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HIPPOCAMPAL ACTIVITY DURING AVOIDANCE BEHAVIOR IN THE RAT

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Hippocampal Activity During Avoidance Behavior in the Rat

Frederick J. Brenner

This thesis submitted in partial fulfillment for the
Doctorate of Philosophy Degree in Psychology at the
University of Massachusetts, Amherst.

May, 1963

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Introduction

Many investigators have systematically probed the functional significance of the hippocampus. Evidence seems to indicate that this phylogenetically old cortex may be the locus of integration for such complex processes as instinct, memory and learning. For instance Penfield and Milner (1958) have reported that unilateral lesions in the dominant hippocampal formation caused a temporary memory loss of verbal material in man. They further indicated that bilateral destruction of the hippocampus caused a permanent loss of memory for recent events. Furthermore, Kaada, Pasmussen, and Kveim (1961) found that bilateral and unilateral hippocampal lesions in rats produced significantly more errors during learning and greater loss in retention of a maze habit than either operated or non-operated controls. In addition, it has been demonstrated by Kimura (1958) that bilateral lesions in the posterior part of the dorsal hippocampus of the rat interfered with the acquisition and retention of an avoidance response in the straight runway. On the other hand, Isaacson, Douglas, and Moore (1961) found that rats with extensive, but subtotal, bilateral lesions of

the hippocampus showed lower latencies in both learning and extinction trials and reached the learning criterion of an avoidance response sooner than did control animals when tested in a shuttle box. Porter, Conrad, and Brady (1959) trained monkeys to self-induce brain stimulation and found that with electrodes placed in the posterior part of the hippocampus, self-stimulation yielded seizure-like oscillographic patterns of sharply rising high amplitude waves followed by no recordable activity. However, after the termination of these patterns and a return to a normal EEG, lever-pressing continued to occur at a high rate. These investigators found, as did Feldman (1961) in the rat, that during post-ictal depression when lever-pressing could not elicit seizure activity there was a corresponding decrease in lever-pressing. This suggested that the animals experienced some reward associated with hippocampal seizures. It is worth noting here that young human patients that are subject to petit mal seizures will sometimes intentionally spread fingers apart and wave hands in front of their eyes while looking at bright lights in order to induce seizure activity.

In a paper more directly related to the present study, Adey, Dunlop, and Hendrix (1960), using an electrophysiological method, discovered a neural correlate of approach behavior in the hippocampus. By correlating EEG patterns from the hippocampus with approach behavior in the cat, they

were successful in determining that the characteristic 5-7 cps theta wave was present immediately before and during "goal directed behavior" in a runway task. Also, Adey and Dunlop (1960) studied the hippocampal effects of the analgesic cyclohexamines, Sernyl and Cl-400, which gave rise to psychic disturbances in man such as hallucinations and echolalia. When injected intraperitoneally in cats these agents abolished the 5-7 cps wave in the hippocampus and induced seizure spiking. At the same time these cyclohexamines interfered with the goal-directed behavior in their subjects in that the animals no longer traversed the runway for a food reward. During recovery from the drug, the theta rhythm reappeared with a return of approach behavior. These findings support their contention that the hippocampal theta rhythm is a correlate of goal-directed motor activity.

There seems little doubt that EEG frequencies of 5-7 cps of approximately sinusoidal form can be detected at the hippocampus. These so-called theta wave patterns have been found in the guinea pig (Green and Morin, 1952) and in the cat, monkey and rabbit (Green and Arduini, 1954). However, their exact relationship to any behavioral end point is not quite clear. For, in contrast to Adey's hypothesis that the hippocampal slow waves are a correlate of goal-directed behavior, Green and Arduini (1954) had earlier demonstrated that they can be produced by purely sensory input. These

latter investigators further suggested that there was a "reciprocal or inverse relationship between the activity of the hippocampus and the cortex" In particular this relationship was characterized by synchronization of the hippocampus and desynchronization of the cortex, and furthermore, that both of these phenomena are related to attention or alerting of the organism. Moreover, they stated that these neuro-electrical phenomena could be initiated by sensory stimulation of all modalities.

On the other hand some investigators (Grastyán, Lissák, Madarász, and Donhoffer, 1959) have considered the hippocampal slow wave pattern as a correlate of an orienting reflex (OR) of the Pavlovian type. It should be noted that in both Pavlov's work and in current Russian psychophysiology the OR has been studied as a response to sensory stimuli. Razran (1961) in a recent review of the Russian literature characterized the OR as an "investigatory" attitude or as a temporary response to novel stimuli. Razran further explained that although the stimulus need not be strange to the animal, at least its relationship to the conditioning situation had to be novel for the animal. Also, the OR adapts quickly, and in classical conditioning decreases as the conditioned response increases. Razran further pointed out that evidence published in the Russian literature indicated that the OR has electrophysiological correlates in the cortex (characterized by desynchronization) and sub-

cortical areas, as in the hippocampal which typically shows synchronous activity.

The above discussion leads to the conclusion that the theta rhythm is related to behavior in some way, either as a correlate of a planned motor act (Adey et al, 1960), as a correlate of the Pavlovian orienting reflex (Grastyán et al, 1959) or as a correlate of arousal or attention (Green and Arduini, 1954).

To complete these introductory remarks, a brief description of the neuroanatomy of the hippocampus and related structures follows. The hippocampus is composed in part of primitive cortex (archipallium), histologically distinguishable from the cortex of most recent origin (neopallium). This primitive cortex is characterized by the absence or poor development of the supragranular layers, the ending of the afferent plexus in the superficial layers, and the relative paucity of cells of short axon, the so-called Golgi Type II cells (MacLean, 1959). Although no direct sensory afferents to the hippocampus are known, there are indirect connections to the hippocampal formation from virtually all parts of the brain stem and cortex (Adey, Dunlop, and Sunderland, 1959; Papez, 1958).

In his comprehensive discussion of the mammalian hippocampus Green (1960) draws attention to the connections between the hippocampus and the ascending reticular activating system (ARAS). These connecting fibers reach the hippo-

campus via the tegmentum and hypothalamus. In addition to these connections from reticular formation and hypothalamus there are afferents from the amygdala, septum, and entorhinal cortex. Besides these extrahippocampal afferents, the hippocampal commissure serves to conduct impulses from one hippocampus to that of the opposite side.

The major efferent projections of the hippocampus have been described for the rat by Nauta (1956). He reported that the fibers leaving the hippocampus via the fornix terminated in the septum, the nucleus of the diagonal band, the preoptic nucleus of the hypothalamus, and the mammillary body. Moreover, he described direct pathways to the hypothalamus and the thalamus. Nauta further described an indirect pathway from the hippocampus to the midbrain via the mammillotegmental tract and medial forebrain bundle.

In particular, the present study was an attempt to discover if in the dorsal hippocampus of the rat there were characteristic theta EEG patterns which correlated with responding in the nondiscriminative Sidman Avoidance Task (SAT). The task typically consists of the subject avoiding short grid shock (ca. 0.5 sec) through a floor grid in a Skinner-box by making a periodic lever-press-and-release. Sidman (1953a, 1953b, 1954, 1955) has reported that when this response has stabilized, the response rate (number of responses per minute) does not vary from session to session more than 0.1 responses per minute. Specifically, although

the animals respond in a random fashion within each day's session, the response rate is virtually the same from day to day. The SAT is designated as nondiscriminative because there is no specific exteroceptive warning signal which serves as a cue to elicit the response; that is, there is no "proper" time to respond, as for example within 5 seconds after a warning signal. Now then, if one considers that the hippocampal slow wave is an accompaniment of the Pavlovian orienting reflex or even a correlate of mere sensory input, then if there were no external warning signal, the EEG records should be devoid of the 5-7 cps theta rhythm. It was the major hypothesis of the present study that hippocampal theta rhythm would appear in rats trained in a Sidman avoidance task in which no warning signal was used. A result such as this would seem to favor an arousal explanation of the hippocampal theta rhythm. Furthermore, if the theta rhythm indeed occurred, it was planned to establish whether or not it was necessary for the performance of an avoidance task by altering it by means of electrical brain stimulation. This was done by simultaneously stimulating the hippocampus and recording the electrical change by means of a contralateral electrode. The advantage of this technique over previous attempts was the greater control over electrical brain stimulation than was possible with a drug or lesion technique. Also, effects of electrical brain stimulation on the EEG can be immediately and accurately recorded in con-

trast to drug or lesion effects. Previous evidence (Adey et al., 1960) led to the prediction that there would be shifts from the normally asynchronous EEG pattern between lever presses to a 5-7 cps pattern correlated with presses, and further, that changes in the EEG pattern due to electrical brain stimulation would correlate with changes in lever-press responding.

Method

Subjects

Fourteen mature male Sprague Dawley rats ranging in age from 120-180 days were used as subjects. Six of these were unoperated and used to standardize training procedures in the Sidman avoidance task and to obtain estimations of response rates. The remaining 8 animals each had 2 bipolar electrodes stereotaxically implanted in deep brain structures. The experimental group consisted of 6 of these implanted animals all of which had at least one electrode in the dorsal hippocampus. Two other animals designated as operated controls had bipolar electrodes stereotaxically placed in brain areas other than the hippocampus; one animal had one electrode in the lateral ventricle and the other in the corpus callosum. The other operated control animal had one electrode in the amygdala and a second electrode in the putamen.

Apparatus

The apparatus for the behavioral task consisted of a Skinner-box with a grid floor wired to a Grason-Stadler shock source and scrambler unit (#E110G4GS). The Skinner

box was placed in an electrically shielded, sound-deadened room. To further reduce possible electrical interference which would contaminate EEG recording, a lever-press interrupted a beam of light activating a photoelectrical cell. This photoelectrical cell activated a circuit which was programmed to delay a 0.5 sec 2 ma ac shock for 30 seconds only after the last lever-press and release. With this program the delays were not cumulative. Also, the apparatus provided records of lever-pressing behavior.

The EEG potentials were recorded with a 5P5 Grass EEG pre-amplifier and a Model SE driver amplifier which attenuated a Grass Model 5 oscillograph. The bipolar electrodes were made of two stainless steel enamel-coated wires twisted together and bared at the tips and set in a miniature socket as described by Valenstein, Wodos, and Stein (1961). The greatest distance across both wires of these bipolar electrodes was .026 inches. Special cable which reduces wire flexion artifacts was used within the Skinner-box to connect the brain electrodes to the recording and stimulating system.

These cables leading from the brain electrodes were connected to a slip ring (Lehigh Valley Electronics # 1373) fastened to the ceiling of the rat chamber and from there the leads passed to a pair of relay contacts. This relay permitted the experimenter to switch from the recorder to the stimulator or from the stimulator to the recorder from

outside the experimental chamber. All brain stimulation was done with a Grass Model S4 stimulator.

Procedure

The 6 unoperated control animals were used to develop a rapid and efficient training technique for the SAT. This technique consisted of using a hand-operated switch to deliver short duration (110 V. ac, 0.5 sec, 2 ma) grid shocks to the feet of the animal to train the animals by successive approximation. At first shocks were given to goad the animal to move until it was in the vicinity of the lever. Next the short shocks were delivered until the animal pressed the lever. After a lever-press and release the animal was given a 30 second delay at which time another automatically programmed shock occurred unless lever-pressing intervened. Occasionally, the animals had to be goaded by using the hand-operated switch to induce more lever-pressing, but eventually the use of this switch was discontinued as the animals' performance improved. The subjects were trained in this fashion until they avoided at least 70% of the due grid shocks without the use of the hand-operated switch. All sessions were 5 hours in duration and all animals reached the avoidance criterion within 2 sessions.

Following the electrode implanting surgery the 8 animals implanted were rested for one week. The 6 animals that were supposed to have one or more hippocampal electrodes were then given a 5 sec biphasic electrical stimulation in

the hippocampal lead (100 cps, 1.0 msec pulses) increasing the voltage stepwise from 2 to 10 volts to try to elicit hippocampal seizure discharges. All hippocampus implanted rats showed the typical seizure patterns described by Liberson and Akert (1955); thus, it was assumed that the electrodes were accurately placed. The 8 implanted animals were then trained in the SAT as described above without lead wires attached to the animals. On the day after the avoidance criterion was reached the contacts of the implanted electrodes were connected to the lead wires leading to the recording or stimulating apparatus. After the subjects had reached the 70% criterion, EEG recordings were made periodically during the last 2 hours of the next session. On the following 2 sessions in addition to EEG recording, trains of biphasic electrical pulses were delivered to the hippocampus on one side while recordings were made from the other side. Brain stimulation (biphasic 3 and 6 cps at 1.0 msec duration) was used in an attempt to alter the hippocampal activity and disrupt the animal's avoidance behavior. The experimenter began these stimulations when he perceived a transition from irregular hippocampogram to the regular theta pattern. Since it was possible that errors in judgment and timing might be responsible for a lack of effects, a second series of stimulations was given in which the stimulation was initiated without regard to appearance of the theta rhythm. In the second series the

3 best performing animals (numbers 8, 10, and 15 which averaged over 80% avoidance) were stimulated with 1.0 min trains of 1.0 msec pulses at 3 and 6 cps. In either series the voltage was gradually increased from 1.5 volts to the subseizure tolerance level of the individual subject - - usually below 6 volts. These trains were initiated when the subject appeared quiet but were terminated if either seizure-like behavior or seizure spiking was observed on an oscilloscope monitor prior to the end of the one minute period of stimulation.

Following the completion of the experiment all 8 implanted subjects were sacrificed. The animals were first anesthetized with nembutal and then were perfused with 10% formalin. The whole brain was then removed and stained with thionin using a technique reported by Chang (1936). Microtome sections were made at 15 μ to find the deepest point of electrode penetration.

Results

SAT performance

All 14 animals were successfully trained to avoid 70% of the grid shock. The mean percent avoidance for the 6 unoperated control animals during the fourth and fifth hours of the last 4 sessions was 76.6% while that for the animals with hippocampal electrodes including sessions with brain stimulation was 80%. A t-test showed no significant difference between the 2 groups ($P > .10$). The 2 operated control animals had a mean percent avoidance of 71.4%.

Histology

Figure 1 shows the results of histological examinations of the recovered electrode implanted brains, and shows that the electrode placements were situated as intended. It shows that animals 7, 10, 12 had bilateral (bipolar) electrodes located in the dorsal hippocampal arch (plane 54; Krieg, 1946). Animal 8 had the right electrode in the hippocampus and the left in the lateral nucleus of the thalamus; animal 9 had the left electrode in the hippocampus with the right being in the corpus callosum; and number 15 had a left hippocampal electrode while the right was in the

Fig. 1. Representations of transverse sections of the rat brain after Krieg (1946) to show the depth and placement of the electrode tips. The black or open circles represent the position of the electrode tips. AM, amygdala; HIPP, hippocampus; LV, lateral ventricle; NL, lateral nucleus of thalamus; PUT, putamen.

RAT 13

RAT 14

RAT 15

RAT 7, 10, 12

RAT 8

RAT 9

lateral nucleus of the thalamus. The Figure also shows the electrode placement for the 2 operated controls; rat 13 had the left electrode in the putamen and the right in the amygdala. Rat 14 had the right electrode in the lateral ventricle and the left in the corpus callosum.

Neuro-electric correlates of non-discriminated avoidance

Samples of the hippocampal recordings are presented in Figure 2 and 3. In Figure 2 animals 7, 10, and 12 had bipolar electrodes in both hippocampi and, except possibly for rat 7 on the left side, the theta rhythm appeared on both sides prior and during a lever-press. Figure 3 shows samples of the records of animals 8, 9, and 15 which had one bipolar electrode in the hippocampus and the second more superficially, in the corpus callosum, or in a deeper structure, the lateral nucleus of dorsal thalamus. It is quite apparent that as these rats were about to make a response the recordings from the hippocampal electrodes showed a shift from the irregular hippocampogram to one of almost sinusoidal regularity, but this shift did not occur in the other structures. The only other noticeable change was a reduction of amplitude in the record from the lateral nucleus of the thalamus in rat 15.

An analysis of the hippocampograms of these 6 experimental animals was done in an effort to establish whether or not there were any consistent differences between wave forms that occurred independently of a lever-press and those

Fig. 2. Records from the 3 bilateral hippocampal animals. Paper speed 30 mm per second. A stimulus marker is shown in the center channel making the small pips 1.0 sec apart and the heavy black marks on the far right of each record indicate a lever-press.

Fig. 3. Records for the 3 animals with one hippocampal electrode and a second electrode in adjacent structures. Paper speed 30 mm per second. Stimulus marker programmed as in Figure 2.

132

133

RAT 8
L.LAT.NUC.THAL.
75 μ V/CM

R.HIPP.
150 μ V/CM

RAT 9

L.HIPP.
50 μ V/CM

R.CORPUS CALL.
100 μ V/CM

RAT 15

R.LAT.NUC.THAL.
30 μ V/CM

L.HIPP.

30 μ V/CM

that occurred just prior to a lever-press. Therefore, for each rat, comparisons were made between a set of 25 samples related to lever-presses and a set of 25 samples lying half-way between 2 lever-presses which were at least 14 seconds apart. An analysis of variance was performed on von Neumann's Mean square of Successive Difference (MSSD) as described by Leiderman and Shapiro (1962). They reported

that the MSSD is a quantitative way of describing the variability of a time-series of observations which is applicable to an analysis of wave data of the type observed in this study. They further pointed out that the MSSD is a more sensitive measure than either the mean or the variance alone.

To obtain the MSSD a transparent grid of vertical lines 2mm apart was placed over each of the one-second samples chosen (recorded at 30 mm/sec) and an amplitude reading made where each vertical grid line first crossed the EEG wave. Sixteen such readings were entered on an IBM card for each second. These data were then processed by an IBM 1620 computer according to the formula for the MSSD,

$$\frac{\sum_{i=1}^{n-1} (X_i - X_{i+1})^2}{n-1}$$
, yielding one MSSD score for each second of data sampled. Fifty such scores were obtained in the same way for each animal, 25 scores related to lever-presses and 25 not related to lever presses. A subjects by treatments by treatments analysis of variance was then done on these scores. The results are shown in Table 1.

Table 1

Results of Analysis of Variance of MSSD Values

Source of Variance	df	SSq	MS	F	p
SS(Subjects)	5	1.2193110	.24386220		
A(Type of score)	1	1.746030	1.746030	8.4855686	.05
M(Measure)	24	1.2044630	5.018588E-02	1.0864960	
<u>SS</u> x A	5	1.090430	.20580860		
M x <u>SS</u>	120	5.5428780	4.6190650E-02		
M x A	24	1.8754120	7.8142166E-02	2.4920	.001
<u>SS</u> x A x M	120	3.7622810	.03135230		
Total	299	16.379791			

As can be seen from Table 1, there is a significant difference between the two types of scores, related and non-related, (A , $P < .05$). There was also a significant interaction of "measures by type of score" ($M \times A$, $P < .001$). This latter effect is illustrated by Figure 4. It shows that the related samples are of lower value and cluster closer together while the non-related samples have a higher mean value and are more diffuse. The results of the analysis indicated one more important fact, namely that even though the samples were selected serially, there were no serial effects (P for measures = .50). This analysis thus supports the contention that the hippocampogram became organized in those samples related to a lever-press.

Referring again to Figure 2 it is apparent that the hippocampogram not only became organized but the frequency consistently falls within the theta range (5-7 cps) as revealed by a simple frequency count. Table 2 shows the result of this frequency count which was made on the same 25 samples that were used to obtain the MSSD values. The operated control animals on the other hand did not show this organizing trend and the frequencies just prior to lever-presses remained irregular and difficult to count.

Electrical brain stimulation

In neither the first nor the second series was there any evidence for response suppression due to brain stimulation as long as seizures did not occur. In the second series

Table 2

EEG Frequencies for Related Samples

	Rat 7	Rat 8	Rat 9	Rat 10	Rat 12	Rat 15
Mean	6.65	7.00	7.08	7.64	6.96	6.92
SD	.757	.707	.837	.491	.456	.400
Grand Mean - - - -	7.04					
Overall Standard Deviation	.755					

Fig. 4. Distribution of mean MSSD values for non-related and related measures.

with stimulation over longer intervals (one minute), similar lack of effects was noted and this was substantiated by the analysis made possible by this method. Table 3 shows results of the more systematically investigated second series using trains of 3 and 6 cps pulses. The tabulation shows the number of lever-presses during 10 one-minute brain stimulations periods and 10 randomly picked one-minute samples for each subject, chosen by using a table of random numbers. Since neither 3 nor 6 cps stimulations yielded any difference in responding, the brain stimulation samples were picked regardless of stimulus frequency. It can be seen that there is virtually no difference in response rate under conditions of stimulation or non-stimulation. Only in the case of rat 10 was there any appreciable difference but a t-test showed that this difference was not significant ($P > .10$).

Figure 5 shows records taken during contralateral brain stimulation at 6 cps, 2 volts, for rat 10 with bilateral hippocampal electrodes, and for rat 15 which had one thalamic and one hippocampal electrode. Rat 15 was stimulated (3.0 volts) at the thalamic electrode and recordings taken from the hippocampal electrode. It can be seen that theta activity occurred prior to and during lever-pressing, and no driving of the hippocampal record appeared. At the highest voltages driving did occur but seizures quickly ensued and the stimulation was halted.

However, Figure 6 shows recordings at the hippocampus dur-

Table 3

The Effect of Electrical Brain Stimulation (EBS)
on Conditioned Response Rate

Sample	Rat #10		Rat #8		Rat #15	
	CR's/min	CR's/min	CR's/min	CR's/min	CR's/min	CR's/min
	during	with no	during	with no	during	with no
	EBS	EBS	EBS	EBS	EBS	EBS
1	3	4	7	5	3	3
2	3	4	6	6	2	2
3	5	4	5	6	2	6
4	5	4	7	6	3	4
5	6	6	7	5	4	4
6	6	6	5	9	4	2
7	11	5	2	7	5	5
8	13	7	6	6	4	2
9	5	5	5	7	1	4
10	7	6	5	5	3	2
Mean	6.4	5.1	6.1	6.2	3.1	3.4

Fig. 5. Hippocampograms recorded from the left hippocampi of rats 10 and 15 during 6 cps electrical brain stimulation. Rat 10 was stimulated in the right hippocampus, whereas rat 15 was stimulated in the right lateral nucleus of the thalamus. The electrical brain stimulation was already on and continued throughout the period shown.

703

704

RAT 10

L.HIPP.

75 μ V/CM

R.HIPP.

STIM. 6cps

2 VOLTS

168

169

RAT 15

L.HIPP.

30 μ V/CM

R.LAT.NUC.THAL.

STIM. 6cps

3 VOLTS

Fig. 6. Hippocampograms recorded from the left hippocampi of rats 10 and 15 during 3 cps electrical brain stimulation. Rat 10 was stimulated in the right hippocampus while rat 15 was stimulated in the right lateral nucleus of the thalamus.

RAT 10

L.HIPP.

75 μ V/CM

R.HIPP.

STIM. 3cps

3 VOLTS

576

578

577

RAT 15

L.HIPP.

30 μ V/CM

R.LAT.NUC.THAL.

STIM. 3cps

4 VOLTS

206

207

208

ing contralateral 3 cps stimulation for both of these rats in which it can readily be seen that hippocampal areas under the recording electrode were being driven by the impressing stimulation with a most predictable wave pattern occurring when the contralateral hippocampus was stimulated in rat 10. This Figure also shows an unexpected phenomenon for rat 10 that occurred when stimulated at 3 cps; the impressed 3 cps rhythms were replaced by the theta rhythm just prior to a leverpress. It is felt that a similar phenomenon occurred for rat 15 although the evoked pattern was more complex (probably due to the stimulus site being the thalamus) and it was somewhat obscured by a movement artifact. Also, when the site of stimulation for rat 15 was reversed -- stimulating the hippocampus and recording from the thalamus -- no theta rhythm was ever seen from the thalamic electrode nor was there evidence of suppression or an impressed 3 cps rhythm. This was also true of rat 8 which had electrodes placed similarly to those of rat 15.

Discussion

With respect to the demonstration that hippocampal theta rhythms are correlated with overt behavior in the rat, the results of the present study support the findings obtained on the rabbit, cat and monkey (Green and Arduini, 1954; Adey et al, 1960; and Grastýan et al, 1959). However, the results reported here do not support the hypothesis of Grastýan et al (1959) that the theta rhythm is a correlate of the orienting reflex. This is so because they assumed that an exteroceptive warning stimulus is a necessary antecedent for the OR. The present study demonstrated that the theta rhythm was present even when no warning stimulus was used. Furthermore, the theta rhythm did not show adaptation or extinction as the OR does (Razran, 1961). In the present study the theta rhythm continued to be present during hundreds of avoidance conditioning trials. Also, Adey et al (1960) showed that the theta rhythm correlated with responding even after "many thousands of trials" during approach responding conditioning trials in their animals, and this further militates against an OR hypothesis. Rather, they attributed the theta rhythm to the effect of "goal-directed behavior." This

does not appear to be far different from the statement of Green and Arduini (1954) that the hippocampal theta rhythm is a correlate of attention or arousal. In the studies of Adey and others (Adey and Dunlop, 1960; Adey et al, 1960) the animals were placed in the start chamber of a "T-box" 1.5 meters long and had to determine the correct side by locating an illuminated rectangle. The animals certainly had to be attentive to the cues in order to respond correctly in this 2-choice situation; therefore, in this case the difference between the arousal concept and the goal-directed behavior concept seems to be a semantic one.

However, there is additional evidence to support the arousal concept. The present study demonstrated that electrical stimulation of the hippocampus that was below seizure threshold did not disrupt the animals' behavior. This supports the results presented by Holmes and Adey (1960) who applied electrical stimulation at 6 and 100 cps to the entorhinal cortex, which has a direct anatomical relationship with the hippocampus and found that 2-choice runway behavior was not disturbed. This suggests that the theta rhythm which can be monitored in the hippocampus is initiated by arousal but is not itself a necessary concomitant of arousal or responding, at least with respect to well established habits. It is recalled, however, that the cyclohexamines did depress the hippocampal theta rhythm, elicited seizure discharges and depressed runway behavior (Adey and Dunlop,

1960). This apparent contradiction can be explained by the suggestion that excessive stimulation or seizure activity can effect adjacent structures that are more directly related to arousal. Also, since it is known that analgesics and psychotropic drugs affect the thalamus, hypothalamus and brain stem reticular formation (Berger, 1960) it is very possible that cyclohexamines primarily affected the ARAS and this in turn altered the hippocampal theta rhythm and responsivity. This arousal hypothesis becomes even more inviting when one considers the neural connections of the hippocampus. As was previously mentioned Nauta (1956) demonstrated that there are direct thalamic and hypothalamic connections to the hippocampus, and also, that there are indirect midbrain-reticular connections to this structure. This anatomical evidence that is well supported by the studies of Green and Arduini (1954) and Adey et al (1958) is certainly consistent with an arousal explanation of the theta rhythm mediated by the ARAS. Moreover, Kidelberg, White, and Brazier (1959) have demonstrated a direct relationship between the ARAS and the hippocampus. They found that when stimulating various ARAS structures the hippocampogram showed a theta pattern, and that if the neural connections from these structures to the hippocampus were cut, the hippocampogram no longer showed the theta rhythm. In the present study it can be emphasized that the displacement of driven 3 cps hippocampal rhythms by theta activity just prior to a conditioned response (Figure 6)

strongly supports the contention that theta activity in the hippocampus is evoked by other proactive mechanisms. This finding also suggests that there is a strong tendency for hippocampal theta activity to occur during arousal but is not necessary for arousal.

The major hypothesis of the present study was that the hippocampal theta rhythm in the rat could be correlated with responding in a nondiscriminative avoidance task and this was supported by the results. However, the prediction that brain stimulation which disrupted the theta activity would also disrupt responding in an avoidance task was not supported. However, an ad hoc hypothesis would hold that this result is what one would expect if the hippocampal theta rhythm is merely correlated with arousal but not a necessary correlate for on-going learned behavior.

Returning to the behavioral studies mentioned in the Introduction in which lesions were placed in the hippocampus (Kimura, 1958; Kaada et al, 1961; Isaacson et al, 1961; and Isaacson and Wickelgren, 1962) evidence was presented that led to the conclusion that the hippocampus does play an important role in performing a variety of learned responses. While it is not unusual for the results of lesion studies and the results of electro-physiological studies to suggest somewhat different interpretations of the function of any given brain structure, Flynn and Wasman (1960) presented evidence which suggests a reconciliation of these views. They demon-

strated that cats could learn a classically conditioned leg withdrawal during the after-discharge of hippocampal seizures even though they were not capable of performing it during the seizure. They further reported that disruption of previously learned behavior by brain stimulation might be explained by motor deficiency due to the effects of the induced hippocampal seizure on the motor cortex. In particular they found that hippocampal seizures increase the latency of cortically evoked movement: thus, lesions, like seizures might depress learned responses by affecting motor mechanisms. The fact that only bilateral hippocampal seizures disrupt learned responses (Flynn et al, 1961) and that the effect may well be on the motor side (Flynn and Wasman, 1960) of the conditioned relationship agrees with the results of the present study. In the present study stimulating the hippocampus with 6 cps at 2 and 3 volts showed no evoked discharges in the hippocampus on the opposite side although it was quite likely that the stimulated structures were being affected. However, no disruption of conditioned behavior occurred. But, applying 3 cps electrical stimulation of subseizural intensity to the hippocampus disrupted normal neural activity on both sides as shown by the driven hippocampogram (Figure 6), but it did not prevent the subject from making the conditioned response, presumably since the motor centers were not effected. Further, the displacement of the driven rhythm by theta rhythm indicates that the arousal mechanisms were still intact.

In short, when one considers the evidence of the present study with respect to neuroanatomy, electrophysiology, and behavior correlates, the arousal hypothesis of the origins of the theta rhythm seems to integrate more facts than any other single hypothesis.

Summary

In a Sidman avoidance task, rats with bipolar hippocampal electrodes showed that irregular hippocampal EEG activity changed to regular 5-7 cps theta activity just preceding and during an avoidance lever-press. The appearance of these theta rhythms during the Sidman avoidance task suggested that the theta rhythm was not dependent upon exteroceptive stimuli, but rather was a correlate of centrally initiated attention or arousal. Simultaneous stimulation and recording showed that altering the hippocampal rhythms did not disrupt avoidance behavior if stimulating voltage was below seizure inducing levels; this suggested that hippocampal theta activity, though correlated, is not causally related to arousal.

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