A Neuro-Clinical Study of the Spread of Experimentally Induced Epileptiform Discharge from the Amygdala

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A NEURO-CLINICAL STUDY OF THE SPREAD OF EXPERIMENTALLY INDUCED EPILEPTIFORM DISCHARGE FROM THE AMYGDALA IN CATS

by

Daniel Rosen

A Thesis
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in partial fulfillment
of the
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Daniel Rosen
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CHAPTER I

INTRODUCTION

Vivid descriptions of epileptic seizures appear throughout history dating as far back as Biblical literature. Hippocrates recorded the main symptoms of the major epileptic seizure. Later, Greek and Latin physicians gave more detailed descriptions, including premonitory signs and auras that often preceded the onset of convulsive seizures. Then, later accounts by Greek and Latin physicians indicate that different types of attacks were recognized, such as minor seizures associated with petit mal attacks, as they are now known. (The following historical and nosological information was obtained from Penfield & Jasper, 1954.)

It was with the significant contributions made by Hughlings Jackson during the latter part of the 19th Century that the neurophysiological conceptions of brain function, in general, and the phenomenon of the epileptic discharge, in particular, formed a basis for modern day research and understanding of these functions. During the time of Jackson, and the years following, the "epilepsies," as they were first classified by Jackson, were divided into two
major categories: symptomatic and cryptogenic epilepsy (also known as essential and idiopathic epilepsy). Symptomatic epilepsy comprised cases in which causes were discovered. These causes were usually cited as intracerebral abnormality, brain lesion, or extracerebral influences such as toxicity or systemic disturbances. Cases of epilepsy where no causes could be identified were termed cryptogenic epilepsy.

While this coarse classification is still in use today, a more useful nosology can be constructed, using clinicoanatomical classifications. One such set of classifications can be made on the basis of which anatomical region serves as the origin of the epileptiform discharge. Further classification within the major clinicoanatomical divisions are determined by electroencephalographic patterns. Thus, for example, centrencephalic seizures, which exhibit a three per second wave-and-spike pattern that is primarily bifrontal, indicate petit mal epilepsy. Grand mal seizure activity is indicated by generalized bilateral rapid rhythms. Psychomotor automatism shows four-six per second rhythms that are primarily bitemporal or frontotemporal.

However, one should be aware of the fact that in many individual epileptic patients many of these forms are demonstrated within the same patient, either separated in time or occurring sequentially throughout a specific attack. For example, petit mal
seizures may be followed by a generalized convulsion of the grand
mal type. Focal cerebral seizures may also engender generalized
bilateral seizure activity before the attack subsides.

The experimental investigation of epilepsy and its associated
phenomena of epileptiform discharge-spreading activities may yield
at least two important and related classes of data. First of all,
experimental studies may generate further data that can be applied
directly toward clinical diagnosis and treatment of epilepsy. More-
over, the neuroclinical study of epilepsy may yield important in-
sight with regard to other psychopathological entities. For example,
Gibbs, Gibbs, and Fuster (1938) found similar dysrhythmic patterns
between patients with epilepsy (grand mal, petit mal, and psycho-
motor epilepsy) and patients clinically diagnosed as schizophrenic.
These striking similarities were obtained from electroencephalo-
gram records. Erickson (1945) reported a case of a woman, who,
among other symptomatic behaviors, exhibited extreme nymphomania
as an expression of a cortical epileptiform discharge. The cortical
focus was confirmed as an encapsulated hemangioma, which was
surgically removed. Following the removal of the neoplasm, the
patient no longer manifested the nymphomania. Thus, one could
hypothesize that the clinical boundary lines which delimit certain
psychopathological disorders, characterized by recurring dys-
rhythmia are arbitrary, and less important than classifications of
these disorders which are based on physiological parameters.

The second reason behind such experimental investigations is that experimentally induced epileptiform lesions may provide an important experimental technique modality from which data can be derived concerning the relative synergistic effects of various neural structures and pathways in terms of resultant clinical and sub-clinical behaviors. For example, by inducing a self-sustaining discharge in a specific area of the brain in question, investigators may be able to determine the neural structures and pathways, and perhaps even the neurochemical actions that are affected by discharge originating from that given area. Indeed, one of the major problems incurred by investigators in their attempts to ascribe definitive actions and functions of specific neural structures has involved the relative inability to discern the ongoing synergistic activity of surrounding structures. Perhaps the use of experimentally induced epileptiform discharges may provide a fruitful approach for selectively studying the relative functions and contributions of certain brain areas and pathways.

The experimental question of concern in this study involves the issue of how subcortical structures and their pathways function in the "spread" of an epileptiform discharge. More specifically, this study will attempt to explore the functional roles of the thalamic reticular and hypothalamic pathways in the spread of discharge from
epileptiform lesions in the amygdalae of cats. This experimental issue will be explicated after the following review of some of the literature relevant to this study.

Epileptiform attacks involve a complex integrative spread of after-discharge as the seizure proceeds from onset to finish. While the nature of the spreading discharge is not fully explained at present, there is experimental evidence that tends to implicate the function of thalamic and thalamic reticular systems as being crucial in terms of regulating recurring changes in cortical and sub-cortical potentials.

Jasper (1949) presents a classification of thalamocortical systems and discusses the integrative actions of the thalamic reticular system. He distinguishes between specific and diffuse reticular projections. The former system is divided into three main groups: (1) Primary afferent relay systems, (2) secondary afferent relay systems, and (3) specific elaborative systems. These systems relay impulses from the main sensory pathways and provide "conduction" to the sensory cortices. In addition, the secondary afferent relay systems receive their afferent supply from such structures as the cerebellum, corpus striatum, and hypothalamus. The specific elaborative systems have point-to-point projections to frontal, temporal, and parietal cortex.

In addition to local cortical projections of these specific
systems, the thalamic reticular system exhibits nonspecific diffuse projection systems. It has been shown that these diffuse projection systems have the remarkable property of being able to regulate the rhythmic electrical activity of the entire cortex (Chang, 1950; Demsey & Morison, 1942a; Dempsey & Morison, 1942b; Fields, King & O'Leary, 1949; Hunter & Jasper, 1949; Jasper, 1949; Penfield & Jasper, 1954).

Using local stimulation in cats, Jasper (1949) passed a stimulating needle slowly through the thalamus and found a striking disturbance in the spontaneous rhythms of large cortical areas. Moreover, the spontaneous cortical activity was often replaced by responses in time with the thalamic stimulus.

In view of these findings, one may conclude that the thalamic reticular systems have a vital function in terms of conducting the spread of discharge to other brain areas. This proposition may be presented even more cogently if one considers the findings of Hunter and Jasper (1949). These investigators obtained sustained after-discharges in cats, lasting from fifteen to thirty seconds. The after-discharges were obtained from stimulation using bipolar electrodes within the thalamus. In addition, prolonged after-discharges were recorded from the cortex of both hemispheres after stimulation within the intralaminar system near the massa intermedia. With more intense stimulation in this area, there
occurred a generalized tonic-clonic convulsive seizure during post-stimulation, which simulated petit mal and grand mal convulsions.

In addition to thalamic reticular systems above the mesencephalon, there appear to be reticular systems emanating from the lower brain stem that also exert influence on cortical motor activity. Inhibition of cortical motor response was elicited by stimulating the bulbar area. Such stimulation was effective against reflex activity and decerebrate rigidity in cats (Magoun & Rhines, 1946).

Most of the literature concerned with the nature of the spread of epileptiform discharge has been circumscribed around the study of after-discharge of the cerebral cortex. Erickson's (1940) studies of the spread of epileptiform discharge across the cerebral cortex of monkeys, suggested that the spread of discharge occurs by neuronal pathways. However, spread of discharge topographically to adjacent regions of the gray matter, a spread by contiguity, was not precluded, but it was de-emphasized. Erickson concluded that the corpus callosum played a crucial role in terms of the conductance of the discharge. This conclusion was based on his findings, that when the corpus callosum was completely sectioned, epileptiform activity from one area of the cortex was not picked up from its homologous region in the other hemisphere.
However, Jasper (1949) found that unilateral stimulation of certain thalamic reticular regions in cats produced bilateral responses in sensory-motor regions after complete section of the corpus callosum and of the anterior and posterior commissures. Whereas, complete section of the massa intermedia in the cat often prevented the spread of discharge to the opposite hemisphere while the corpus callosum was intact. In fact, Jasper found that in some cases the section resulting in this effect of localizing the spread involved a portion of the subthalamus and hypothalamus.

Thus, whereas Erickson contended that subcortical regions were not very instrumental in the spread of cortical discharge, it should be noted that this assertion was based on his observations of the spread of discharge emanating from a focal point of stimulation on the cerebral cortex alone. Yet, many epilepsies are due to subcortical foci. This applies, in particular, to those cases which are often referred to as idiopathic or cryptogenic epilepsy (Hayne, Belsnson, & Gibbs, 1949). In addition, others have observed that certain epileptiform lesions of the thalamic nuclei may clinically simulate cortical focal epilepsy (Chusid, 1970).

Although sections of the corpus callosum generally confine the spread of cortical focal stimulation to the affected hemisphere, the role of certain subcortical structures in the spread of after-discharge, should not be removed from consideration. At least
two related findings support the proposition that certain subcortical structures have crucial roles in the spread of the epileptiform discharge.

First of all, there tends to be a synchronous, rhythmic pattern in the cerebral cortex which can be readily influenced by stimulation of thalamic reticular structures (Adrian, 1936; Chusid, 1970; Dempsey & Morison, 1942a; Hayne et al., 1949; and Jasper, 1949). Secondly, there is the remarkable phenomenon of "cortico-thalamic reverberation." That is, long sustained after-discharge can be maintained between the cortex and the thalamus. These after-discharges can be abolished by destruction of the cortex or the thalamus, alone, or by the interruption of their interconnections. Furthermore, such an after-discharge can be induced by stimulation of any point along the neuronal pathway circuit between the thalamus and the cortex (Chang, 1950; Dempsey & Morison, 1942b; Jasper, 1958; and Thompson, 1967).

Therefore, it would appear that the corticothalamic reverberating system (involving the same structures which Jasper described as the diffuse projection systems of the thalamus) would have paramount importance in terms of propagating the spread of an epileptiform discharge, whether its origin is cortical or subcortical. Hayne et al. (1949) suggested that the failure of many surgical interventions intended to abolish epilepsy are due to the
fact that the surgical attack has been aimed at a secondary cortical rather than a primary subcortical focus. These investigators concluded from their studies that the cortex and subcortex exhibit comparable normal and abnormal activity in epileptic patients.

The role of the hypothalamus and other subcortical structures in the spread of epileptiform discharge remains relatively obscure. However, certain findings implicate the hypothalamus as being an important structure in terms of modulating the synergistic effects of gross brain activity. It has close proximity, in absolute terms, and via neural pathways to other subcortical areas, in particular, the thalamic reticular systems. This may lead one to speculate that the hypothalamus may indeed have significant modulating effects on the spread of after-discharge (Velasco & Lindsley, 1965).

Studies dealing with the sleep-waking dimensions of the ascending reticular activating system have also generated data which demonstrate that certain areas of the hypothalamus, primarily the posterior portion, exert control over arousal mechanisms, in animals (Feldman & Waller, 1962). Thus, it appears that the hypothalamus may have significant modulating effects on the thalamic reticular systems.

As these studies that have been cited suggest, certain portions of the thalamus can function to spread and sustain after-
discharge. In addition, there is evidence that thalamic-hypothalamic interconnections may function in terms of expressing abnormalities (lesions) in one of these areas, when the origin of the lesion may have its focus in the other area. For example, a case reported by Engel and Aring (1945) revealed hypothalamic "attacks" caused by a confirmed thalamic lesion. The primary hypothalamic symptom in this patient was hyperthermia.

In light of the aforementioned data, the following experimental study was designed. This study will attempt to investigate:

1. **The neuroclinical effects of inducing epileptiform lesions in the basolateral amygdalae of cats.** -This anatomical site was selected because it has been shown that permanent, self-sustaining epileptiform lesions can be readily induced by the application of carbacol (choline carbamate) to the basolateral components of the amygdalae, in cats (Grossman, 1963). Secondly, the amygdala has direct neuronal fiber connections with the hypothalamus and thalamus (Chusid, 1970; Fox, 1949; Papez, 1945; & Thompson, 1967). This provides an opportunity to experimentally study the probable neural pathways that serve to spread the epileptiform discharge.

2. **To determine whether the selective interruption of these interconnecting pathways will have the effect of confining or localizing the spread of discharge from an epileptiform focus in**
the amygdala. -- A survey of the literature did not obtain any data that strongly support any prediction in regard to this aspect of the experimental question.
CHAPTER II

METHODS

Subjects

Three adult cats, two males and a female, were the experimental subjects used in this study. Prior to this study, these animals had never been subjected to any known experimental interventions. The cats received distemper vaccinations upon their arrival at the laboratory. Subsequent to an examination by a veterinarian during the intake procedure for new animals, the cats required no veterinary attention. They appeared healthy and normal in terms of observed feeding and behavioral patterns and there were no apparent infections. Each cat was placed in a 76 cm. x 76 cm. x 76 cm. stainless steel-bar cage. The animals were single-housed in these same cages throughout the experiment. During the entire experiment, the cats had free access to food\(^1\) and water.

\(^1\)Purina Cat Chow, Ralston-Purina Co., St. Louis, Missouri.
Procedures

The first phase of the experiment involved the bilateral implantation of recording electrodes using a stereotaxic apparatus.¹ The target areas of the recording electrodes as determined by the Snider and Niemer atlas (1961) were the basolateral components of the amygdala. In addition, electrodes were implanted on the surface of the frontal-motor cortex, parietal cortex, and the temporal-parietal cortex, for purposes of recording cortical electrical activity. An "indifferent" of silver wire was placed under the surrounding skin tissue. The "free" terminal end-wire of each electrode was connected to a plug,² which was then cemented in place, on the surface of the animal's skull.

Polyethylene cannulas were chronically implanted during this first procedure. They served for the delivery of the carbachol, which would be applied to the designated brain area, later in the experiment. These cannulas were bilaterally implanted, stereotaxically, with the basolateral amygdalae as the target areas. The anesthetic agent that was used in this operation and in the subsequent surgical intervention was sodium pentobarbital.³ Atropine

¹ Manufactured by J. David Kopf, Tujunga, California.
² Amphenol, #DS 15P.
³ Fort Dodge Veterinary Supply.
sulphate\(^1\) was used as a vagolytic agent. Both drugs were injected intraperitoneally.

The first phase of the experiment was accomplished after the implanted electrodes and cannulas were cemented in place on the skull. A 0.5 cc dose of procaine penicillin G\(^2\) was administered for prophylactic purposes against post-operative infection. The animals were then returned to their respective cages.

The three cats were allowed a period during which full recovery occurred. Following complete recovery, it was observed that each of the cats exhibited their normal, characteristic behaviors. They were extremely responsive to human contact, displaying a great deal of affection and desire for persons to attend to them and pet them. Their intake of food and water was normal. In short, there were no abnormal behavioral patterns observed and the cats seemed quite healthy.

The next experimental intervention involved an attempt to induce experimentally an epileptiform lesion in one of the cats. The female cat, a calico, was selected from among the three experimental animals to undergo this procedure. Electroen-

\(^1\)Abbott Laboratories, North Chicago, Illinois.

\(^2\)Squibb Co., New Brunswick, New Jersey.
cephalogram (EEG) samples were obtained from this cat over a period of three days immediately prior to the day in which the attempt to induce epilepsy occurred. (All EEG records in this experiment were obtained while the animals were unanesthetized and fully awake.) These EEG samples indicated no abnormalities in brain activity. After obtaining these normal pre-test EEG samples, an attempt was made to induce epilepsy, experimentally. This procedure involved a technique whereby carbachol\textsuperscript{1} (choline carbamate) was injected into one of the cannulas in the amount of ten micro-liters. Unilateral, rather than bilateral application of the chemical, was carried out to observe both the immediate and the subsequent effects upon the "untreated" hemisphere.

Following a latency period of about three minutes, a dramatic change occurred. Both, the EEG and the concomitant behavioral observations indicated a self-sustained epileptiform discharge which began to manifest in an overt clinical seizure. These, and other observed effects, will be outlined in the presentation of the results.

With the above results obtained by the application of carbachol to the basolateral amygdala, it was decided that a surgical intervention would be attempted on the second cat before

\textsuperscript{1} Manufactured as Carcholin by Merck, Sharp, and Dohme, West Point, Pennsylvania.
the carbachol treatment. It was proposed that an electrolytic lesion be performed on this cat with the intention of attempting to localize or confine the epileptiform discharge within the amygdaloid region.

At this point, a problematic issue was confronted. The amygdala has efferent fibers that "communicate" with the thalamus and the hypothalamus. It is also believed that the amygdala has fiber connections with other subcortical areas. An attempt to localize or confine the epileptiform discharge by means of ablating all known or believed connective tracts that issue from the amygdala would necessitate a relatively gross destruction. Moreover, it would be almost impossible to control or account for the obtained experimental results in terms of drawing conclusions as to how specific fiber tracts function to modulate or conduct the spread of after-discharge from the focus in the amygdala. Therefore, such gross destruction techniques were ruled out. However, since the hypothalamus was suspected of being a primary structure with regard to regulating or mediating the spreading phenomenon from subcortical origins, it was decided that the stria terminalis would become the site for the electrolytic lesion. The stria terminalis is a bundle of neuro-fibers that arise from the amygdala and "loop around" to connect with the hypothalamus. Thus, bilateral lesioning of the stria terminalis at a point dorsomedial from the amygdala was performed by means of stereotaxic techniques.
Immediately following this surgical intervention, an injection of penicillin was administered and the cat was allowed to convalesce for two weeks. After these two weeks, during which full recovery occurred, the carbachol treatment was applied in the same fashion as with the first cat.

It was discovered some time after the stereotaxic implantation procedure had been performed on the third cat that the cannulas were inaccurately placed. Through a miscalculation, the wrong coordinates were used to place the cannulas. However, evidence from a previous experiment in which carbachol was used to induce epileptiform lesions in cats suggested that the epileptogenic properties of carbachol were highly specific to the basolateral amygdala (Grossman, 1963). Therefore, it was felt that negative results obtained from carbachol treatment in this case, if they were to occur, could provide an unanticipated source of control data. Thus, the carbachol treatment was applied to the third cat, notwithstanding the experimental error.

Brain tissues were then to undergo histological procedures in order to attempt verification of the proposed experimental lesions.
CHAPTER III

RESULTS

Figure I reveals the effects of the carbachol on the EEG record of the first cat. In Figure I, the first stage shows the EEG patterns of the left and right amygdalae just prior to the application of carbachol. It can be observed that there is a consistent, normal pattern of low-voltage electrical activity, corresponding to normal patterns of wakefulness. Just below that is the EEG pattern of the frontal motor cortex during Stage 1, which reveals a similar normal pattern of electrical activity.

Concomitant behavioral observations during Stage 1 were made. During the EEG recordings, the cat was held on a person's lap. This close human contact seemed to mitigate against fear and associated gross movements. In Stage 1, it was observed that the animal was somewhat apprehensive and curious. However, the cat did not make any attempts at defying the moderate restraints imposed by the person holding it. The EEG corroborates this fact since there appeared almost no evidence of movement artifact.

The application of carbachol to the left amygdala generated
FIGURE I. --Initial Effects of Carbachol Treatment on EEG Activity in First Cat

Stage 1: 10 Minutes Prior to Carbachol Treatment
- Left Amygdala: 50 MV (bipolar)
- Right Amygdala: 50 MV (bipolar)
- Left Frontal Cortex: 50 MV (bipolar)
- Right Frontal Cortex: 50 MV (bipolar)

Stage 2: 10 Minutes After Carbachol Treatment
- Left Amygdala: 50 MV (bipolar)
- Right Amygdala: 50 MV (bipolar)
- Left Frontal Cortex: 50 MV (Clonic Phase)
- Right Frontal Cortex: 50 MV (bipolar)
almost immediate abnormal EEG patterns. Referring to Figure I, Stage 2 illustrates the effects of the carbachol after less than two minutes following its application to the left amygdala. It can be observed that while the EEG record of the left amygdala reveals the sharp spikes that are characteristic of epilepsy, the activity of the right amygdala, at this point, is comparable to that which was observed in Stage I. The first observable sign of behavioral change was the occurrence of a frothy, viscous flow of saliva. Also, a steady moaning sound started. The salivation and moaning began two minutes after the carbachol had been administered. It also corresponded in time to the initial onset of the epileptiform spiking pattern that was observed in the left amygdala (Stage 2).

After approximately three minutes had elapsed, subsequent to the carbachol treatment, the cat was placed on the floor. The animal remained in a standing position but did not move away from this spot, even though there was enough slack in the EEG recording cable to permit some free locomotion. The animal's right eye and ear were twitching and then the right side of the mouth region began to exhibit paroxysmal tremors. At a point of about six minutes into the seizure, there was loss of control over the anal sphincter. Also at this point, clonic motor activity began to appear in the right limbs. A portion of the EEG reading obtained from the left cortical hemisphere during this time is shown in
Figure I, Stage 2. It reveals the high-voltage, dysynchronous spiking discharges associated with this clonic episode.

The cat manifested clonic motor activity for about twenty minutes although it decreased in intensity over time. The flow of saliva persisted but it similarly became attenuated. The animal continued to moan during the remainder of the clinical seizure.

The next day, approximately twenty-four hours after the carbachol treatment, further clinical observations were made. The cat exhibited bilateral facial grimaces and eye twitches. Upon removing the cat from the cage, two features became apparent. First, it was noted that the cat was startled by the initial tactual contact. However, once the contact was established, the animal appeared receptive. Then, as the cat was being lowered to the floor, it was observed that the limbs were not extended toward the floor as the cat approached it. Normally, the cat extended its limbs in anticipation of assuming a standing position. Thus, it appeared that the animal was blind. The eyes were glazed-over and the pupils were abnormally large.

The second behavioral feature to be noted was that the cat exhibited extreme ataxia. The animal's attempts at walking seemed to engender great difficulty, as the cat revealed a staggered gait. Finally, the cat resigned itself to remaining stationary, in a kneeling position.
An EEG record was then obtained, of which a portion is presented in Figure II. This electroencephalogram indicated that the epileptiform activity was now present in both hemispheres. Both the left and right amygdalae exhibited the epileptiform high-voltage wave and spiking patterns. The record of the left and right cortex are also shown. Some abnormal waves and spiking was also present there.

Follow-up observations on this cat were made over several subsequent weeks. The clinical features that were noted over this period can be enumerated as follows. The cat continued to manifest clinical seizures. The frequency of these seizures decreased over this period of time. Food and water consumption was abnormally high and defecation and urination were also found to occur at an abnormally high level—perhaps due in large part to the increased intake of food and water. The ataxic features disappeared after about two or three days and, subsequently, the cat revealed no difficulties in proprioception or locomotion. However, the apparent extreme visual deficits persisted, as evidenced by startled responses to initial tactual contacts, complete inhibition to jumping out from its cage on its own (which was not at all the case prior to carbachol treatment), and failure to circumvent obstacles in its path.

Approximately three weeks after the carbachol treatment
FIGURE II.--Bilateral Effects of Carbachol Treatment After 24 Hours

Left Amygdala

Right Amygdala

Left Frontal Cortex

Right Frontal Cortex
had been performed on the first cat, a surgical intervention involving the bilateral electrolytic lesion of the stria terminalis was carried out on the second cat. This experimental intervention was done in an attempt to localize the epileptiform lesion that was anticipated to occur following carbachol treatment. There were no apparent behavioral changes resulting from this experimental intervention. Attempts to obtain pre-carbachol treatment EEG measures were fraught with difficulties. In contrast to the first cat, who remained relatively docile during occasions which imposed physically restraintive measures, the second cat had always been extremely defiant during such occasions in the experiment. Thus, the EEG record that was obtained from this second cat was inundated with movement artifact and, therefore, in this case, the EEG was of limited use. However, there was no reason to speculate that any anomalies in brain activity were present.

The subsequent carbachol treatment applied to this second cat failed to generate any clinical seizure activity. In fact, there were no observable behavioral changes. The animal was allowed to walk around the laboratory. The cat's activities included exploring various areas of the laboratory and attempts at eliciting tactual contact from the experimener. Yet, there were no clinical signs of epilepsy.

However, about twenty-four hours after the application of
the carbachol, it was noted that the pupils of both eyes were ab-
normally large. Upon further examination, it was found that this
cat, too, was blind. Thus, while there did not appear to be any
spread of epileptiform discharge from the left amygdala, the
resultant blindness suggested that there was, in fact, a lesion
produced by the carbachol.

The second cat was observed for the next few days. It
appeared healthy except for the blindness. The cat's behavioral
patterns such as feeding, evacuating, responses to humans and
conspecifics, and etc., remained normal. In short, there were
no observable behavioral changes, aside from those that were
directly associated with the animal's blindness. Moreover, no
seizure activity occurred.

With regard to the third cat, since it was discovered that
through miscalculation, the cannulas were not implanted accurately
in terms of the intended target areas, it was not expected that the
carbachol treatment would generate any epileptogenic properties
in this animal. This assumption was made in view of previous
data. However, it seemed that this situation still offered experi-
mental utility in terms of providing experimental control data.
Thus, carbachol was applied to the brain, through the misplaced
left cannula. As expected, there was no evidence of clinical
epilepsy following carbachol treatment. Furthermore, there were
no behavioral changes in the cat that could be observed.

The animal was under observation for the next forty-eight hours to determine if any changed occurred—especially those concerned with vision. There were none observed.

Further observations could not be made immediately after this forty-eight hour period since the experimenter was unable to schedule any observations in the laboratory for the next two days. Upon returning to the laboratory, it was learned that this third cat had succumbed in its cage. There had been no indications that the cat was unhealthy during previous observations. Histological examination was precluded since enough time had elapsed after death to permit deterioration. Although it was open to speculation that the carbachol treatment proved fatal, the cause of death remains an enigma.

Verification of the epileptiform lesion that was presumed in the second cat was attempted by means of histological staining techniques. A section of tissue in which a lesion appeared to exist was prepared for histological staining procedures. Although it can not be said with certainty, it appeared that the tissue in question included the basolateral nuclei of the amygdala. This tissue section was prepared using normal histological procedures and subjected to hematoxylin staining techniques. Figure III is a photograph of one of these prepared cross-sections of lesioned
tissue. One can observe the lesion-affected portion of tissue at the top of the photograph. The bottom half appears to show tissue that had remained relatively unaffected in contrast to that which is seen in the upper portion of the photograph. Figure IV presents a photograph of an area of tissue that appears to have been ablated quite severely by the effects of the carbachol. A large number of degenerated neurons and fiber tracts have given way to the appearance of collagenous-fiber scar tissue and glial cells.
FIGURE III. --Photograph of Tissue Section Showing a Relative Contrast Between Lesion-Affected and Normal Tissue

FIGURE IV. --Vivid Demonstration of Neural Damage Caused by Carbachol Lesion
CHAPTER IV

DISCUSSION

The results obtained in this study from the experimental treatment of carbachol application to the basolateral amygdalae in cats provide confirmation of those effects obtained by this chemical in Grossman's (1963) investigations. That is, a permanent self-sustaining epileptiform discharge can be experimentally induced in cats by means of carbachol administered at the site of the basolateral components of the amygdala. Furthermore, the epileptogenic properties of carbachol seem to be specific to the basolateral amygdala. Grossman found no evidence of the epileptogenic effects of carbachol when it was introduced into other brain regions. The findings in this study tend to support this notion since it was found that not all brain tissue can serve as a suitable substrate for expressing the epileptogenic properties of carbachol. That is, in the case of the third cat, in which it was known that the carbachol was delivered to a site other than the basolateral amygdala, there was no evidence of epileptiform activity after the carbachol treatment.

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The EEG records demonstrated that cortical dysfunction in terms of electrical activity can be attributed to various states of subcortical activity. As Hayne et al., (1949) suggested, many clinical cases of epilepsy and perhaps even other classes of pathanomic behaviors which suggest cortical dysfunction may have etiological origins in primary subcortical areas.

The results of this study suggest that the spread of after-discharge from the amygdala to other brain areas is dependent on conductance via fiber tracts leading from the amygdala to the hypothalamic nuclei. However, this conclusion must be tempered by the consideration of possible experimental error with regard to the experimental interventions introduced in this study. The verification of the intended carbachol lesions in terms of their presence, and then, in terms of their appearance within the proposed anatomical sites, were obtained by means of histological procedures and deduced from clinical evidence. The clinical findings offered an independent source of verification of the carbachol lesions. In the first cat, almost instantaneous spiking was recorded from an electrode implanted to measure such activity in the basolateral amygdala. The epileptiform discharge spread to other areas only after a momentary latency period, following this initial focus in the amygdala. The subsequent clinical seizures demonstrated further evidence that the carbachol
treatment did, in fact, induce a self-sustaining epileptiform discharge.

The absence of such a vivid clinical demonstration of seizures following the carbachol treatment in the second cat may lead one to speculate that experimental artifact could have accounted for the absence of clinical epileptic seizures rather than the surgical intervention prior to the carbachol treatment. That is, perhaps the cannula was not in the requisite position to allow the chemical to effectively reach the specific area in question. Another issue in this regard concerns the question of whether the proposed electrolytic lesion of the stria terminalis was actually accomplished.

Therefore, it is important to consider carefully the results obtained in this experiment before expounding on these results for interpretive conclusions. In considering the issue of whether the carbachol treatment effectively rendered a lesion in the basolateral amygdala of the second cat, two major findings are crucial. One such finding is the histological evidence which suggested that the experimental treatment of carbachol produced a lesion at the intended site. The other finding was the clinical observation of blindness in the second cat, as well as in the first cat, which in both cases occurred approximately twenty-four hours after the carbachol treatment. Thus, in the first cat, one of the effects of the carbachol lesion in the basolateral amygdala was blindness. The eyes seemed glazed-over and the pupils were abnormally
enlarged. This same syndrome appeared in the case of the second cat following carbachol intervention. However, in the case of the third cat, in which the cannulas were misplaced through known experimental error, the application of carbachol failed to cause blindness. Therefore, one could deduce that a lesion in the basolateral amygdala of the second cat was accomplished by means of the carbachol treatment.

Also, in the case of the second cat, the issue of experimental error or artifact should be explored with regard to the surgical intervention which ostensibly localized the effects of the carbachol lesion. In order to effectively lesion the stria terminalis, using stereotaxic techniques, one must concede to the fact that to some extent the tissue immediately surrounding the target-point of the lesion would inadvertently be included within the resultant lesion. Yet, even if one were to maximize the implications of this concession upon evaluating the results, the following statement could still be made. That is, the experimental intervention that did occur, did in fact, localize the effects of the epileptiform lesion. Such data, even at this level of analysis, could have significant implications for applied clinical treatment of epilepsies in which the origin of the focus is believed to be within the temporal lobe region. Thus, for example, it may be possible to surgically control the occurrence of clinical seizures when the focus is
believed to be contained in that area by means of interrupting fiber tracts leading to the hypothalamic structures. Whereas, presently the surgery associated with abolishing epilepsy in that area usually necessitates the complete removal of the temporal lobe.

However, for the experimental purposes which gave impetus to this study, it may be fruitful to attempt a determination of the physiological elements that may have been involved in terms of the experimental outcome.

The proposed site of the electrolytic lesion was at a point dorso-medial from the basolateral amygdala. This site was selected with the intention of interrupting the stria terminalis without involving the basolateral component of the amygdala in the electrolytic lesion. The clinical observation of blindness suggests that the electrolytic lesion was accomplished without involving the basolateral amygdala itself in this lesion. That is, the animal did not develop any visual deficits after the electrolytic lesion but blindness did occur after the carbachol treatment.

The experimental data at present indicate that a self-sustaining epileptiform discharge can only be localized or abolished by either effective interruption of the neural pathway systems necessary for the conductance of the discharge, or by effective ablation of the focus itself (Thompson, 1967). Thus, if
one asserted the notion that perhaps those cells that may have been inadvertently ablated in the electrolytic lesion were accountable for localizing the effects of the carbachol lesion, then one would confront the task of demonstrating the existence of amygdaloid fiber tracts at the site other than the stria terminalis. A review of the literature relevant to this question failed to note the existence of any fiber tracts in the proposed area of the electrolytic lesion, other than the stria terminalis, which have direct connection to the amygdala. Therefore, while exact certainty cannot be established, the aforementioned considerations suggest that the carbachol lesion was localized by means of interrupting the stria terminalis at a point between its issue from the amygdaloid region and the hypothalamic nuclei.

The blindness which developed in the first two cats seems to be associated with the experimental lesion in the basolateral amygdala. Speculation as to the cause of this phenomenon brings into consideration the anatomical aspects of this region of the brain. Papez has conducted many investigative studies concerned with the amygdala and its neural connections. In one such study (Papez, 1945), the fiber tracts of the amygdaloid region were delineated. There appears to be a fiber tract which has been referred to as the "loop of Archambault and Meyer." These fibers arise from the lateral geniculate and loop around the amygdala.
and transverse across into the pretectal region and then to the visual cortex. Lesions of the amygdala in monkeys have often resulted in visual or visual association deficits (Schreiner & Kling, 1953). Ostensibly, such visual abnormalities have been a result of interrupting this fiber tract at its point within the amygdaloid region. Therefore, the resultant blindness in the first two cats may have occurred because the carbachol lesions engendered interruption of this fiber tract at the site of the amygdala.

Papez (1945) also was able to trace the course of the stria terminalis. The stria terminalis is composed of five bands of fibers that issue from the lateral, basal, accessory, medial, and central nuclei of the amygdala. These fibers "loop" around to connect with the hypothalamic nuclei. The stria terminalis comprises the only direct efferent system from the amygdala to the hypothalamus.

The existing amygdalo-thalamic connections that have been found include fiber tracts from the amygdaloid area to the anterior portions of the thalamus (Chusid, 1970; Fox, 1949; & Papez, 1945). In addition, the temporo-thalamic fasciculus of Arnold which enters the posterior thalamus and then spreads medially into the nucleus pulvinaris medialis is believed to have its issue from the amygdala or the hippocampal gyrus (Fox, 1949).

One of the hypotheses that was promulgated in this study
concerned the function of the thalamic reticular systems with regard
to the spread of epileptiform discharge. A large body of data in-
dicated that a long, sustained after-discharge could be maintained
within what has been called the cortico-thalamic reverberating
circuit (Chang, 1950; Dempsey & Morison, 1942a, 1942b; Fields et
al., 1949; Hunter & Jasper, 1949; Jasper, 1949; Penfield & Jasper,
1954). Moreover, if sufficient stimulation was applied within
these systems in cats it was found that generalized convulsive
seizures occurred (Hunter & Jasper, 1949).

In this study, the epileptiform lesion which was experi-
mentally induced in the basolateral amygdala remained localized
in the second cat in which the stria terminalis was experimentally
interrupted. However, the amygdalo-thalamic connections osten-
sibly remained intact. Yet, there were no clinical manifestations
of epileptic seizure activity. Although these results may appear
to be at variance with the aforementioned data, careful considera-
tion of the data may suggest that no actual incompatibility of the data
exists. In the attempt to construct a viable interpretation of the
experimental outcome in this study, two major inter-related
elements of the epileptogenic phenomenon will be considered. First
of all, the notion of the intensity or magnitude of the epileptiform
discharge will be considered. Secondly, the manner in which the
local discharge spreads from one area of the brain to another will
be examined.

Generalized convulsive seizures are often characterized as states of excessive electrical voltage, or hypersynchrony. Hypersynchrony may occur in two forms. One form is demonstrated by a large number of unit discharges where each unit discharge is of normal voltage but the aggregate effect of so many units firing in synchronous rhythm appears as excessive voltage on the electroencephalogram. The other form of hypersynchrony occurs when a lesser number of unit discharges are involved but in which each unit discharge is of extremely high voltage. Generally the excessive voltage associated with epileptiform discharge involves the combined features of both of these forms of hypersynchrony (Penfield & Jasper, 1954). Thus, there may be a large number of units firing in synchronous activity and each unit may be much more than normally active. Therefore, while the primary focus of an epileptiform discharge may involve localized unit discharges of excessive voltage, the manifestation of the epileptic spread may depend on the recruitment of many more synchronously firing units.

Thus, in this experiment, the epileptiform lesions in the basolateral amygdalae of both affected cats commanded an intrinsic high-voltage discharge. However, in the case of the second cat, it is suggested that the spread of the localized high-voltage discharge did not occur because of some failure in recruiting a
sufficient number of synchronous-firing units in other brain areas.

If credence is given to the supposition that the diffuse projection systems of the thalamic reticular structure are crucial in terms of recruiting synchronous discharge from a large number of units in the brain, then the results of this study can be interpreted in the following manner.

Although the amygdaloid region seems to have fiber-tract connections with certain thalamic nuclei, it does not necessarily follow that the amygdala has direct issue within the thalamic reticular system. It has been shown that only certain portions of the thalamus potently affect the spontaneous resting rhythms of the cortex (Chusid, 1970; Machne, Calma, & Magoun, 1955; Penfield & Jasper, 1954). These portions include the nonspecific projection nuclei which receive afferent fibers from the ascending reticular system. The nuclei of the midline, the central medial nucleus, and portions of the ventralis medialis have been included within this reticular projection system. These areas have been included in the reticular system because widespread recruiting responses occurred following their local stimulation. It appears that the amygdalo-thalamic interconnections do not directly involve these specific areas of the thalamic reticular systems.

The amygdala does, however, have close relations with the hypothalamus in terms of known fiber-tract connections (stria
terminalis). Moreover, experimental data suggest that the activities of the amygdala are integrated within the hypothalamus and that the former may modulate the activities of the latter. Electrical stimulation of the amygdala has elicited respiratory changes, cardiovascular changes, pupillary changes, salivation, and piloerection (Gloor, 1960). Feeding behavior, attention, fear, and rage appear to be the most common integrated patterns of behavior following stimulation of the amygdala (Goddard, 1964). All of these behavior patterns can be demonstrated by differential stimulation of the hypothalamic nuclei (Thompson, 1967). Papez and others have asserted the notion that the hypothalamus serves as the central integrating structure for such activities. Therefore, it would appear that the hypothalamus could be a primary receiving area of amygdala discharge activity.

Feldman and Waller (1962), and others, have shown that certain hypothalamic lesions will dramatically affect the arousal mechanisms of the ascending reticular system. Such integrated patterns of behavior as fear, rage, and feeding, require the facilities of attention and readiness for action. The recruitment of the many sensorimotor modalities and other brain regions seems to be accomplished through the diffuse projection systems of the thalamic reticular structures. Therefore, this would indicate that the hypothalamus has direct and significant "input"
within the thalamic reticular systems.

The results of this study suggest, therefore, that the hypothalamus may function as a crucial secondary focus from which the spread of an epileptiform discharge originating in the amygdala may be extended to the thalamic reticular systems. Interruption of the spread of the epileptiform discharge from the amygdala to the hypothalamus by means of a lesion to the stria terminalis may have the effect of confining or localizing the epileptiform discharge.

The results of this study can only yield speculative data. In this vein, it is hoped that future experiments in this area of investigation will place the results of this study in perspective either through confirmation or disconfirmation. Thus, at this point, one can only conjecture as to the implications of this study. Perhaps future research will be able to demonstrate more clearly the synergistic effects of certain neural systems in the brain. One of the major implications derived from this study is that the physiological functions of a given area in the brain may be better understood in terms of its connections to other areas. So that the functions of a discrete anatomical area in the brain are best described as the emergent functions of those other neural structures from which it receives fiber-tract connections. Thus, it would seem that the epileptic seizure is a dramatic manifestation of neural synergy.
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A NEURO-CLINICAL STUDY OF THE SPREAD OF EXPERIMENTALLY INDUCED EPILEPTIFORM DISCHARGE FROM THE AMYGDALA IN CATS

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This study attempted to investigate the spread of epileptiform discharge from an experimentally induced epileptiform lesion in the basolateral amygdalae in cats. Previous experiments have demonstrated that carbachol (choline carbamate), when applied to the basolateral amygdalae in cats, produced a self-sustained epileptiform focus. Three adult cats were used in this experiment. Carbachol was delivered through a chronically implanted cannula to the basolateral amygdala in the first cat. Clinical epileptic seizures resulted from the carbachol treatment. The stria terminalis was electrolytically lesioned in the second cat, prior to carbachol treatment. This second animal did not exhibit any clinical epilepsy following the application of carbachol. The third cat provided an unanticipated source of control data since it was discovered that the cannulas were misplaced. Carbachol treatment failed to produce any seizures in this cat.

A discussion of the results and their implications are presented.