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Electrical stimulation (88 Hz) of the lateral hypothalamus elicits a sustained theta response at hippocampal recording sites of rats immobilized with succinylcholine. By pairing this unconditioned stimulus with a 10-second presentation of a light, conditioned theta responses consistently appear after 40 trials. When the conditioned rats were tested in the absence of a neuromuscular blocking agent, the conditioned stimulus elicited a theta response that was associated with slow locomotor

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20. ABSTRACT (continued)

activity in 70 percent of the trials. This paper provides an assay for hippocampal bioelectric activity that is useful in understanding radiation-induced behavioral incapacitation.

PREFACE

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INTRODUCTION

While there have been many studies showing changes in hippocampal bioelectric patterns associated with changes in performance of learned tasks,[1,4,5,8,9] there have been very few studies of neuronal plasticity of the hippocampus itself.

Segal et al.[12] showed that hippocampal unit responses to a stimulus could be modified through experience by using both positive and negative contingencies. Black et al.[3] showed that it is possible to condition paralyzed dogs to modify the frequency of occurrence of trains of hippocampal theta waves by making the occurrence of theta instrumental in the avoidance of shock. Glazer[7] has demonstrated a similar effect in freely moving rats.

In our laboratory, hippocampal theta could be reliably produced with high frequency electrical stimulation of the lateral hypothalamus (medial forebrain bundle). The plasticity of this induced hippocampal theta response in animals immobilized with succinylcholine was evaluated by a Pavlovian conditioning paradigm. After a stable conditioned response was established, the freely moving subjects were tested to determine what particular behavior patterns are emitted in conjunction with conditioned hippocampal theta patterns.

METHODS

Subjects. Six male Sprague-Dawley rats weighing approximately 300 grams were used. The animals were housed in individual cages and maintained on an *ad libitum* food (Purina Chow) and water schedule in a room that was illuminated from 0730 to 1600 daily. Ambient temperature was set at 68°F. An electrode assembly consisting of two pairs of hippocampal recording electrodes, a pair of cortical recording electrodes and a pair of lateral hypothalamic stimulating electrodes were chronically implanted in the rats anesthetized with pentobarbital (50 mg/kg).

Two weeks after electrode implantation, the animals were reanesthetized, and a PE 10 catheter was implanted in the external jugular vein. The catheter was brought out between the shoulder blades and was attached to a 30-gauge disposable hypodermic needle that was cemented to the cranial implant. The catheter was flushed with a sodium heparin solution (25 units/ml) every evening.

Conditioned stimulus. Illumination of the conditioning chamber by a small 6-candlepower light bulb (G. E. #82) for 12 seconds served as the conditioned stimulus (CS). The light was presented alone to provide a behavioral and EEG base line in the freely moving animal. Although it produced a startle reaction on the first few trials, after 40 habituation trials, the CS no longer had any consistent effect upon EEG and behavior.

Unconditioned stimulus. Stimulation of the lateral hypothalamus served as the unconditioned stimulus (UCS) for hippocampal theta (88 Hz, 4-6 volts). The stimulation was sufficient to maintain bar pressing for self-stimulation in an operant chamber. When it was applied continuously for 5 seconds, continuous hippocampal theta waves and vigorous running appeared.

Conditioning procedure. After habituation to the CS, the rats were given training sessions of 20 trials per day in which the CS was followed immediately by the UCS. The rats were immobilized by a continuous intravenous infusion of 0.2 mg/ml succinylcholine injected at a rate of 60 μl/min. Artificial respiration was provided by means of a respiratory pump.

The presentations of CS and UCS were controlled by a tape programmer and assorted timers. The trials were presented on an aperiodic schedule at a rate of approximately 1 trial every 3 minutes. During extinction, the programming equipment operated normally; the stimulator was turned on but was disconnected from the subject's hypothalamic electrodes.

After a reliable conditioned hippocampal theta response (CR) was established to the CS in the immobilized rat, behavioral changes associated with conditioned hippocampal theta responses were studied in the freely moving subject.

Controls for CS specificity and pseudoconditioning. In two rats, the CS and UCS were applied independently in a random sequence to determine whether temporal contiguity between CS and UCS was necessary for the establishment of "a response" to the CS. An additional test for the specificity of the CS was to substitute an 80 dB tone (3000 Hz) for the light and test for transfer in rats previously conditioned to the light.

Data analysis. The EEG data were recorded on magnetic tape. After analog to digital conversion, discrete 5-second epochs taken before, during and after each trial were analyzed by means of a fast Fourier analysis carried out by a PDP 11-45 computer. Behavior in the presence of the light before and after conditioning was monitored on videotape.

RESULTS

Rapid development of a classically conditioned theta response can be seen in sample recordings obtained from rat 457 (Figures 1 and 2) and in a spectral analysis of this rat's hippocampal bioelectric activity (Figure 3). Samples of 5 seconds length were taken immediately before the CS, 5 seconds during the CS, during the UCS, and 5 seconds after the UCS. Each spectrum in Figures 3 and 4 is an average of the indicated number of epochs for that session. Before conditioning, the CS had no discernible effect on the hippocampal activity. This can be seen in both Figure 1 (top, trial 1) and in Figure 3 (A versus D). When the UCS was applied to the hypothalamus, the immediate appearance of a 12-Hz synchronous pattern was seen at the hippocampal recording sites. (See Figure 1 (UCS interval) and Figure 3B.) This pattern was followed by a poststimulation theta response of 8 Hz (Figure 1, post-UCS response interval and Figure 3C). Rat 457 began to show an altered hippocampal response to the light by the middle of the second training session. By the end of session 3, the response to the CS was an instantaneous appearance of synchronous 8-Hz theta persisting throughout the CS interval, as can be seen in the bottom tracing of Figure 1. This progressive frequency shift associated with training can be seen quantitatively in Figure 3D-G. Note the similarity of the fully developed CR spectrum (G) to that of the late UCR spectrum (C) in Figure 3.

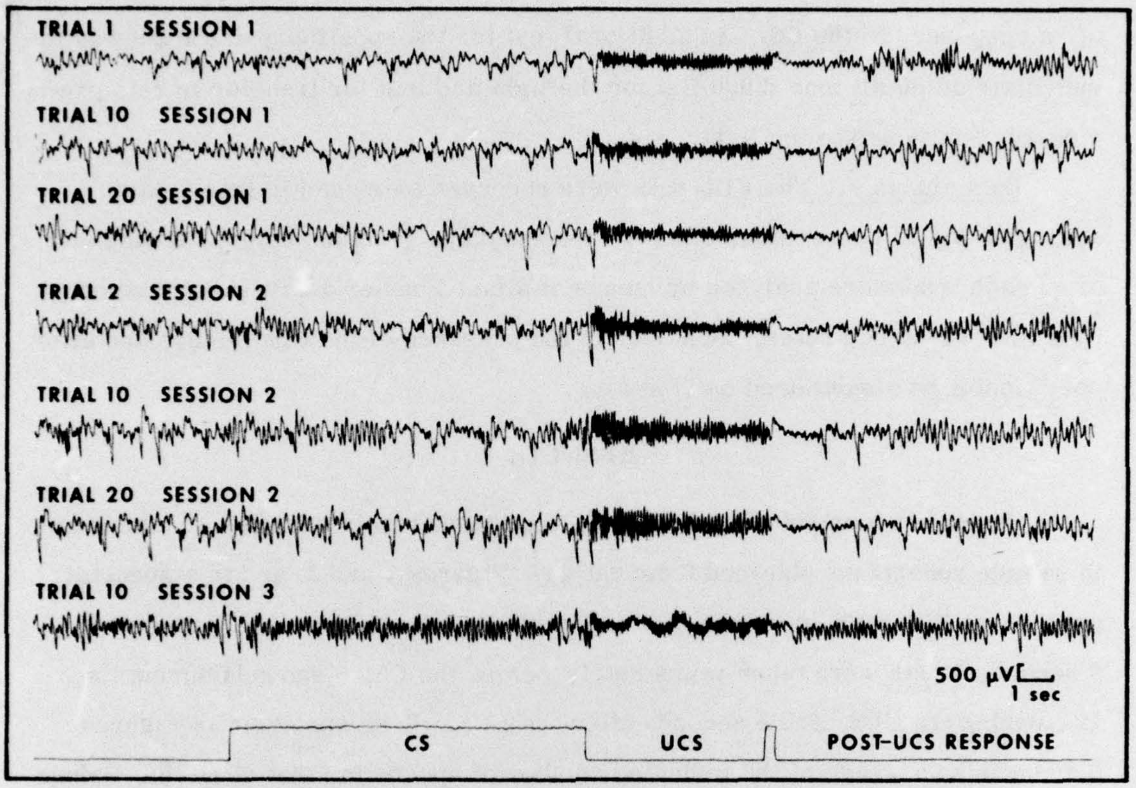

Figure 1. Acquisition of a classically conditioned hippocampal theta response (rat 457)

Tests of extinction and reconditioning for rat 457 are shown in Figure 2 and in Figure 3, sections H and I. Extinction of the response was obtained by trial 15 of the first extinction session (session 4). The response was reestablished in two reconditioning sessions (lower trace, Figure 2). These changes are reflected in the spectra of Figure 3H, I. Note that the extinction spectrum, Figure 3H, is very similar to the spectrum obtained in the 5-second epoch preceding the CS (Figure 3A) and to the spectrum obtained when the CS was presented alone, before conditioning (Figure 3D).

Rat 457 was the fastest to acquire the conditioned response, extinguish and recondition. It is interesting to compare the development of a conditioned EEG response in this subject to that of rat 499, the slowest of the group. Spectra of

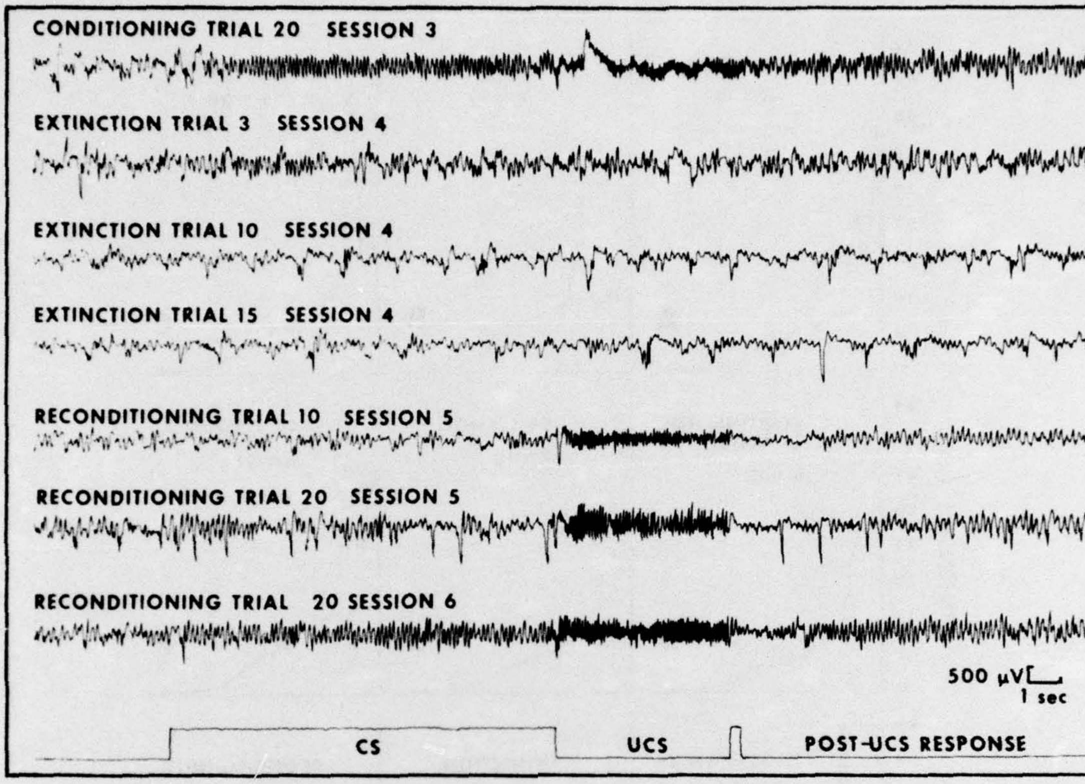

Figure 2. Extinction and reconditioning of a classically conditioned hippocampal theta response (rat 457)

the latter animal are shown in Figure 4. Despite the difference in acquisition rate, it is apparent from Figures 3 and 4 that the first change as a result of conditioning is a marked reduction in power for the frequencies below 8 Hz, followed by progressive development of a distinct peak in the frequency spectrum at 8 Hz. With extinction, the lower frequencies return but disappear with reconditioning. A pseudoconditioning procedure proved ineffective in influencing the hippocampal EEG in the presence of the CS. Transfer tests showed that when an auditory stimulus was substituted for the CS, the CR was not evoked. In addition, a layoff for as long as 7 weeks does not affect performance of this habit.

Behavioral findings. On 70 percent of the nonsuccinylcholine test trials, conditioned theta was associated with a very slow, deliberate locomotor response

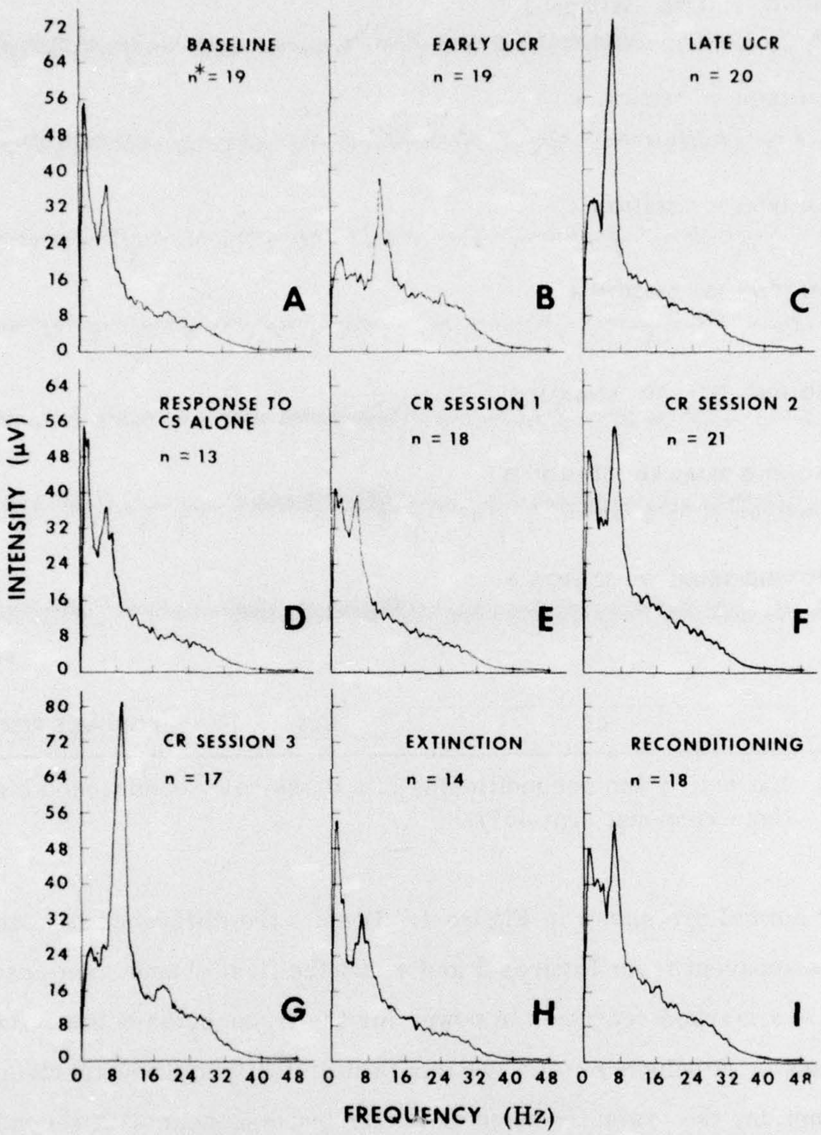

Figure 3. Average frequency spectra on development of a conditioned hippocampal theta response for rat 457 (succinylcholine). * Each spectrum is an average of the number of trials indicated for each condition.

that started with the onset of the CS and persisted throughout the CS interval. As soon as the UCS was triggered, the subjects exhibited a much more vigorous "forced" running response that was very stereotyped.

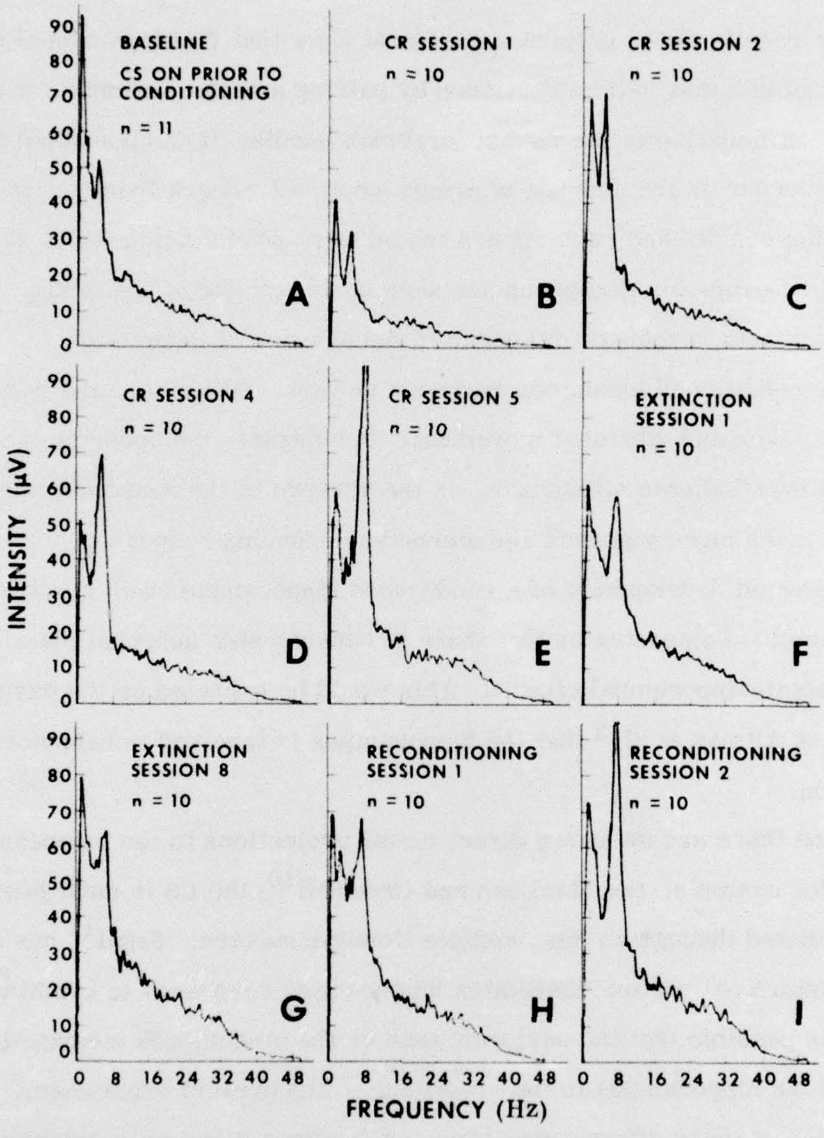

Figure 4. Average frequency spectra on development of a conditioned hippocampal theta response for rat 499 (succinylcholine)

The theta response to the CS on the 30 percent of the trials when no movement was evident was identical in frequency and amplitude to movement related conditioned hippocampal theta responses.

DISCUSSION

The results of the present experiment show that (1) hippocampal theta responses can be classically conditioned by pairing a neutral stimulus with high frequency stimulation of the medial forebrain bundle; (2) hippocampal theta patterns can occur in the absence of proprioceptive feedback from active muscles; (3) when the conditioned subjects are tested when not immobilized or physically restrained[3] locomotor responses are seen on 70 percent of the trials; however, conditioned theta responses did occur in the absence of locomotion.

The conditioned locomotor response is slow, deliberate, and is associated with head, neck and vibrissal movement. In contrast, the unconditioned response to lateral hypothalamic stimulation, in the absence of the neuromuscular blockade, is a much more vigorous and stereotyped running response.

The rapid development of a conditioned hippocampal theta response to a visual stimulus demonstrates that there is considerable neuronal plasticity in the medial septal-hippocampal circuit. This would be expected on the basis of the proposal of Altman et al.[2] that the hippocampus is involved in behavioral maturation.

Since there are no known direct visual projections to the hippocampus and septum (for example, see MacLean and Creswell[10]) the CS is most probably being mediated through an intermediate limbic structure. Segal[11] has suggested that the entorhinal cortex "facilitates hippocampal responses to conditioned stimuli". It is possible that the perforant path or the cingulum is mediating the response of the hippocampus to the visual cue in the present experiment. Selective interruption of these afferent projections after the CR has been established will provide an answer to this intriguing question.

Because the conditioned response is time locked, and can be localized to a distinct cellular layer in the hippocampus,[6] classically conditioned hippocampal theta responses, as established by this procedure, should provide a valuable method for studying cellular alterations associated with learning and memory in a distinct brain region.

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