed, since a lesion of the FTA would eliminate performance of pavlovian conditional fear responses, it would be impossible to determine, using lesion techniques, whether the FTA plays a role in encoding the value of the US. An alternative approach that can give us leverage on this question is to temporarily inactivate the amygdala. If we prevent FTA activity just during the inflation procedure we should prevent the animal from encoding the altered hedonic value of the shock. This procedure should not affect the CS-US association, as the FTA would be functional during initial training with the tone and mild shock. Performance of the original fear memory should not be compromised during testing, because FTA activity will have returned to normal.

We conducted just such an experiment.[22] Rats with bilateral chronic indwelling cannulas implanted into the FTA received pairings of tone with a mild shock, but the amygdala was not manipulated at this time. The next day the rats received a series of strong shocks, but no tone was presented. All rats received an infusion of the GABA agonist muscimol to inactivate cells within the FTA. For half the rats the infusion was performed just before the inflation manipulation, so that the amygdala was shut down at the time of memory inflation (INFL/Before). The other half of the rats received the same muscimol infusion, but immediately after the inflation shock (INFL/After). For this control group the amygdala would have been able to encode the inflation shocks, so they served as a control for any nonspecific effects of muscimol. Two other control groups (CNTRL/Before and CNTRL/After) were treated like the two inflation groups, but they did not receive the inflation shocks; they were merely placed in the inflation context. The next day the rats were placed in a novel context and presented with the tone. No infusions and no shocks were given, and freezing to the tone was assessed (FIG. 6).

A comparison of the INFL/After and the CNTRL/After groups of FIGURE 6 shows a replication of Rescorla's inflation effect. However, from a comparison of the INFL/After and the INFL/Before groups, it is obvious that infusing muscimol immediately before inflation treatment completely abolished the enhancement of fear. Thus, the FTA is essential for the inflation of fear. Because all animals received muscimol infusions, the effects cannot be attributed to some nonspecific or long-term consequence of muscimol infusion. These data provide strong evidence that the amygdala encodes the hedonic memory of the US. The effects cannot stem from disruption of some postinflation consolidation processes, because animals that received infusions immediately after inflation showed enhanced fear.

CONCLUSIONS

The amygdala pays a crucial role in pavlovian fear conditioning. The series of experiments summarized in this chapter go a long way towards pinpointing what this crucial role is for the frontotemporal amygdala. Taken together, the data suggest that the FTA permanently encodes the hedonic value of aversive stimuli and makes that memory available to other structures that encode other aspects of the fear-conditioning experience. For example, the hippocampus can connect that affective information to particular contexts and episodes.[23,24] The medial geniculate connects it to specific auditory stimuli.[25] Projections to the striatum may provide affective tone to instrumental behavior.[26] The affective information stored by the FTA may be able to influence the storage of declarative memories mediated by other structures.[27] Certainly the FTA is not the only part of the amygdala important for fear. For example, the ability for affective memories to activate the central nucleus via its connections with the FTA is critical for initiating the constellation of behaviors that allows a species to effectively defend against threats that jeopardize its survival.[2,4,5] A greater appreciation of the interrelations between these regions and other components of the fear circuitry will enhance our understanding of fear processes and will be critical for advancements in the treatment of fear-related disorders.

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