The effects of hippocampal electrographic seizures on one-way active avoidance and visual discrimination in rats: State-dependent effects*

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Rats were trained on a one-way jump avoidance task prior to and following electrically induced hippocampal electrographic seizures. Rats trained when normal did not perform a jump avoidance response following seizures, but avoidance recovered within 24 h. When rats were trained immediately following seizures, the response did not transfer to the normal condition. Performance on two-choice brightness and pattern discriminations were not disrupted by seizures. It is suggested that seizures disrupt normal hippocampal function in the control of movement and that disruption and hence "state-dependent" effects occur on tasks in which the primary measure of performance involves movement.

A number of studies have shown that the electroencephalographic activity (EEG) of the hippocampus is correlated with movement (Bland & Vanderwolf, 1970; Vanderwolf, 1969, 1971; Whishaw & Vanderwolf, 1970). During voluntary movements (walking, running, jumping), rhythmical slow activity (RSA or theta) occurs, while during automatic activities (facewashing, chewing food), a large-amplitude irregular activity is recorded from the same electrode sites. MacLean, Flannigan, Flynn, Kim, & Stevens (1955-1956) have demonstrated that both electrical stimulation and drug administration which disrupt the normal activity of the hippocampus also disrupts the performance of animals on learned tasks. The object of the present study was to replicate the kind of effect demonstrated by MacLean et al (1955-1956) and to compare the severity of the effects on different kinds of tasks. Studies have been made on the learning and performance of animals during afterdischarges produced by hippocampal electrical stimulation (Delgado & Sevillano, 1961; Flynn & Wasman, 1960). A second purpose of the present study was to determine if animals could learn a response during the suppressed hippocampal EEG condition following the termination of afterdischarges.

METHODS

Experiments were performed upon 32 male hooded rats weighing 300-400 g. Three intracerebral electrode pairs were implanted in each rat: one pair each in the right and left

hippocampus plus one pair in the anterior neocortex. Hippocampal electrodes were implanted 3 mm posterior to bregma, 3 mm lateral to the midline, and 3.5 mm ventral from the surface of the skull, with the dorsal skull surface horizontal. An electrode pair (Plastic Products, No. MS-303) consisted of two 0.010-in.-diam steel wires insulated to within 0.5-1.0 mm; one tip was cut 1.0 mm shorter than the other. Steel screws (one serving as a ground) and dental cement were used to fix the electrodes firmly to the skull. Behavioral testing began following a 1-week recovery period. At the conclusion of the experiments, the rats were sacrificed under deep pentobarbital anaesthesia and the brains removed. Following fixation in formalin, brains were sectioned at 40 microns while frozen and stained with thionin.

Electrical activity was monitored with a Gilson polygraph, and electrical stimulation was delivered with a Heathkit Impscope stimulator. Electrical stimulation consisted of a 0.3-sec train of 10-V 60-Hz 3-msec-duration monophasic pulses. The activation of electrical stimulation was recorded on the chart with the event marker pen. Recordings were made from one hippocampal electrode and from the neocortical electrode, while stimulation was delivered through the contralateral hippocampal electrode.

Avoidance Test

The active avoidance apparatus was an unpainted plywood box, 12 x 12 in., with walls 11 in. high. The floor was a grid of 1/8-in. stainless steel rods set about 1/2 in. apart and could be electrified by a Harvard

Inductorium powered by a 1.5-V dry cell battery. A plywood shelf, 2 1/2 in. wide, ran around the outside of the apparatus 1/2 in. from the top. Training consisted of placing an animal in the apparatus and administering footshock after 5 sec had elapsed. Administration of footshock continued at intervals of about 1/2 sec until the animal escaped from the apparatus or until 60 sec had elapsed. An animal could escape by jumping up, grabbing the edge of the box, and pulling itself onto the outside ledge of the box. If the animal failed to escape, it was placed on the ledge. In either case, the animal remained on the ledge for 30 sec until the next trial began. Daily sessions of training were given in which an animal was required to make five avoidance responses. Criterion consisted of the rat making five consecutive avoidances on the first five trials of any training session.

Discrimination Tests

The two-choice visual discrimination apparatus was similar to that described by Thompson & Bryant (1955). For pretraining, animals were given a 15-min adaption to the apparatus in squads of four. Following the adaption, the animals were trained singly to run from the start to goalbox to avoid 1-mA footshock. Animals were allowed 5 sec to leave the startbox and 20 sec to clear the choice chamber before footshock was administered. Once the animals were avoiding, two unpainted plywood doors were placed ajar in the opening to the goalbox and gradually brought to a fully closed position with successive trials. Pretraining was usually complete within 15 trials when the animals were knocking down the fully closed doors. On the following day, training on a discrimination problem began, and on this and subsequent days, the animals were given 10 trials per day with a 30- to 60-sec intertrial interval. Training was complete when animals reached a 9/10 criterion on one day.

Stimulation Procedure

In all tests in which electrical stimulation was applied to the hippocampus, stimulation resulted in an electrographic seizure. Immediately after the period of stimulation, large-amplitude afterdischarges were recorded in the hippocampus and were followed by a period during which hippocampal EEG was depressed. Testing and training began immediately following the termination of afterdischarges.

General Results

Electrical stimulation resulted in three successive phases of hippocampal EEG change (Fig. 1): (1) high-amplitude afterdischarges which lasted from about 10 to 60 sec; (2) suppressed hippocampal EEG

*This research was supported by Grant 28-1108 from the University of Lethbridge, Lethbridge, Alta., Canada

Fig. 1. Hippocampal electrographic seizure. Note: (1) rhythmic slow activity during walking prior to electrical stimulation, (2) large amplitude afterdischarges during which the rat sits motionless, and (3) suppressed EEG stage during which rat begins to move again.

which lasted for about 1-5 min in different animals; (3) recovery of amplitude which was complete in about 15 min. Abnormalities in the EEG could be seen as long as 1 h later. At this time there was a greater incidence of fast activity and less slow activity during immobility and automatic activities such as grooming, facewashing, or chewing food. During voluntary movements such as walking or rearing, 6- to 10-Hz rhythmic slow activity was recorded and was comparable in amplitude and frequency to preictal EEG.

During afterdischarges, animals were usually immobile but did not show behavioral signs of convulsion. An occasional animal would groom during the initial occurrence of afterdischarges or walk rapidly for a short distance. With the termination of afterdischarges, the animals would move about, sniffing, turning, and rearing.

To visual inspection, the activity of the neocortex returned to normal at the termination of afterdischarges and did not show the depressed activity typical of the hippocampal EEG.

Both the pattern of EEG activity (the occurrence of rhythmic slow activity during voluntary movement) and histological verification indicated that the electrodes were located in the hippocampal-dentate gyrus complex.

EXPERIMENT 1

It has previously been reported (Whishaw, 1971) that rats which are well trained on a jump avoidance do not avoid following a stimulation-induced hippocampal electrographic seizure. The purpose of Experiment 1 was to replicate the results and to determine the time required for the avoidance response to recover. Eight rats (four with implanted electrodes) were trained on the jump avoidance response until they met criterion. On the following day, all the rats were tested. The implanted rats received an electrographic seizure, and all were given five test trials at intervals of 10 sec, 1 h, and 24 h. During the test

sessions, no footshock was administered and the rats were allowed 60 sec in which to avoid. The same animals were then trained with footshock in both preictal and postictal conditions until criterion was met in both conditions. When this procedure was completed, animals were given 1 further training day on which the seizure was induced from the hippocampus contralateral to that previously used.

Results

The effects of an electrographic seizure on a well-learned active avoidance response are shown in Table 1. It can be seen from Table 1 that performance was severely disrupted at 10 sec but had recovered by 24 h. Latencies for control and experimental animals were significantly different at 10 sec and 1 h (10 sec, $U = 0$, $p < .05$; 1 h, $U = 1$, $p < .05$) but were not significantly different at 24 h.

Attempts to retrain the animals in the postictal phase were successful. Animals made a mean of 5.5 errors on original learning, 1 error during preictal training, but made 12.3 errors acquiring the avoidance response in the postictal condition. This difference between original and postictal training was significant ($U = 0$, $p < .05$). Thus, the avoidance response does not transfer from the preictal to postictal stage but can be relearned in the postictal stage.

Stimulation of the hippocampus contralateral to that stimulated during training resulted in a seizure; however, there was no affect on performance, since all animals in the postictal condition avoided footshock on the

first five test trials. Thus, once an animal has learned in the postictal phase, the response is transferable to the postictal phase of electrographic seizures induced from other hippocampal loci.

EXPERIMENT 2

The results of Experiment 1 indicated that rats which had met criterion on an avoidance response when normal could relearn in the postictal phase of a seizure. The object of Experiment 2 was to see if naive rats could learn the avoidance response in the postictal stage. In addition, an attempt was made to determine if the postictal learned response would transfer to the normal condition. Four rats with implanted electrodes received an electrographic seizure and were then trained; four control rats were given training only. On the day following the first training session and on subsequent days, the rats were given five test trials (no footshock) prior to the seizure to see if they would avoid. The animals were allowed 60 sec in which to avoid on each trial. When the animals reached criterion in the postictal stage, they were retrained without preceding seizures.

Results

The experimental animals were able to learn the avoidance response in the postictal condition; however, they made significantly more errors than did control animals (Experimental = 39 vs Control = 12.5, $U = 0$, $p < .05$). Prior to the day on which they met criterion, none of the experimental animals avoided when normal within the 60-sec test periods. On the day on which they met

Table 1
Mean Pre- and Postictal Latencies in Seconds for Performance on an Active Avoidance Task at Different Intervals Following an Electrographic Hippocampal Seizure

Group	Original Training	10 Sec	1 H	24 H
Experimental (N = 4)	1.3	51.6	20.7	9.5
Control (N = 4)	1.6	2.1	1.8	2.0

Fig. 2. The mean latency of control ($N = 4$) and experimental ($N = 4$) rats on a jump avoidance task. Experimental rats were trained first in the postictal condition and then in the preictal condition. Each point summarizes the mean latency of two daily trials.

criterion, two animals avoided when normal; the other two required three and one errors, respectively, to meet criterion.

Thus, animals could learn in the postictal stage without preictal training, and the response transferred with few or no errors to the normal condition.

EXPERIMENT 3

In Experiment 2, animals trained in the postictal condition of a seizure began to avoid as soon as the avoidance response was acquired in the postictal stage, or else they learned the avoidance response in only a few errors when trained in the preictal stage. During testing in Experiment 2, it was noted that the hippocampal EEG often recovered its amplitude prior to the completion of training. Animals, therefore, received part of their training when the hippocampal EEG was flat and part of their training when the hippocampal EEG was recovering amplitude. The object of Experiment 3 was to train the animals in the postictal phase only when the EEG was flat. Therefore, eight animals (four with implanted electrodes) were given two trials of training each day until a criterion of five successive avoidances were obtained. The animals with implanted electrodes received training after the seizure was induced.

Once these animals reached criterion in the postictal phase of training, they were retrained without preceding seizures.

Results

A summary of the results of Experiment 3 is given in Fig. 2. It can be seen from Fig. 1 that daily training on the jump avoidance task during the period when the hippocampal EEG was flat resulted in an impaired acquisition of the avoidance task as compared to performance of control animals (experimental errors = 26.5 vs control errors = 8.0, $U = 0$, $p < .05$). When the animals were retrained without preceding seizures, they required as many trials and errors as did the control animals (mean errors control = 8.0, experimental = 10.5, $U = 9$, $p < .05$). Thus, the results of Experiment 3 indicate that the acquisition of an avoidance response in the postictal stage of a seizure can be considered state-dependent when compared to acquisition in the normal condition.

EXPERIMENT 4

The results of Experiments 1-3 indicated that learning in a jump avoidance task is state dependent in both the preictal and postictal stages of an electrographic seizure. The object of Experiment 4 was to determine if similar state-dependent effects could

be obtained on a more complex task such as a two-choice visual discrimination. Thus, eight rats (Group I) which had shown state-dependent effects in the jump avoidance task were trained on a black vs white discrimination task. Four animals were trained to run to the white card, and four were trained to run to the black card. On the day following completion of training, the animals were given 10 test trials, a seizure, and then 10 training trials. This procedure was continued daily until a criterion of 9/10 correct in the postictal stage was obtained. The procedure was then repeated on a horizontal vs vertical pattern discrimination (vertical stripes positive). Eight naive rats (Group II) were then trained on the discrimination tasks and retrained following seizures in the same way. When this group had completed testing on the visual discrimination tasks, they were trained to criteria on the jump avoidance task in the preictal condition and then retrained in the postictal condition.

Results

The results of Experiment 4 are summarized in Table 2. The animals in both Groups I and II required fewer postictal errors to reacquire the brightness or pattern discriminations

than was required in original training. The animals in Group II which had not previously experienced electrographic seizures did make more errors than Group I animals on both discrimination tasks in the postictal stage. This difference was not significant on the brightness task ($U = 16$, $p > .05$), but was on the pattern task ($U = 11$, $p > .05$). One explanation of the difference in errors made by the two groups is that Group II animals were more disturbed by the electrographic seizures than Group I animals. When placed in the apparatus following an electrographic seizure, all animals required footshock to initiate the discrimination response. Group I animals reacted violently to the footshock, raced around the box, and subsequently made errors. Animals which had experienced electrographic seizures previously responded less violently. Thus, errors may not have been due to a failure to discriminate, but rather may have been due to interference effects. When animals in Group II were subsequently trained on the jump avoidance task, they required more errors and trials to learn in the postictal training stage than in the preictal stage (mean preictal errors = 4.6; trials = 18.7; mean postictal errors = 34.4; trials = 77.7; $U = 1$, $p < .05$). Thus, the state-dependent effect was obtained from these animals in a condition similar to that reported in Experiment 1, but not on the visual discrimination tasks.

DISCUSSION

The results indicate that learning occurring prior to a hippocampal electrographic seizure is partly independent from learning during the suppressed EEG activity of the postictal stage. On an active avoidance response which required rats to jump out of an 11-in. box within 5 sec in order to avoid footshock, learning appeared to be state dependent (Overton, 1968). Such state-dependent effects were not obtained on a two-choice pattern or brightness visual discrimination.

Similar state-dependent effects on a conditioned emotional response have previously been reported following bilateral stimulation of the amygdala (Lidsky, Levine, Kreinick, & Schwartzbaum, 1970). Major differences between the Lidsky et al study and this is that bilateral stimulation was used in the former study and behavioral as well as electrographic seizures were produced. Amygdaloid seizures also impaired performance as long as 24 h after the seizure; in the present study, it was demonstrated that performance was normal 24 h after the hippocampal seizures. The demonstration of

Table 2
Mean Errors on Brightness and Pattern Discriminations in Preictal and Postictal Electrographic Seizure Conditions

	Task	Initial Training Mean Errors	Preictal Test Mean Errors	Postictal Training Mean Errors	Initial vs Postictal Training
Group 1	Brightness	25	1	1	$U = 0$, $p < .05$
	Pattern	11	1	1	$U = 0$, $p < .05$
Group 2	Brightness	21	1	3.5	$U = 0$, $p < .05$
	Pattern	17	1	5	$U = 3$, $p < .05$

state-dependent effects following hippocampal seizures unaccompanied by behavioral seizures reinforces the point made by Lidsky et al, that such effects may have important implication for studies in which performance is altered by treatments such as electroconvulsive shock and drugs. Electroconvulsive shock produces hippocampal afterdischarges followed by suppressed activity (personal observation), and injections of puromycin which do not affect learning but disrupt performance 3 h later (Barondes & Cohen, 1966, 1967) also produce a suppression of hippocampal EEG (Cohen, Ervin, & Barondes, 1966). There are probably differences in the effects of various treatments in different tasks and experimental species. For example, electrographic seizures did not impair performance by rats on a two-choice visual discrimination in this study, but Adey & Dunlop (1960) obtained impairment on a similar problem when cats were injected with cyclohexamines which produced abnormal hippocampal EEG.

One interpretation of the finding that well-trained rats will not avoid in the postictal condition is that the electrographic seizure impairs the movement ability of the rat. Such impairment could be caused by a descending barrage of impulses from the hippocampus to the hypothalamus via the fornix. However, following the termination of afterdischarges, rats usually begin to move about spontaneously, often becoming quite active. If an electrographic seizure is induced in a rat running in a motor-driven wheel, running actually appears to be improved during the suppressed EEG stage (Whishaw, 1971). Hyperactivity following limbic system seizures has also been reported in other studies (Delgado & Sevillano, 1961; Lidsky et al, 1970). Finally, the interpretation that the seizure results in a general impairment of motor ability is not supported by the finding that animals will learn to perform the jump avoidance response in the postictal condition.

A second possible interpretation of the effects reported here is that the electrographic seizure spreads to other

areas such as the neocortex and disrupts their function. Afterdischarges were recorded in the anterior neocortex and have been recorded in a number of limbic sites (Creutzfeldt & Meyer-Mickeleit, 1953; Kaada, 1951). Whether such widespread propagation implies active involvement is uncertain. Involvement seems unlikely, since behavioral convulsions are not observed, and electrical activity in these structures appears normal with the cessation of afterdischarges. It seems possible, therefore, to consider the state-dependent effects a result of hippocampal dysfunction.

It has been suggested that the induction of seizures either electrically or chemically in the hippocampus result in a "functional ablation" (MacLean et al, 1955-1956). Olton (1970) has presented evidence which does not support this interpretation. In the latter study, penicillin injections were used to produce functional abnormalities characterized by spike discharges. In agreement, Flynn & Wasmann (1960) report that learning (but not performance) can occur during hippocampal afterdischarges. Nevertheless, it may be important to distinguish between hippocampal EEG dysfunctions which involve afterdischarges and those which involve complete suppression of EEG activity. The former may be conducted to other neural structures causing such effects as arrest of movement (Delgado & Sevillano, 1961; Flynn & Wasmann, 1960), while complete suppression may be more similar to complete ablation. The results of the present study, obtained during the suppressed EEG condition, support the functional ablation hypothesis. Tendencies toward hyperactivity during suppressed EEG may be similar to hyperexploratory activity following hippocampal lesions (Glickman, Higgins, & Isaacson, 1970; Kim, Choi, Kim, Chang, Park, & Kang, 1970). Also, findings of no deficit on a visual discrimination following electrographic seizures is similar to that reported to follow hippocampal ablation (Thompson & Massopust, 1960).

Studies on hippocampal EEG

suggest that the hippocampus is part of a movement-triggering mechanism (for reviews, see Vanderwolf, 1971; Vanderwolf, Bland, & Whishaw, 1971). During the initiation of a voluntary movement such as jumping out of a box, hippocampal RSA precedes the jump, reaches its highest frequency at the point of the jump, and then decreases in frequency and amplitude as the animal comes to a stop at the edge of the box. Stimulation of the hypothalamus, which produces RSA, can make an animal jump out of the box, while stimulation of the dentate gyrus, which suppresses RSA, can arrest initiation of such a movement. The results of the present study show that a seizure which disrupts normal electrical activity disrupts the concomitant behavioral response. Although RSA also occurs with movement in a visual discrimination task, the performance measure in this task is the selection of the appropriate card, not whether or not footshock is required to initiate the response. A similar dissociation effect has been found following medial thalamic lesions which also disrupt RSA. Medial thalamic rats are unable to perform an active avoidance response, but show no deficit in a three-choice visual discrimination (Vanderwolf, 1971).

Whether or not the dissociation between performance in the jump avoidance and visual discrimination task could be accounted for in terms of other factors is uncertain. Although the jump avoidance task could be considered easier than a visual discrimination task, the rats in the visual task still required footshock following seizures to initiate movement, but they made the correct choice. Amount of prior practice would not seem to be a factor, since in an earlier study (Whishaw, 1971), a similar disruption of jump avoidance was observed in rats which had

received hundreds of pre-seizure trials over a period of weeks. In summary, the effects of seizures on performance of a jump avoidance response may be due to a dysfunction of a diencephalic-hippocampal movement-triggering system.

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