1961

Effects of Some Enzyme Inhibitors on the Threshold to Cortical Desynchrony by Reticular Formation Stimulation

Seward A. Ridlon
Loyola University Chicago

EFFECTS OF SOME ENZYME INHIBITORS ON THE THRESHOLD TO CORTICAL DESYNCHRONY BY RETICULAR FORMATION STIMULATION

by
Seward A. Ridlon

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September

1961

LIFE

Seward A. Ridlon was born June 14, 1927 in Montpelier, Vermont.

He graduated from Concord, N. H. high school in 1945 and from the University of New Hampshire in February, 1953, with a degree of Bachelor of Science. He returned to the University of New Hampshire in 1955 and was awarded the degree of Master of Science in June, 1956. He continued his graduate studies at Loyola University, Stritch School of Medicine, entering the Pharmacology department in September, 1958.

During the period from 1945 to 1947, he served in the U. S. Navy. He was employed as a medical service representative by The E. L. Patch Co. from 1953 through 1955. From 1956 through 1958 he was employed by Armour Pharmaceutical Co. as a junior scientist.

He was married in 1955 and has three children.

ACKNOWLEDGEMENT

Many people have been of help to me during the research and writing of this dissertation. The advice and criticisms of my work given by Dr. Y.T. Oester, Dr. C. D. Proctor and my advisor, Dr. A. G. Karczmar have been especially appreciated. Also, the help of Dr. W. Wellband of the Anatomy department in the preparation of the gross sections has been invaluable.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The pharmacology and physiology of the reticular formation has been the subject of many investigations since its role in producing cortical desynchronization was elaborated by Moruzzi and Magoun (1949).

Moruzzi and Magoun showed that electrical stimulation of the reticular formation with a high frequency, low voltage train of impulses produces an electrical pattern in the cortex similar to that seen when the sciatic nerve is stimulated or when the animal is behaviorally aroused. The resultant electroencephalographic pattern has been termed an arousal or alert pattern.

Further work by Magoun and Moruzzi showed that lesions placed bilaterally in the reticular formation produced an animal which was both behaviorally and electroencephalographically incapable of being aroused.

Acute experiments in which the spinal cord was transected at C-1 (encephale isole preparation of Bremer, 1934), showed that these spinal animals could be easily behaviorally alerted even though the brain was, in effect, disconnected from most of the peripheral sensory pathways. Because it can produce this cortical

effect, the reticular formation is called the Ascending Reticular Activating System (ARAS).

A. Anatomy and Physiology of the Reticular Formation.

The ARAS is essentially a multineuronal sensory relay pathway which receives collaterals from all primary sensory systems. It also receives input from the cerebellum and the cerebral cortex. The ARAS has many projections to the cortex and is intimately connected to the thalamus through the intralaminar nuclei and the center median nucleus, (Nauta and Kuypers, 1958; Scheibel and Scheibel, 1958; Olszewski, 1954; French, 1958; cf. also Fig. 1). The use of the Nauta technique (Nauta and Gygax, 1954) which consists of studying the degeneration of axons after placing a small lesion in the reticular formation, has resulted in much information regarding the efferent connections of the reticular formation. Nauta and Kuypers (1958) showed that the reticular formation in cats contains axons originating at its medullary and pontine levels, which are intermingled with other diffuse fiber systems including the ascending spinal and trigeminal pathways.

Scheibel and Scheibel (1958), using the Golgi staining method to delineate the synaptology of the reticular formation, have shown that the fibers in a long ascending sensory system, such as the medial lemniscus, send collaterals at right angles from its path into the reticular formation. Before these fibers reach the dense neuropil in the center of the reticular core, they send

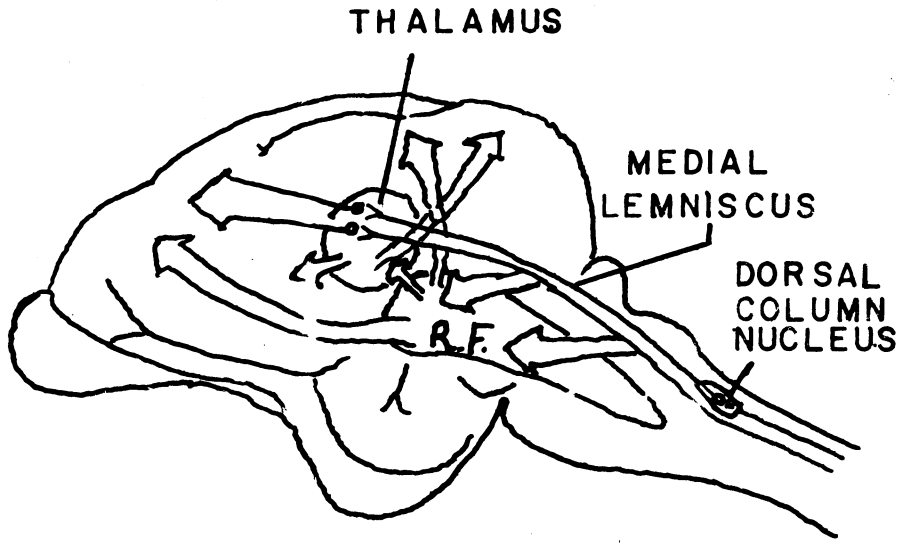

Figure 1.

The afferent and efferent connections of the ascending reticular activating system (ARAS) (Redrawn from Killam et al., 1958).

collaterals in all directions within the reticular mass. The degree of overlap between collaterals entering the reticular formation is so great Scheibel and Scheibel (o.c.) say "that it is difficult to see how any specificity of input can be maintained." By some interesting mathematics, these investigators calculated that 4125 cells are within the area of potential interaction of the soma and dendrites of any one element. It is not difficult, then, to imagine why the reticular formation is so susceptible to electrical stimulation and to drug action.

Olszewski (1954) has described the reticular formation as not a morphological unit but as being composed of many different (structurally) nuclei. He has described nearly 100 different nuclear aggregates in the brain stem reticular formation and proposed that one should, wherever possible, describe the particular nuclei in question rather than use the general term "reticular formation".

The reticular formation has been shown by Magoun and Rhines (1946), Henneman (1949), and Kaada (1950) to have both a facilitatory and an inhibitory effect on the monosynaptic reflexes of the spinal cord. Stimulation in the anterior lateral portion of the reticular formation will increase the response of the patellar reflex while stimulation in the posterior medial portion will inhibit this response. Granit and Kaada (1952) and Eldred and Fujimori (1958) have clearly demonstrated that the reticular forma-

tion controls not only the alpha efferent pathway but also the gamma efferent pathway.

Himwich (1957) described this descending extrapyramidal pathway as being involved in producing smoothly functioning motor activity by coordinating the activity of voluntary muscles. Drugs which stimulate the reticular formation to hyperactivity may produce muscle tremors by increasing or disorganizing the descending reticular pathways and at the same time produce an alert or desynchronized EEG pattern in the cortex. On the other hand, drugs which depress the corticifugal and corticopetal influences of the reticular formation may be useful in Parkinson's disease and may produce a sleep-like or synchronized EEG pattern.

Rinaldi and Himwich (1955) and Himwich (1959) have combined the two diffuse projection systems (ARAS and the diffuse thalamic projection system of Jasper, 1949) into one system, the mesodiencephalic activating system (MDAS). These workers claim that, because the ARAS is intimately connected with the thalamus, and because impulses arising in the ARAS are mediated over the diffuse thalamic projection system, the two systems should be regarded as one.

It has been shown (Morison and Dempsey, 1942; Jasper, 1949; Starzl and Magoun, 1951) that stimulation of the diffuse thalamic projection system by a low frequency train of impulses produces, not cortical desynchrony, but high voltage, slow fre-

quency activity in the cortex. This is termed the recruiting responce. A low frequency stimulus applied to the ARAS will not produce the recruiting response, and, in order to obtain cortical desynchrony upon ARAS stimulation, a high frequency stimulus (200-300 cps) must be used.

A further differentiation of the two systems can be seen by the effect of the barbiturates and the muscle relaxants of the mephenesin type which will be discussed in the next section.

B. Pharmacology of the Reticular Formation.

1. Polysynaptic Blocking Agents.

Several workers (Henneman et al., 1949; Kaada, 1950; King and Unna, 1954; Domino, 1955) have studied the effects of polysynaptic blocking agents whose action can be demonstrated by their ability to suppress withdrawal reflexes, crossed extensor reflexes and in the main, the motor reflexes which are polysynaptic in nature. These drugs will also depress the effect of stimulation of the inhibitory and facilitatory areas of the reticular formation. These data do not indicate whether these compounds act directly on the reticular formation of the brain stem or if they are acting primarily on the spinal internuncial circuits. Zoxazolamine, as reported by Domino (1958), reduces the inhibition produced by reticular formation stimulation but does not affect the desynchronization of the cortical EEG produced by ARAS stimulation.

Funderburk et al. (1953), Morison and Dempsey (1942), King (1956) and Domino (1955) report that pentobarbital, as a representative of the barbiturates, depressed the cortical desynchronization produced by ARAS stimulation at doses which not only have no depressant effect on the recruiting response, but actually enhance the response to stimulation of the thalamic nuclei. As reported by Domino (1958) diethyl ether had a non-specific poly- and mono- synaptic depressant action and it did depress both ARAS provoked desynchronization and the thalamic recruiting response. Mephenesin, however, did not affect the EEG desynchronization produced by ARAS stimulation, but did reduce the amplitude of the recruiting response obtained at the threshold voltage.

French et al. (1953) and Arduini and Arduini (1954) had earlier demonstrated that the reticular formation response to stimulation was greatly depressed by the sedative-hypnotic and anesthetic drugs. These investigators showed that a sub-anesthetic dose of pentobarbital would block the cortical desynchronization due to ARAS stimulation, while the diffuse thalamic projection system was not appreciably affected. They pointed out that the cortical desynchronization produced by ARAS stimulation directly or indirectly, by stimulation of its afferents such as the sciatic nerve, could be blocked by ether or pentobarbital at levels which did not affect other sensory pathways, e.g. the auditory system.

2. Tranquillizers

Killam and Killam (1958, 1959) have reported that the phenothiazine derivative, chlorpromazine, only slightly increases the threshold of EEG desynchrony produced by ARAS stimulation at doses of the drug which markedly depress the behavior of a normal, intact cat. However, these workers did find that the threshold for behavioral arousal by stimulation of the diffuse thalamic projection system was markedly increased.

Hiebel et al. (1954) as reported by Killam and Killam (1957), stated that, in addition to an increase in threshold, chlorpromazine shortened the duration of desynchronization after ARAS stimulation.

Killam and Killam (1958), investigated the effects of drugs on both the afferent inflow and the rostral-caudal conduction of the reticular formation. These investigators studied the potential evoked by single shocks applied to the sciatic nerve and to the diencephalic portion of the reticular formation with recordings being made from two sites in the reticular formation. They found out that chlorpromazine enhanced the conduction time (decreased the latency) of evoked potentials produced by stimulation of both the sciatic nerve and the diencephalic reticular formation. From this, they concluded that chlorpromazine increases the reticular input and conduction and by doing so increases the "filtering" ability of the ARAS to sensory stimulation with no appreciable effect on the

ARAS cortical desynchronization ability.

Rinaldi and Himwich (1955 a,b) reported that the rauwolfia alkaloid, reserpine, had no effect on the MDAS of rabbits in doses up to 0.5 mg/Kg. At doses of 1-2 mg/Kg. of reserpine the cortical EEG pattern was continuously desynchronized and the MDAS was stimulated (o.c.). These results occurred after a latent period of only 20-30 minutes after injection of the drug. This time period, however, does not correspond with usual delay of onset of two to four hours for the resultant behavioral effects seen in normal, intact animals. However, in accordance with these observations, Killam et al. (1957) reported that reserpine in doses up to 0.1 mg/Kg. did not alter the EEG in cats and at higher doses EEG desynchronization was more apparent than in the control period. Berger et al. (1957) reported that reserpine at high doses (2.0-2.5 mg/Kg.) given to intact, unanesthetized, curarized cats caused some slowing and spindling in the cortical and thalamic EEG tracings. They report that these effects were not as pronounced as those seen after treatment with chlorpromazine or meprobamate.

3. Neurohumoral Agents

Bremer and Chatonnet (1949) showed that acetylcholine (ACh) given intracarotidly, caused a reduction in voltage and an increase in frequency of the cortical EEG, indicative of a desynchronized pattern. These investigators also showed that physostigmine had a similar effect but which was, however, more prolonged

than that of ACh. These results have been corroborated by numerous other investigators.

The use of an irreversible cholinesterase (ChE) inhibitor such as DFP (di-isopropyl fluorophosphate) has allowed a more thorough investigation of the cholinergic mechanism in the brain. Rinaldi and Himwich (1955 a,b) have shown that DFP induced cortical desynchronization and that this effect was not prevented by transection of the brain stem at the mid collicular level. An isolated slab of cortical tissue did not respond to injected DFP while the remaining intact cortex did respond in the typical manner to the DFP. This indicated to them that the site of action of DFP was at a sub-cortical level.

Bradley et al. (1953) showed that doses of 1-3 mg/Kg. of DFP produced a low voltage, high frequency EEG pattern which could be antagonized by atropine. This confirmed an earlier report by Wescoe et al. (1948), that atropine protected against the toxic effects of DFP.

Pfeiffer (1959), in reviewing the effects of the proposed ACh precursor, dimethyl aminoethanol (Deanol), concluded that after prolonged treatment with Deanol, previously depressed patients became more lucid and more energetic. Medical students included in a Deanol test, claimed to need less sleep and to feel more refreshed than during the control periods. However, Pepeu et al. (1960) found that Deanol failed to increase the brain con-

tent of ACh when given in a single dose of 500 mg/Kg. to rats or when animals were given Deanol in their drinking water for a period of 45 days. These results do not indicate that ACh is not necessary for central transmission but only that a significant increase of ACh content in neural tissue is not accomplished using this proposed ACh precursor.

Marazzi and Hart (1953, 1955, 1961), using the transcallosal technique, have reported that ACh or anticholinesterases enhance the evoked potential across the callosal pathway when injected via the carotids. Epinephrine, norepinephrine and serotonin all decrease the amplitude of the evoked potential, with serotonin being the most effective of the three compounds. The question of whether these effects might be due to a local anoxia or the result of stimulation of the carotid receptors was answered by Marazzi in a paper presented at the Federation Meetings in April, 1961. He showed that same quantity of drug injected caudally through the carotid artery had no effect on the transcallosal response. The amount of drug that Marazzi injected was minute and not likely to cause a local anoxia for the period of time during which these effects on the transcallosal response are noted. These results provide indirect evidence that these three compounds may be central neurotransmitters, but direct evidence is still lacking.

Giarman (1959) outlined the requirements necessary to implicate a compound as a central neurotransmitting agent. These

criteria are as follows: 1. That the compound in question be present in a discrete pattern of distribution, not present uniformly throughout the brain; 2. That the enzymatic mechanisms necessary for the metabolism should be present in the CNS; 3. That the pharmacological effects resulting from an increase or decrease in the proposed neurohumor be clearly demonstrated; and 4. That agents which block the effects of the proposed neurotransmitter peripherally should produce a CNS effect.

Several workers (Feldberg and Vogt, 1948; Perry, 1956; Hebb and Silver, 1956) have described the occurrence of ACh, choline acetylase and ChE in various regions of the brain. These workers have shown that ACh and the enzymes necessary for the production of ACh are found in the highest concentrations in the gray matter of the brain, particularly in the caudate nucleus and some areas of the brain stem. Sensory nuclear masses have a much higher choline acetylase activity than do the fibers themselves but the activity of the sensory areas is not as great as is found in the motor nuclei of the cord and medulla.

The problem of the type (pseudo- or true) of the cholinesterase that is present in the brain has been approached by Desmedt and La Grutta (1957). These investigators studied the effects of the two types of cholinesterase inhibitors in the brain. DFP was used as representing an inhibitor of pseudo ChE, and Ro2-1205 (N-p-chlorophenyl-N-methyl carbamate of 3-hydroxy phenyl

trimethylammonium bromide) and BW 284-C51 (3-oxo-1:5-diphenyl-pentane-pp'-bis (allyl dimethyl ammonium) dibromide.) as inhibitors of true ChE. Desmedt and La Grutta (o.c.) found that the DFP was much more effective in producing cortical desynchrony than were the inhibitors of true ChE. One might wonder, however, if the true ChE inhibitors used by these investigators would pass the blood-brain barrier as easily as the lipid soluble DFP.

Exley et al. (1958) have tried to determine whether the cholinergic receptors in the ARAS proposed by Rinaldi and Himwich (1955 a,b, 1957) were of the "muscarinic" or the "nicotinic" type. These investigators showed that cortical desynchronization as produced by ARAS stimulation, could be blocked by atropine and hyoscine but not by the quartenary atropinium compound, methanthelinium. These compounds are all of the muscarinic blocking type. Dihydro beta-erythroidine and mecamylamine which are nicotinic blocking agents, had no effect on ARAS stimulation. Yet nicotine, by itself, is known to produce a change in EEG pattern. In a large dose, 50 mg/Kg. of the base, Exley and co-workers found that nicotine produced a low voltage high frequency EEG pattern with some convulsive spikes. Because of these results, Exley et al. concluded that the cholinergic mechanism acting in the brain was muscarinic in nature. Longo (1954), using ganglionic blocking agents, showed that these compounds would also block ARAS stimulation as does atropine. These compounds are "nicotinic" in action.

Longo (o.c.) described this as a cholinergic mechanism and did not try to delineate the nature of the mechanism further.

4. Adrenergic Compounds

Many investigators (Bradley and Elkes, 1953 a,b; Bradley and Key, 1958; Bradley, 1958; Bonvallet et al., 1954; Dell and Bonvallet, 1956; Dell, 1960; Exley et al., 1958; Rothballer, 1956, 1957, 1959; Domino, 1958) have reported on the effects of sympathomimetics on cortical excitability. Rothballer (1959) reviewed the evidence pertaining to the central actions of the adrenergic compounds. He classified these compounds as follows:

1. Compounds having fast onset of action and causing brief activation of the cortical EEG. To this group belong epinephrine, norepinephrine, and phenylepinephrine.
2. Compounds having no immediate effect but a profound lowering of the threshold to epinephrine. Cocaine is an example of this group.
3. The drugs in this group have the combined properties of the first two groups. An example is methamphetamine.

Bonvallet and her co-workers (1954) have proposed, a result of their investigations, that a part of the ARAS could be considered as epinephrine sensitive. They found that in animals which were transected at C-1, epinephrine, given intravenously, caused an intense desynchronization of the EEG. If an intracollicular transection was made which passed ventrally rostral to the pons, epinephrine would not produce desynchronization. When the

intracollicular section passed caudal to the pons, epinephrine was still effective in producing desynchronization. Bonvallet et al., (1954) concluded therefore, that the activation of the cortical EEG by epinephrine was not due to direct action of that drug on the cortex but to an increased activity of this "pie-shaped" wedge in the mesencephalic portion of the ARAS (cf. Fig. 2).

Rothballer (1956, 1957) has extended this work to include studies in which the carotid receptors were denervated to prevent stimulation of the brain stem from these sources. He concluded from his results that epinephrine exerts its effects directly on the neural elements in the ARAS rather than through a vascular reflex or sensory mechanisms. He postulated that the only neurons primarily responsive to adrenergic compounds are in the mesencephalic tegmentum while the cholinergic mechanism may be located more rostral between the mesencephalon and the centrum ovale (center median nucleus of the thalamus).

DeMarr and Martin (1956) reported that repeated doses of epinephrine produced increasingly feeble activation and finally an increase in spindles. Norepinephrine, which has been shown by Vogt (1954) to be the predominant sympathetic amine in the brain, did not produce desynchronization and repeated administration of norepinephrine caused slowing and the appearance of 6-12 cps spindles in the EEG of the cat (DeMarr and Martin, o.c.).

A search of the literature has revealed only two papers

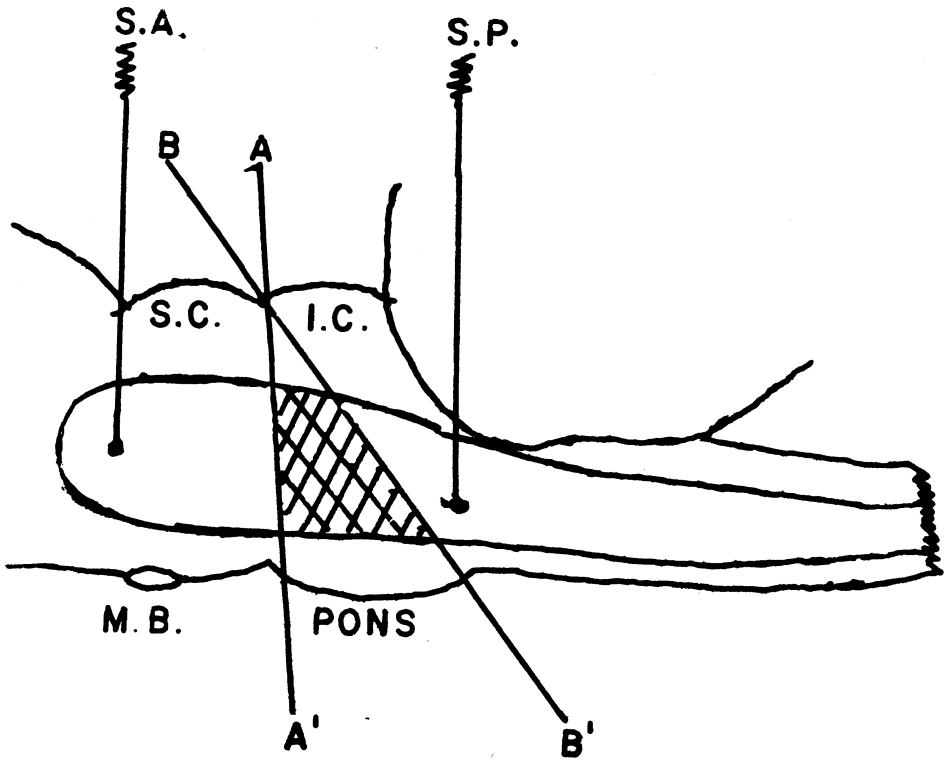

Figure 2.

Schematic representation of the reticular formation. The cross-hatched area between lines A-A' and B-B' represents the epinephrine sensitive portion of the ARAS as described by Bonvallet et al., (1954). S.A. and S.P. represent the anterior lateral and the posterior medial stimulating sites used in the experiments being reported here. S.C. - superior colliculus; I.C. - inferior colliculus; M.B. - mammillary bodies.

concerned with the effects of monoamine oxidase inhibitors on ARAS stimulation. Kuehn and Schallek (1958) and Schallek and Kuehn (1959) reported that iproniazid (20 mg/Kg. I.P. for 5 days or 150 mg/Kg. I.V. acutely) had no effect on the ARAS threshold nor did this compound alter the control EEG.

Vogt (1954) investigated the central localization of epinephrine and norepinephrine in dogs and found that the highest concentrations were in the hypothalamus and the area postrema. The gray striatum around the aqueduct and the vasomotor centers in the midbrain also showed a high concentration of the sympathetic amines. All myelinated fiber tracts, the cerebellum and most of the telencephalon contained negligible amounts.

Amin et al. (1954) and Crawford (1958) presented evidence that, with the exception of the caudate nucleus, the distribution of serotonin paralleled that of epinephrine and norepinephrine. The enzymes necessary for the metabolism of the serotonin are known to occur widely in nature (Dalgleish, 1958 and Blaschko, 1958) and are found in the central nervous system in parallel with serotonin.

C. Purpose of this Research.

From the evidence presented above, it can be suggested that more than one compound could be considered for the role of a transmitter substance in the ARAS and the diffuse projection

system. The distribution of ACh, serotonin and the catechol amines in the CNS also indicate that it is possible that there could be interactions between these and other naturally occurring substances.

There is also indirect evidence that the ARAS contains both cholinergic and adrenergic sensitive elements. Serotonin has not yet been implicated in the transmission in the ARAS and its projections, but its distribution in the CNS and the proposal that it is implicated in the mechanism of action of reserpine, as well as other pharmacologic data to be discussed later (cf. Discussion), makes it too, a likely candidate for neurotransmitter activity.

In consideration of the possibility of at least two neurotransmitter mechanisms active in the ARAS and its diffuse projection system, it was decided that an investigation of the response to threshold stimulation of the ARAS in the presence of altered levels of endogenous substances should be undertaken. Rothballer's (o.c.) suggestion that a part of the ARAS consisted primarily of epinephrine sensitive neurons and also his proposal that the anterior ARAS was cholinergic while the posterior ARAS might be adrenergic, led us to select two widely separated ARAS sites for stimulation. The choice of the two sites depended mainly on the distance between them and on the dependability of the cortical response to stimulation.

Substances such as the anticholinesterases (anti ChE) and monoamine oxidase inhibitors (MAOI) have been used to analyze

transmission systems by many investigators. These agents, when used in sufficient dosages, do cause an increase in the endogenous neurotransmitter substances, and it was felt that the use of these enzyme inhibitors would be more suited to our purpose than would the use of those compounds which mimic the action of the proposed transmitter but do not affect the normal brain levels of these agents. Consequently, we would be able to investigate the effects of increasing the levels or decreasing the rate of enzymatic decreasing the rate of enzymatic destruction of endogenous substances in the brain and the resultant effect on the cortical response to stimulation of the two sites of ARAS by the use of these enzyme inhibitors.

CHAPTER II

METHODS AND MATERIALS

A total of 73 high spinal (C-1 section) cats were used in these experiments. The animals were prepared under ether anesthesia and two hours elapsed between the time ether was discontinued and control threshold values were obtained.

The routine operative procedure carried out under ether consisted of cannulating the trachea, femoral vein and femoral artery, and of the cord section.

It was found that by instilling a small amount (0.5 ml) of 2% xylocaine subdurally at the C-1 level* most of the consequences of spinal shock were avoided. For instance, the blood pressure of the cats after sectioning under a local anesthetic usually was between 90 and 110 mmHg systolic, there was no massive spinal reflex movement during the section, and the monosynaptic (knee jerk) reflex was readily evoked immediately after the section was completed. Xylocaine (2%) was instilled in all cut edges and around the ear bars.

Phonograph needle electrodes (Hoagland, 1940), were used to record the EEG from the anterior sigmoid and supra sylvian gyri. In order to minimize the stimulus artifact, bipolar recordings were

* When respiration ceased, the section of the cord was performed.

made with an intraelectrode distance of 3-5 mm.

The stimulating electrodes used were of two types, one being the bipolar coaxial type supplied by Lehigh Valley Electronics (#1435-6" with 1 mm spacing between electrode tips), the second consisting of #24 gauge nichrome wire with a Formvar enamel coating which was twisted and cut to give a smaller (0.5 mm) tip spacing. These latter electrodes were fitted inside an 18 ga. needle tubing* which was insulated. It was found that there was an inverse relationship between the threshold voltage and the electrode tip spacing.

All recordings were made on a Grass 111 D six channel EEG with an added seventh channel for recording blood pressure. Five of the six channels were utilized in recording the EEG while the sixth monitored Lead 11 of the animals' EKG. The blood pressure was recorded from the femoral artery via a Statham P 23A strain gauge. This was fed to the EEG through a separate Grass low level DC preamplifier (Model 5 PIE) and Grass DC Driver Amplifier (Model 5D).

A Grass Model S4 stimulator with a stimulus isolation unit served for stimulation of the reticular formation. A five second train of square wave impulses, frequency of 300 cps and a pulse duration of 0.5 msec, was used. The voltage was the only

---

* Kindly supplied by C. A. Roberts Co. of Franklin Park, Illinois

variable.

Recordings of the EEG of spinal, unanesthetized cats show predominantly a low voltage, high frequency or desynchronized pattern. This type of EEG in a normal, intact, untreated cat is indicative of a behaviorally awake, aroused animal. A normal, intact cat which was behaviorally asleep would show an EEG pattern consisting of high voltage, low frequency waves interspersed with short periods of low voltage, fast activity. The transition of a normal cat from behavioral sleep to an aroused state is accompanied by an increase in desynchrony (low voltage, fast activity) in the EEG. By injection of atropine, the EEG pattern becomes synchronized with a preponderance of high voltage, low frequency waves with very few, if any, periods of desynchrony evident.

Atropine sulfate (0.5 mg/Kg.) was injected I.V. at least one hour before control threshold readings were determined, to produce a stable, hypersynchronous EEG tracing, and also to alleviate any peripheral effects of the anticholinesterases used. Gosselin et al. (1955) showed that atropine given I.V. to mice was rapidly excreted in the first hour; subsequently, the rate of excretion is reduced so that the difference in the amount excreted in the next 4-8 hours is small. Wikler (1952) reported that atropine sulfate given to dogs produced a stable, sleep-like EEG for several hours. 1 mg/Kg. I.V. of atropine sulfate in a normal, intact cat produces a hypersynchronous EEG pattern which is not significantly altered

in 24 hours (Bastian and Ridlon, unpublished). In the present investigation, 3 control atropinized cats were tested for threshold variations over a period of three to five hours. There was no appreciable change during this time (Figure 8).

The stimulating electrodes were placed stereotactically in two points in the reticular formation with the aid of a Stoelting stereotaxic apparatus. The points selected represent the anterior lateral and posterior medial portions of the reticular formation. Rhines and Magoun (1946); Henneman (1949) and Kaada (1950) demonstrated that stimulation of the anterior lateral site had a facilitatory and the stimulation of the posterior medial site an inhibitory effect on the monosynaptic patellar tendon reflex in anesthetized animals. It was found in preliminary studies that these two areas produced good cortical desynchronization at low voltages. The animals remained in the stereotaxic apparatus for the duration of the experiment. Body temperature was controlled by heating the animal with a G.E. 250 watt infra red reflector lamp.

At the conclusion of the experiment, a high voltage D.C. pulse was applied to the stimulating electrodes to coagulate the track so that the electrode track could be easily determined on sectioning. The animal was then perfused with a 10% formalin solution through both carotids. The brain was removed and kept in formalin (10%) until sectioned. The sectioning was done by hand

and the stimulation point identified. In a few animals whose thresholds seemed to be inordinately high, the stimulation point was found to be at least 0.5-1 mm above the expected site in the reticular formation. However, variations of less than 0.5 mm made no significant change in threshold. The response to stimulation and drugs was similar irrespective of the threshold voltage.

Among cholinergic and adrenergic agents of interest for this investigation, those drugs were chosen which could be expected to exhibit their peak activity within the time course allowed by the use of atropine.

The anticholinesterases used were EPN (ethyl-p-nitro phenyl thionobenzene phosphate), malathion (S-(1-2dicarboxyethyl) o-o-dimethyl dithiophosphate), DFP (diisopropyl fluorophosphate), and physostigmine. With the exception of physostigmine, all the anticholinesterases, because of their instability in aqueous or saline solution, were solubilized in carbo wax (PEG 200). Whenever possible, microliter quantities of these drugs were injected intravenously.

The monoamine oxidase inhibitor, JB-835 (1 phenyl, 3 hydrazino-butane)* was chosen because it has no amphetamine-like actions and, as shown by Spector et al. (1960), produces 75-90% inhibition of the central monoamine oxidase in 30 minutes. In

---

* Kindly supplied by Dr. H.H. Biel of Lakeside Labs., Milwaukee, Wisconsin.

order to increase the level of serotonin in the brain small doses (1 mg/Kg.) of the serotonin precursor, 5-OH tryptophan (5HTP) were given I.V. fifteen minutes after the JB-835. These drugs were all soluble in saline to the extent that it was rarely necessary to give a total amount of fluid over 2 ml.

The threshold for cortical desynchronization was checked at 15, 30, 60, 90, and 120 minutes after the administration of each drug or after the administration of the second drug in those experiments where the precursor was used.

Following qualifications were set to delineate the control threshold:

1. Cortical desynchrony should begin immediately on stimulation.
2. Cortical desynchrony should continue throughout the 5 second stimulation period.
3. Control pattern should return as soon as the stimulation is completed.

In the following text, the term threshold is meant to indicate that voltage necessary to produce a cortical desynchrony meeting the criteria outlined above when applied to the two ARAS sites.

Figures 3-6 represent typical brain sections and electrode placements seen in our experiments.

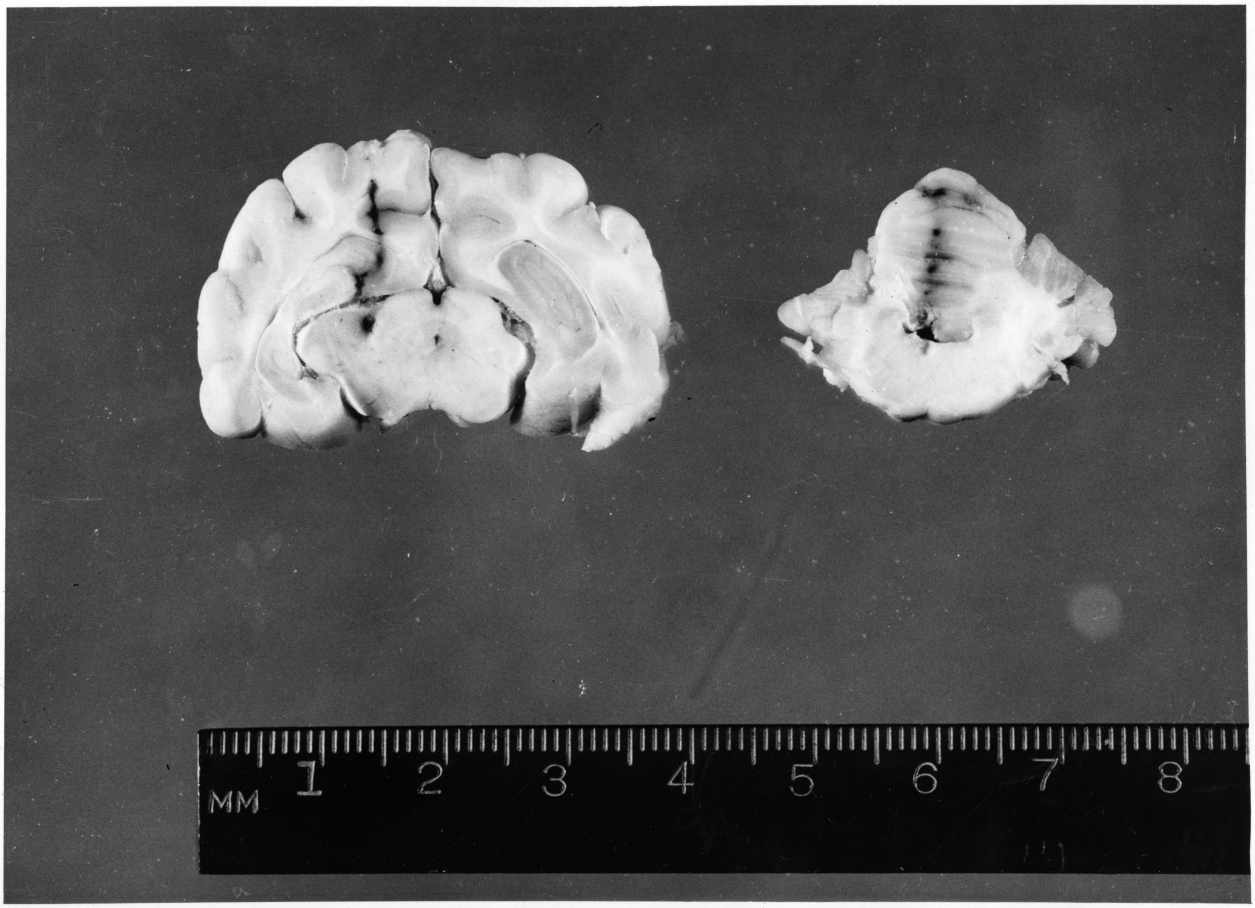

Figure 3.

Photographs of gross sections of cat brain showing the electrode tracks and the variations in stimulating sites.

Figure 4.

Photographs of gross sections of cat brain showing the electrode tracks and the variations in stimulating sites.

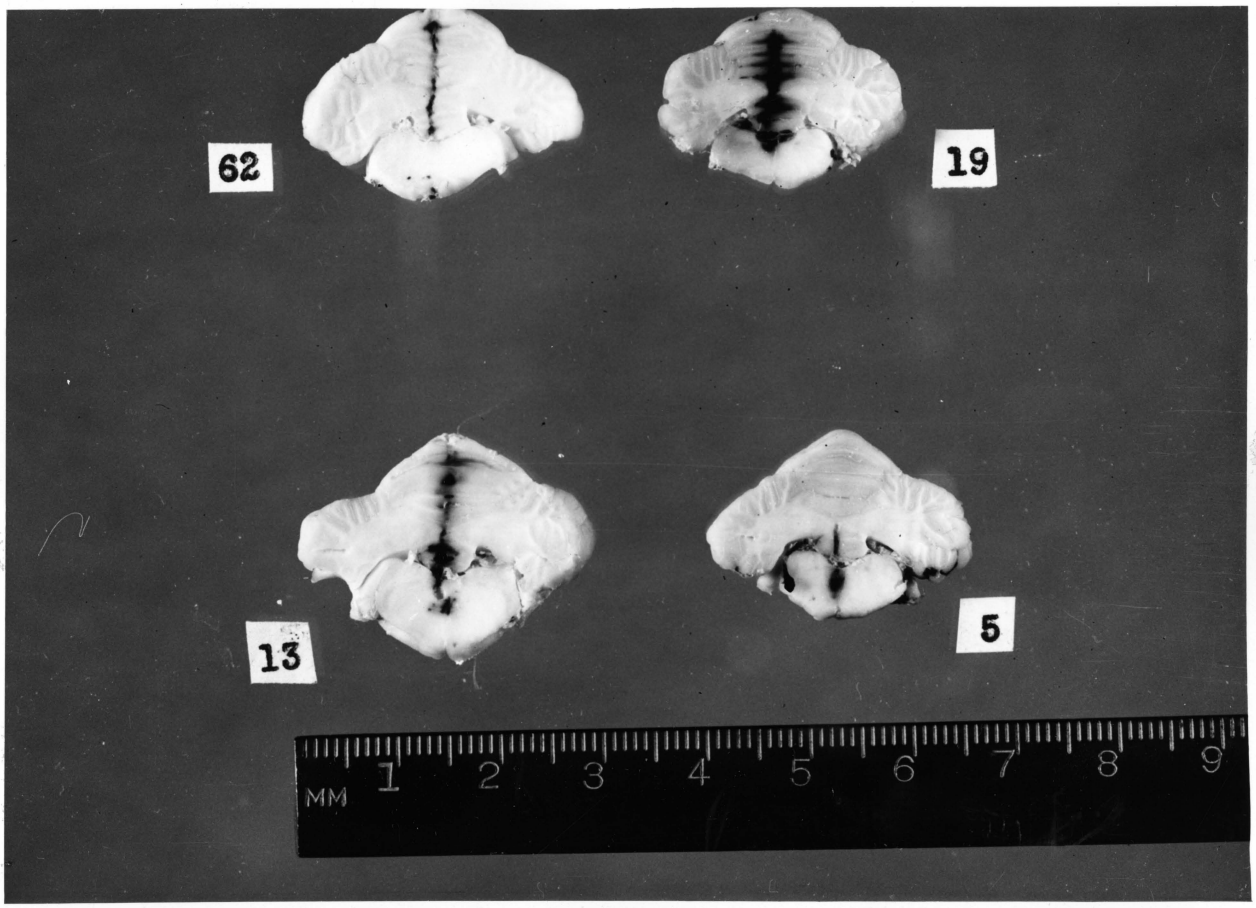

Figure 5.

Photographs of gross sections of cat brain showing the electrode tracks and the variations in stimulating sites.

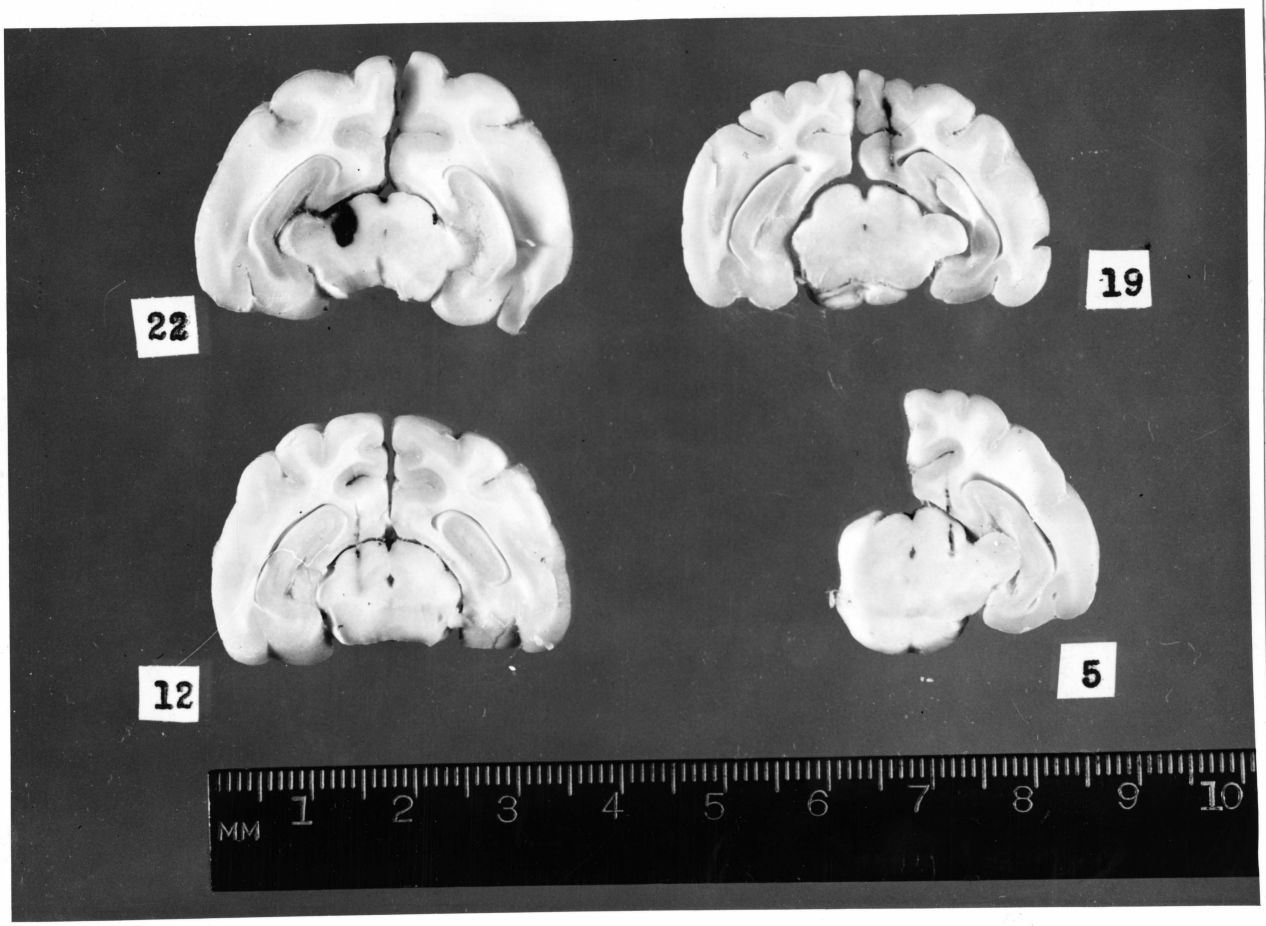

Figure 6.

Photographs of gross sections of cat brain showing the electrode tracks and the variations in stimulating sites.

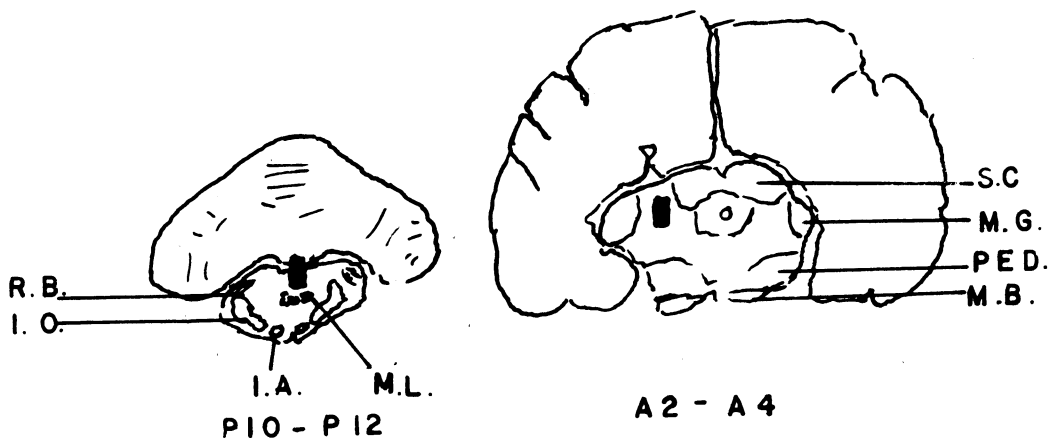

Figure 7.

Drawings of the typical sections with the stimulating sites represented by the filled rectangles. R.B.- restiform body; I.O.-inferior olive; I.A.-pyramidal tract; M.L.- medial lemniscus; S.C.- superior colliculus; M.G.- medial geniculate; Ped.- peduncle; M.B.- mamillary body; P 10 - P 12 and A 2 - A 4 represent the stereotaxic frontal planes in Jasper and Ajmone-Marsan (1957).

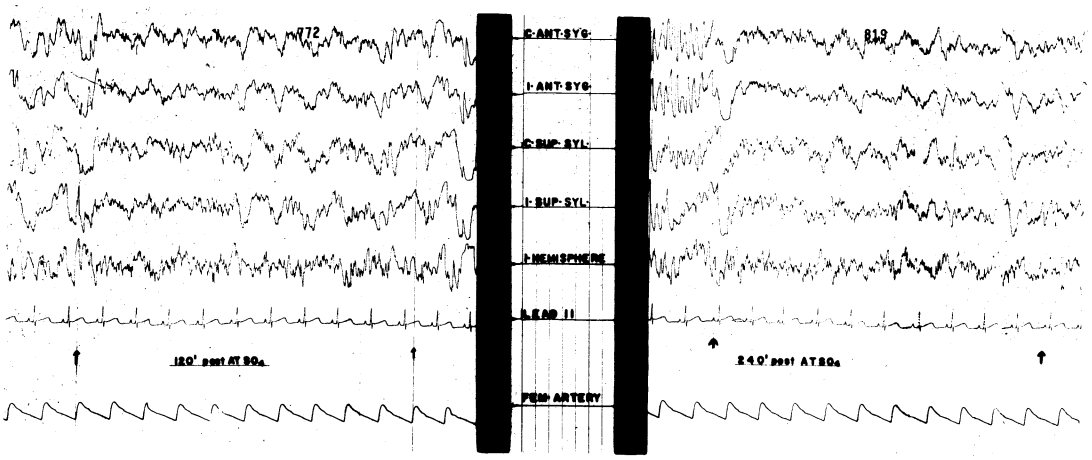

Figure 8.

Atropine control record. Threshold stimulation of 1.5 volts showed no change in resultant desynchronization pattern over a period of 3-5 hours.

CHAPTER III

RESULTS

A. Anticholinesterases.

Previous reports by Karczmar et al. (1959), Awad et al. (1959), and Ridlon and Karczmar (1961) have shown that in atropinized cats, the two organic phosphorus containing anticholinesterase insecticides, EPN and malathion, have little or no effect on the EEG at dose levels approximating their LD_{50} while combinations of the two agents readily antagonize an atropine EEG pattern in cats at levels of each which show no anti-ChE activity (Figure 9). This prompted the investigation of the effects of small doses of anti-ChE on ARAS stimulation. As mentioned in the methods, all experiments being reported here were carried out in atropinized, spinal cats.

DFP at a dose of 50 mcg/Kg. I.V. produced a continuously desynchronized cortical EEG in two animals so that the threshold was essentially reduced to zero. At a dose of 20 mcg/Kg. I.V., DFP, in five animals, did not appreciably lower the threshold at either site of ARAS stimulation although there was a definite prolongation of desynchrony at control threshold and submaximal voltages (Figure 10).

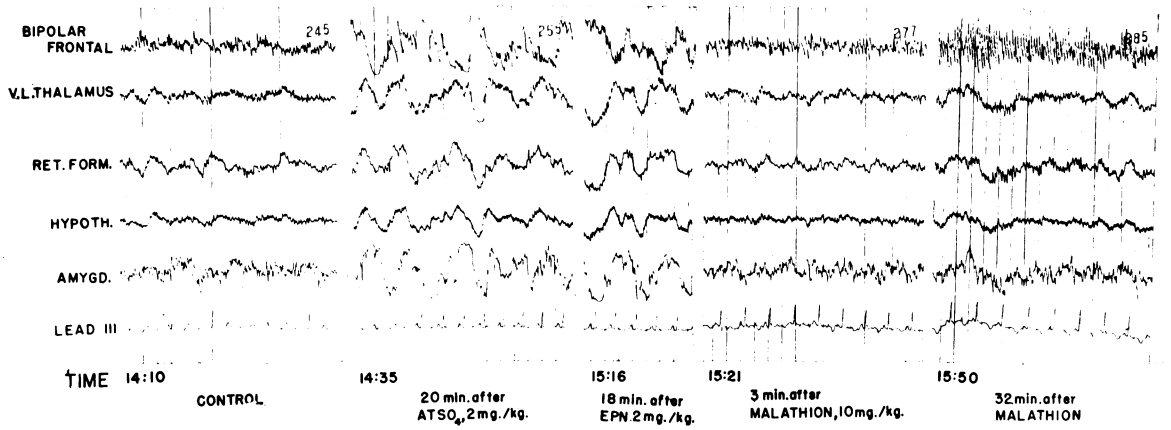

Figure 9.

2.3 Kg. male cat. Combination of 2 mg/Kg. EPN followed in 20 min. by 10 mg/Kg. I.V. malathion produced a spontaneously desynchronized EEG pattern within 3 min. No stimulation.

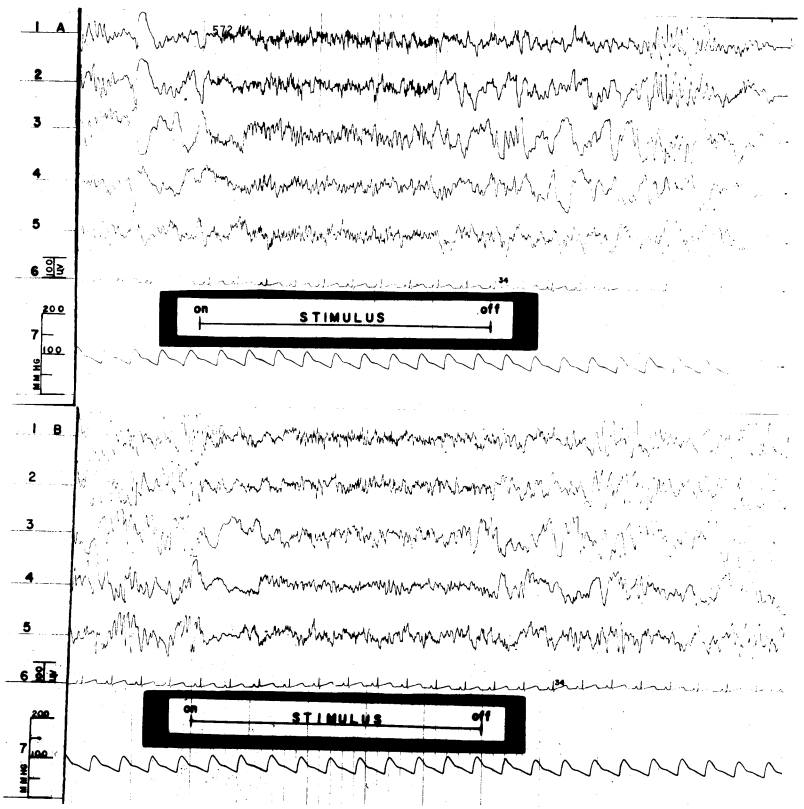

Figure 10.

Cat #34. 2.5 Kg. male. DFP 0.02 mg/Kg. I.V. Anterior lateral ARAS stimulation. A. Control threshold stimulation of 3.0 volts. B. Stimulation of 3.0 volts 30 min. after the injection of DFP produces a prolongation of desynchronization but no change in threshold. 1.-Contralateral anterior sygmoid gyrus; 2.-ipsilateral anterior sygmoid gyrus; 3.-Contralateral suprasylvian gyrus; 4.-Ipsilateral suprasylvian gyrus; 5.-Ipsilateral hemisphere; 6.-Lead ll of the EKG; 7.-Blood pressure as recorded from the femoral artery. The channel alignment is the same in all the following figures. Chart speed is 30 mm/sec. The EEG channels are calibrated to give a 1 cm. deflection to an input of 100 u volts.

There was no apparent difference in the duration of the desynchrony evoked from either ARAS stimulating site. Ten mcg/Kg. I.V. of DFP had no effect in two animals.

Using physostigmine at a dose of 50 mcg/Kg. I.V. in five atropinized spinal cats, the threshold was lowered about 30-35% at both sites in 30 minutes and returned to near control levels in 60 minutes. Prolonged desynchrony was noted also with physostigmine but this was not as well defined as with the irreversible anti-ChE compounds.

It was found that it was necessary to use dose levels of 10-15 mg/Kg. I.V. of EPN to antagonize an atropine pattern in unanesthetized curarized cats. This dose of EPN is in the range of the LD_{50} (11.5 mg/Kg.) of the compound and has been shown (Awad, 1960) to inhibit about 65% of the cortical and 90% of the medullary ChE. Figure 11 shows the results obtained 60 minutes after a dose of 15 mg/Kg. I.V. EPN.

Five mg/Kg. I.V. of EPN produced a definite lowering of threshold from 1.5 to 0.75 volts in the posterior ARAS site and from 1.0 volt to 0.75 volts in the anterior ARAS site 30 minutes after injection. Prolonged desynchrony was noted from both sites in the same magnitude of duration after the end of the stimulation. (Figure 12). Similar results were seen in a total of seven cats.

Six cats treated with 1 mg/Kg. I.V. EPN did not show any decrease in threshold from stimulation in either of the two ARAS

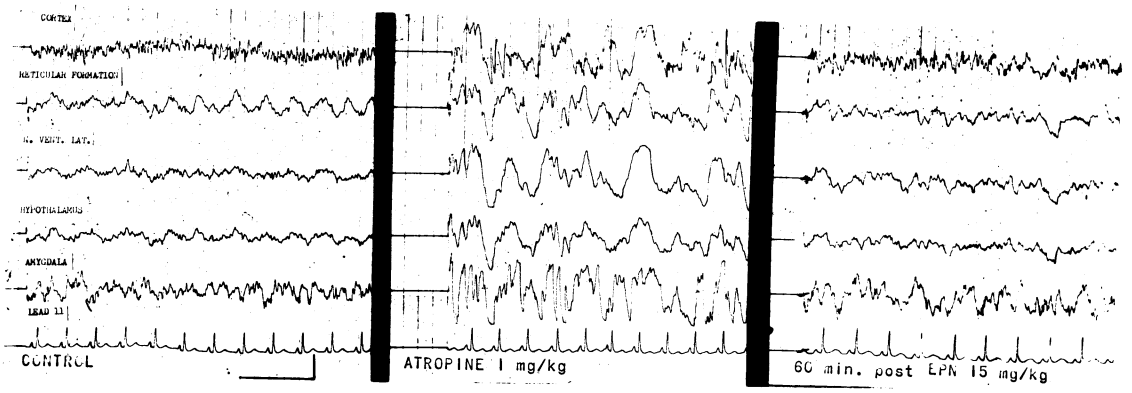

Figure 11.

2.1 Kg. female cat. EPN 15 mg/Kg. I.V. produces in 60 minutes, good antagonism of the atropine control pattern. No stimulation.

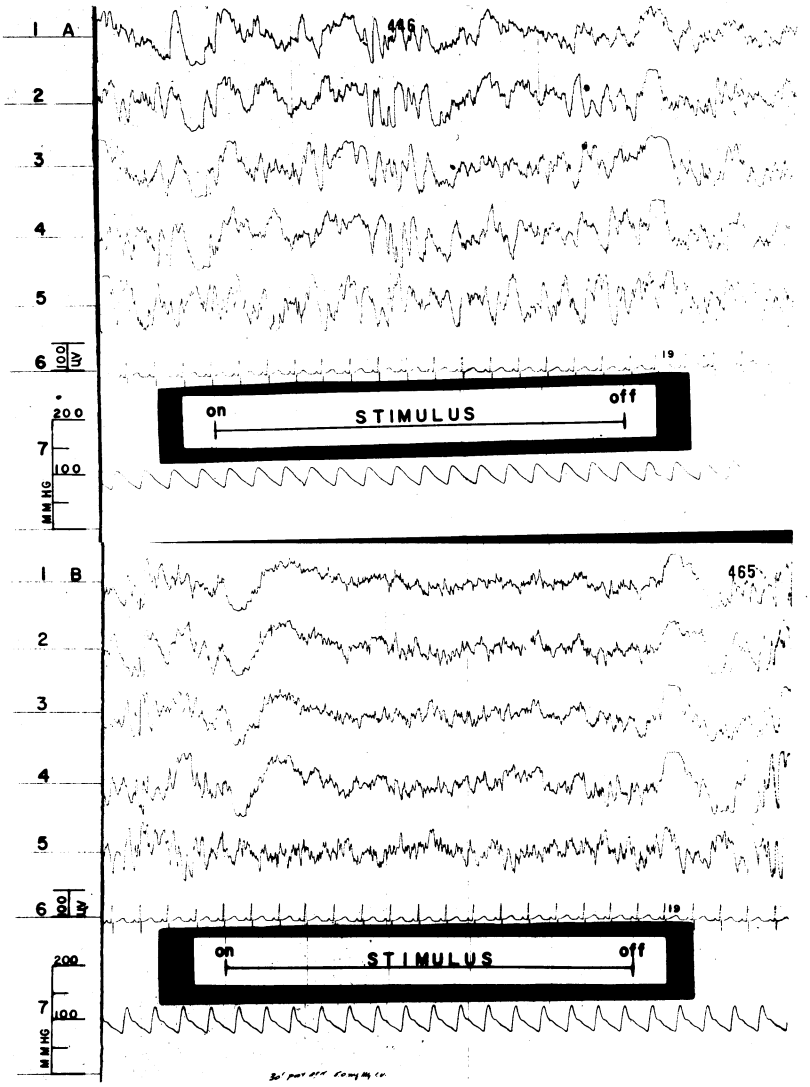

Figure 12.

Cat #19, 2.4 Kg. male. EPN 5 mg/Kg. posterior medial ARAS stimulation. A. Control stimulation of 0.75 volts is subthreshold. B. 0.75 volts stimulation 30 minutes after injection of EPN, 5 mg/Kg., threshold has been reduced from 1.5 volts to 0.75 volts. Channels are the same as listed in Figure 9.

sites; there was however, a definite prolongation of desynchrony at control threshold voltages elicited from both stimulating sites (Figure 13).

Malathion showed a somewhat similar type of response. Fair cortical desynchronization was produced in four atropinized cats only at doses at or above, the LD_{50} (300 mg/Kg.) level. Figure 14 shows the EEG response 30 minutes after 350 mg/Kg., I.V., of malathion. Fifty-to-one hundred mg/Kg. of malathion, I.V., produced a decrease in threshold from stimulation at both sites in the ARAS in all the animals (4 at each dose level) tested (Figure 15). Ten mg/Kg. produced a slight prolongation of desynchrony in 3 out of the 5 animals tested.

B. Monoamine Oxidase Inhibitor (MAOI).

JB-835 (3-hydrazinol phenyl butane) had a somewhat different effect than did the anti-ChE's. At a dose of 3 mg/Kg. I.V., which produces 75-90% inhibition of MAO in the brain in 30 minutes (Spector et al., 1960), there was no significant antagonism of the atropine by 25-30% at both sites in the ARAS. Stimulation at the anterior ARAS site 30 minutes after JB-835 showed a latent period of 1-2 seconds before the onset of desynchrony at the control threshold voltage. Prolonged desynchrony was noted after stimulation of both ARAS sites. The latent period was seen in all eight cats tested with JB-835 but it was particularly pronounced in three

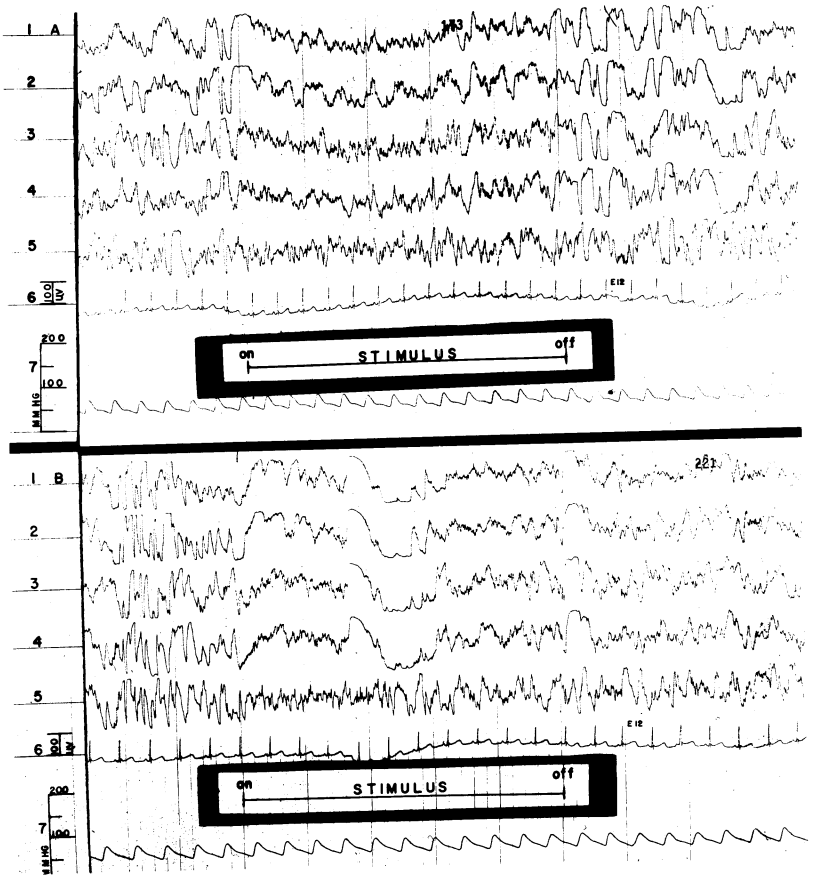

Figure 13.

Cat #12. 2.25 Kg. female. EPN 1 mg/Kg. I.V. produces no change in the threshold. At 60 minutes, there is a prolongation of desynchronization. A.- Control threshold of 0.75 volts. B.- Stimulation of 0.75 volts 60 minutes after injection of EPN. Posterior medial ARAS stimulation.

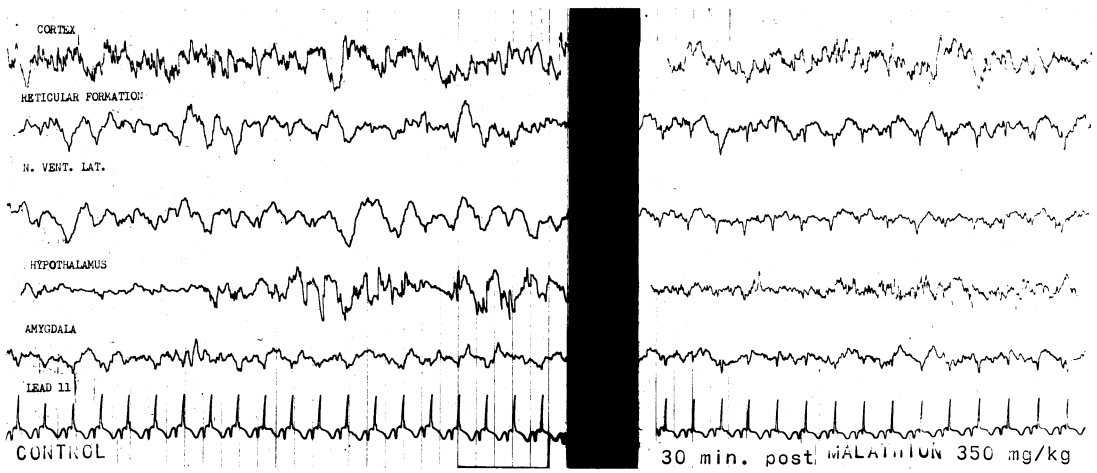

Figure 14.

Cat, male 2.0 Kg. Fair desynchronization noted 30 minutes after 350 mg/Kg. I.V. malathion.

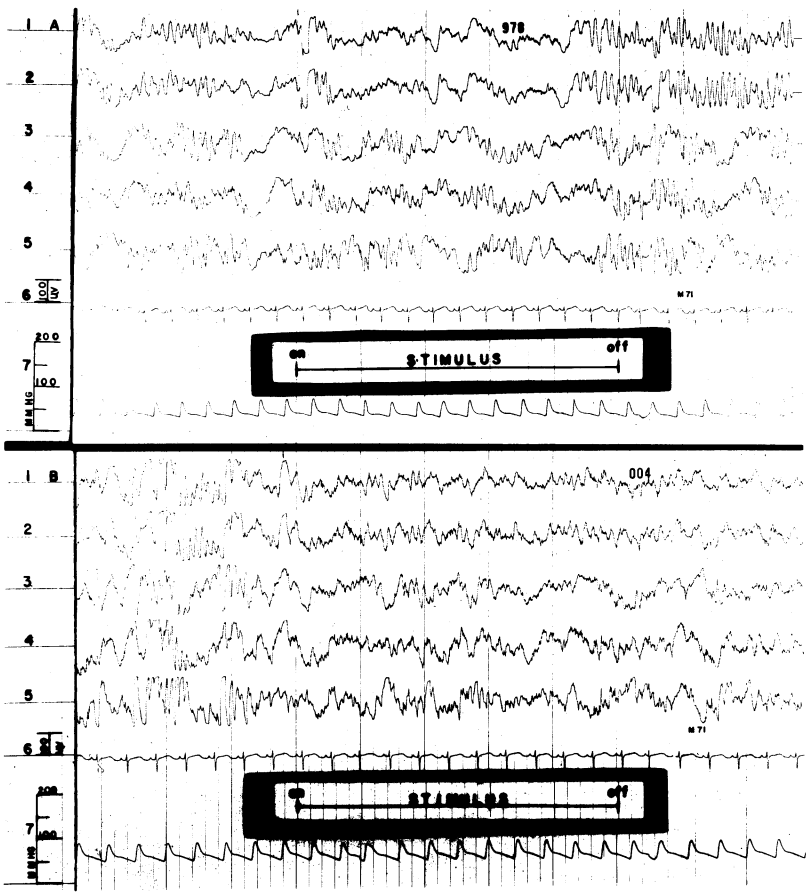

Figure 15.

Cat #71. 1.8 Kg. female. 50 mg/Kg. malathion I.V. Posterior medial ARAS stimulation. A.- Control stimulation of 1.0 volts. B.- Stimulation of 1.0 volts 30 minutes after 50 mg/Kg. malathion produces prolonged desynchronization. Threshold has decreased from 1.5-1.0 volts. Channel selections are the same as in Figure 9.

animals.

Figure 16 shows that stimulation in the anterior lateral ARAS produces, at the control threshold value, an increasing amount of high voltage, slow frequency activity before the onset of desynchrony. However, the threshold has decreased at this time (Figure 17) and, once desynchronization occurs, it is quite marked in all cortical leads at 2.0 volts compared to the control threshold of 3.0 volts.

The threshold in the posterior medial site of the ARAS has also been lowered from 7.0 volts to 5.0 volts. However, latency was not produced with threshold stimulation at the posterior medial site contrary to what was noted after the stimulation of the anterior lateral ARAS.

Figure 18 again shows the extended latent period at the control threshold voltage in the anterior lateral ARAS which is especially prominent in the ipsilateral suprasylvian gyrus and carried into the ipsilateral hemisphere. Figures 19 and 20 show that the threshold in the posterior medial ARAS decreased from 1.5 volts to 1.0 volts with no latent period evident, but there is evidence of prolonged desynchronization. Figure 21 represents again a long latent period and prolonged desynchrony from anterior ARAS site stimulation.

C. MAOI and 5 HTP.

Ten to fifteen mg/Kg. I.V. of 5-HTP given fifteen minutes

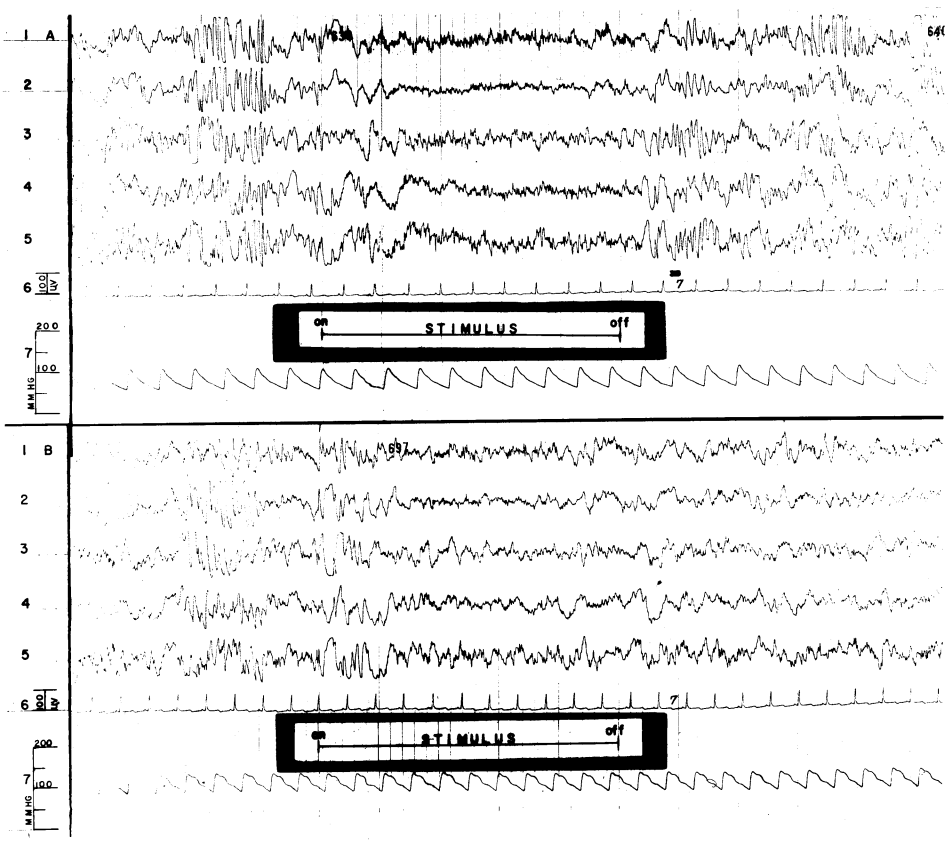

Figure 16.

Cat #7. 2.6 Kg. female. JB-835, 3 mg/Kg. I.V. Anterior lateral ARAS stimulation. A.- Control threshold of 3.0 volts. B.- 30 minutes after injection of JB-835 a stimulation of 3.0 volts shows a short latent period before onset of the desynchrony which continues after stimulation has ceased.

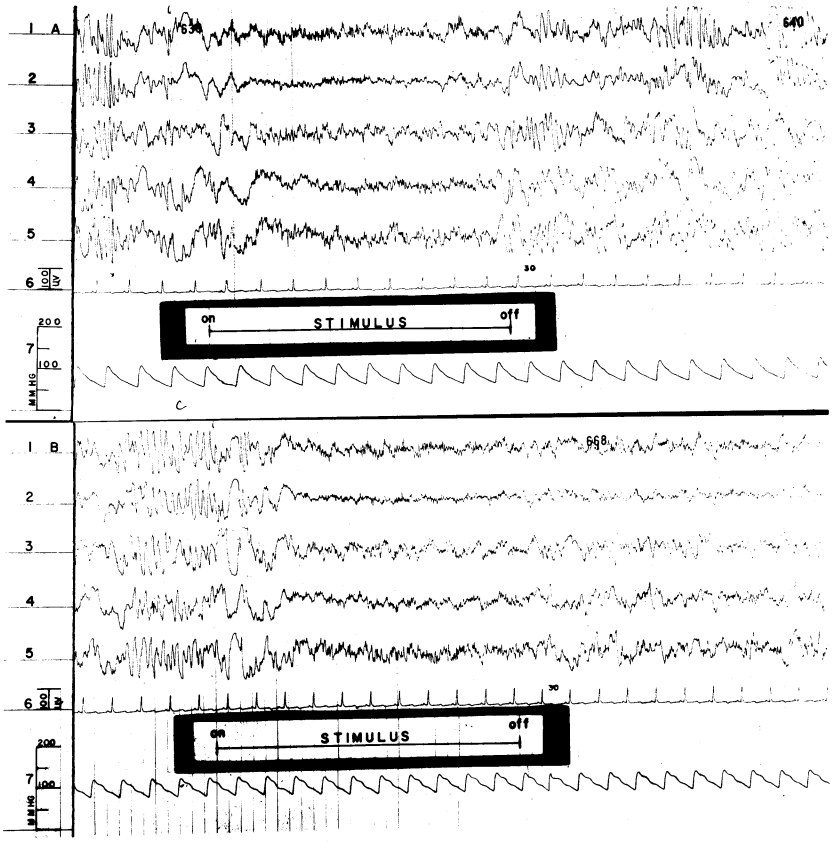

Figure 17.

Cat #30. 2.65 Kg. female. JB-835, 3 mg/Kg. I.V. Anterior lateral ARAS stimulation. A- Control threshold of 3.0 volts. B- 90 minutes after JB-835 2.0 volts has become the threshold. A prolongation of desynchrony is also shown.

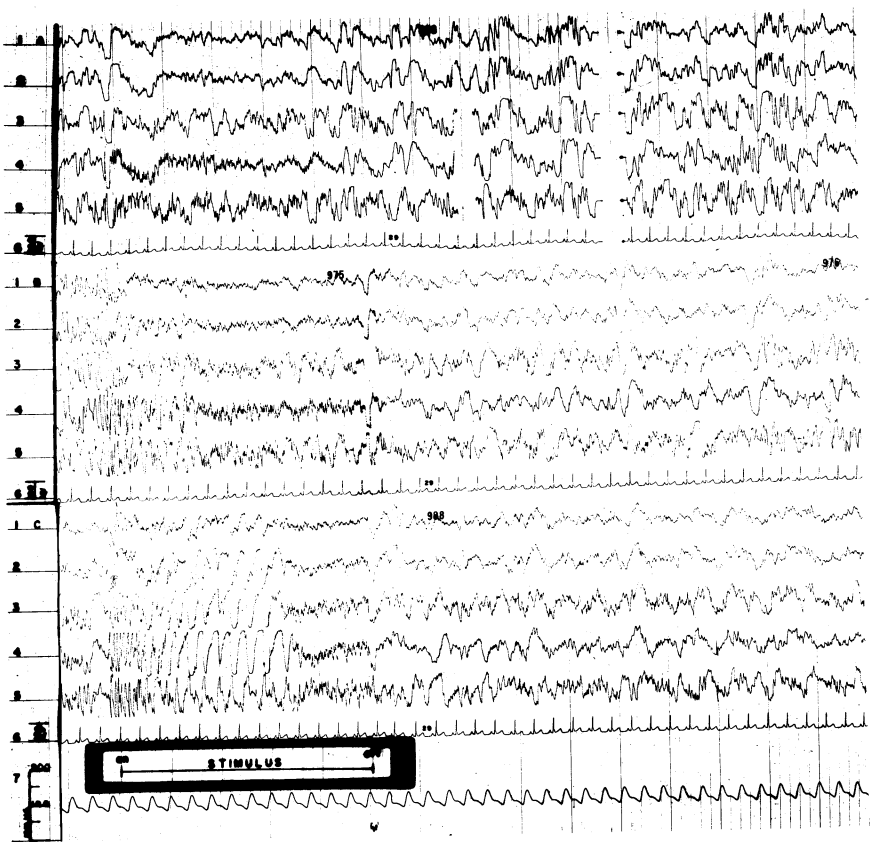

Figure 18.

Cat #29. 3.1 Kg. male. JB-835, 3 mg/Kg. I.V. Anterior lateral ARAS stimulation. A- Control stimulation of 7.0 volts is the threshold. B- 60 minutes after JB-835 there is a two second latent period before desynchronization. This latent period is followed by a prolonged period of desynchrony. C- 90 minutes after JB-835 there is a 3-4 second latent period and evidence of prolonged desynchrony.

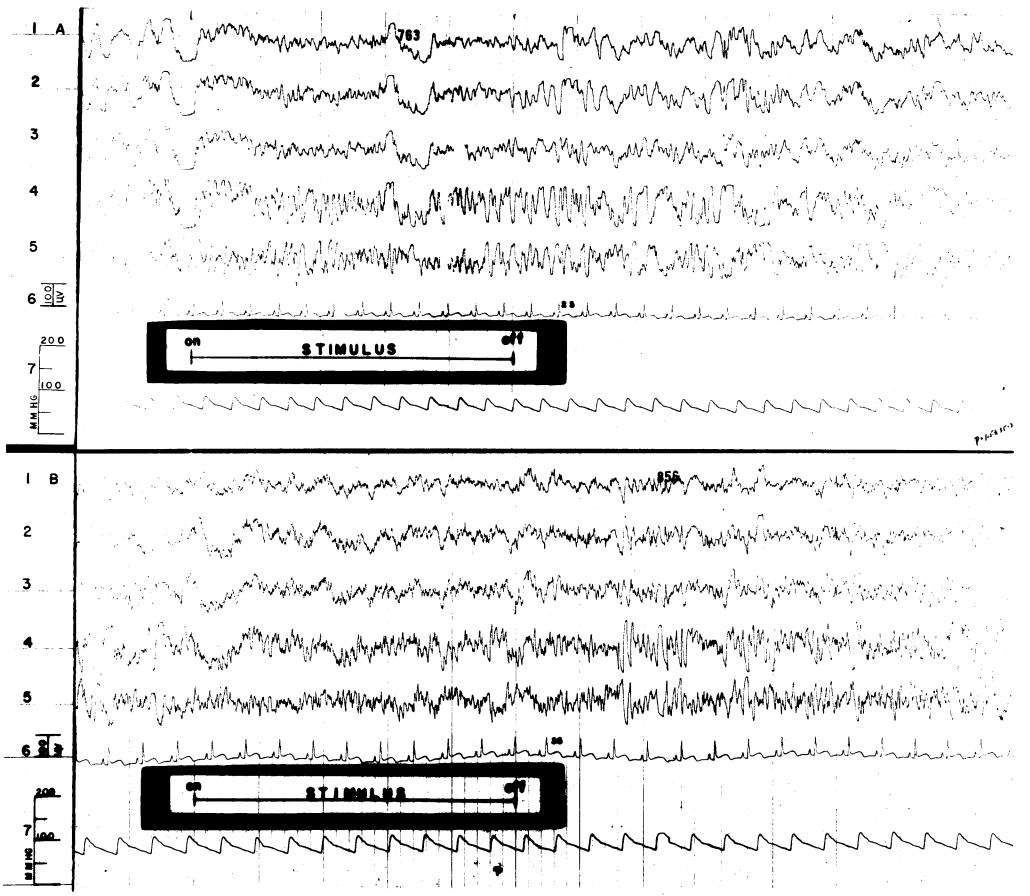

Figure 19.

Cat #25. 3.0 Kg. male. JB-835, 3 mg/Kg. I.V. Posterior medial ARAS stimulation. A- Control, threshold stimulation of 1.5 volts. B- Stimulation of 1.5 volts, 90 minutes after injection of JB-835, produces prolonged desynchrony. Threshold has decreased from 1.5 to 1.0 volts. See Figure 9 for channel organization.

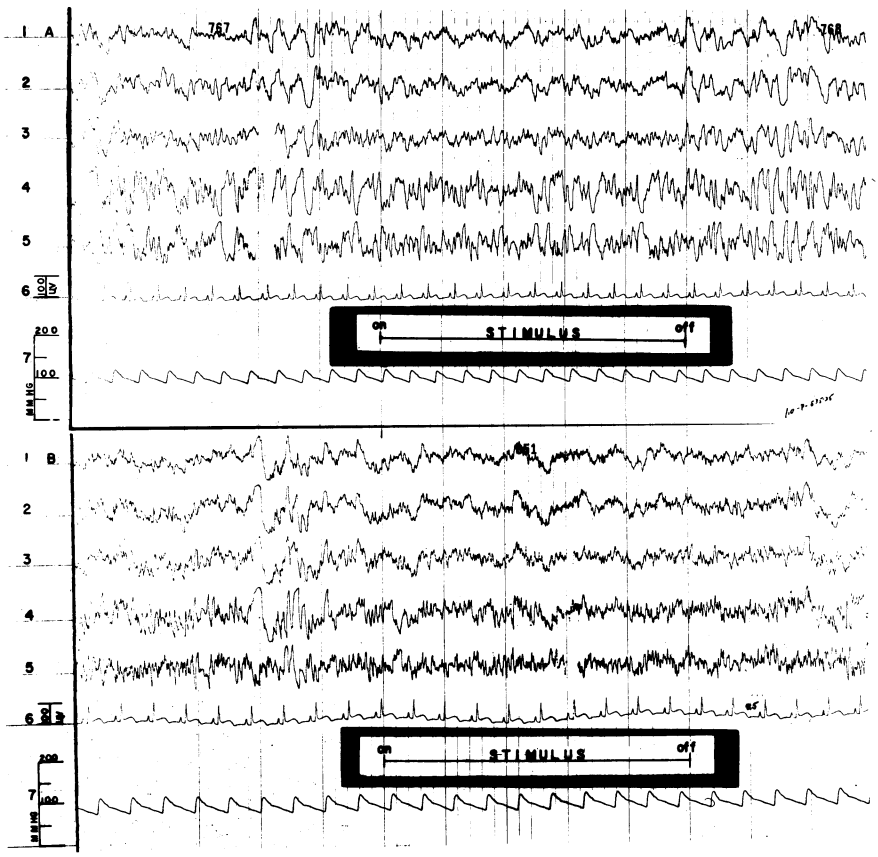

Figure 20.

Cat #25. 3.0 Kg. male. JB-835, 3 mg/Kg. I.V. Posterior medial ARAS stimulation. A- Subthreshold control stimulation of 1.0 volts. B- 90 minutes after JB-835 the threshold is 1.0 volts; and there is also some prolongation of desynchrony.

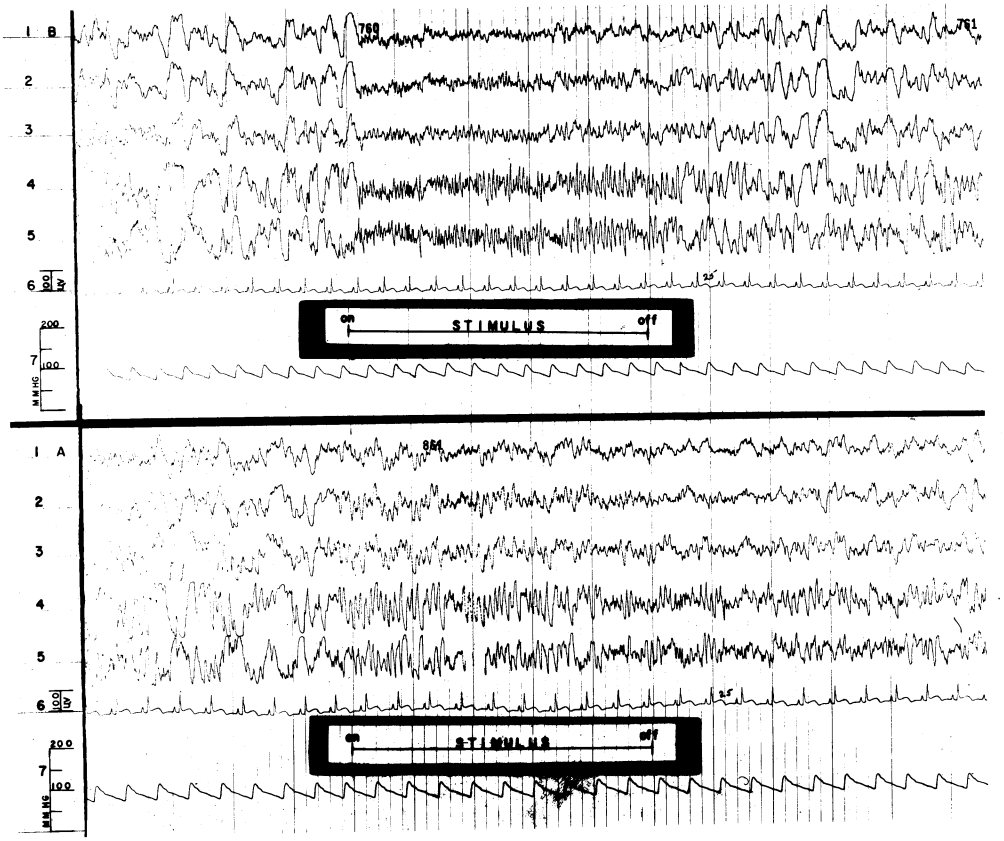

Figure 21.

Cat #25. 3.0 Kg. male. HB-835, 3 mg/Kg. I.V. Anterior lateral ARAS stimulation. A- Stimulation of 4.0 volts is the threshold in the control period. B- At 90 minutes, 4.0 volts produces a long latent period, then a prolonged desynchrony.

after JB-835 caused tachycardia, hypertension and in three animals there was electrical silence in the EEG. Very small doses of 5-HTP, the serotonin precursor, had to be used in conjunction with the JB-835 to avoid the cardiovascular changes arising from the combinations of these two agents.

Figure 22 shows the results obtained with 5-HTP, 1 mg/Kg. I.V. fifteen minutes after 3 mg/Kg, I.V., JB-835. The suprathreshold 5.0 volt stimulation shows a short latent period followed by the prolonged desynchrony. The threshold in the anterior lateral ARAS decreased by 25% and a latent period was noted at 3.0 and 4.0 volts. Sixty minutes following the injection of 5-HTP, no latent period was evoked on anterior lateral ARAS stimulation but the threshold remained decreased.

Figure 23 shows the decrease in threshold from 1.5 volts to 0.9 volts seen on stimulation of the posterior medial ARAS. No latent period was seen but there is a definite increase in the duration of desynchrony. Figure 24 shows that a sub-threshold stimulation of 0.75 volts in the control period is converted to threshold 60 minutes after treatment with the combination of 5-HTP and JB-835.

Of the seven cats treated with the combination of a monoamine oxidase inhibitor and the serotonin precursor, all showed similar effects though the intensity of the effect varied from animal to animal as would be expected when dealing with slight alterations of endogenous compounds.

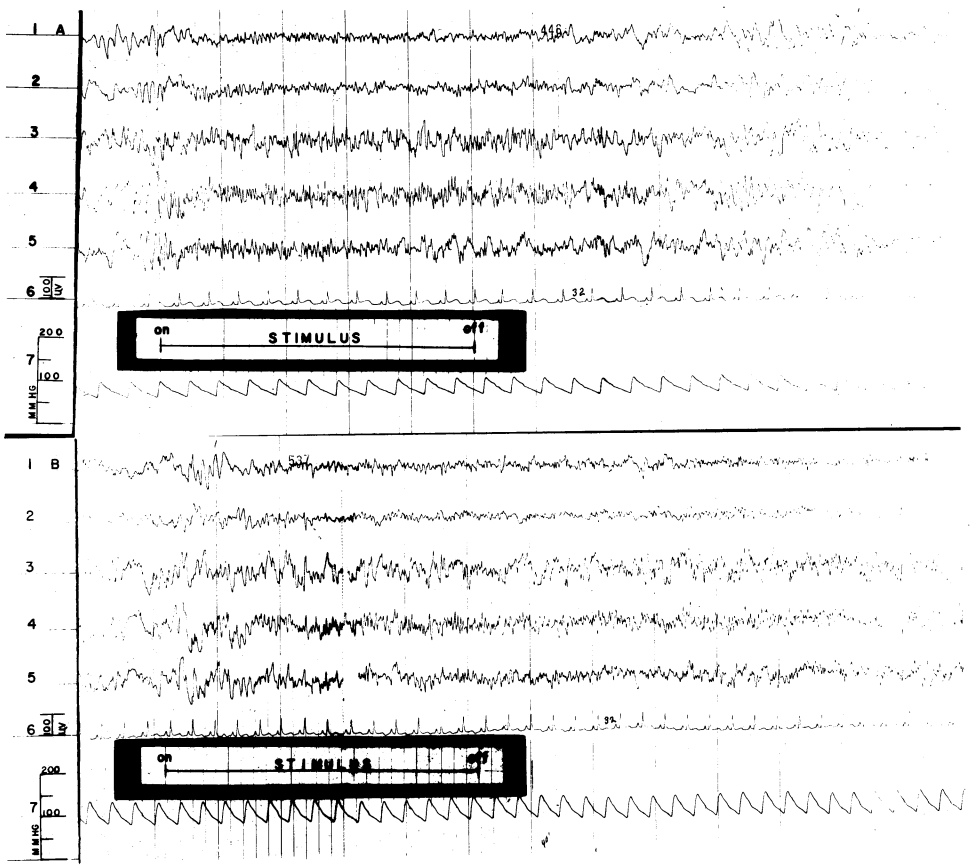

Figure 22.

Cat #32. 2.2 Kg. male. JB-835 with 5-HTP fifteen minutes later. Anterior lateral ARAS stimulation. A- Control supra-threshold stimulation of 5.0 volts. B- 5.0 volts, 45 minutes after 5-HTP, shows prolongation of desynchronization and a short latent period.

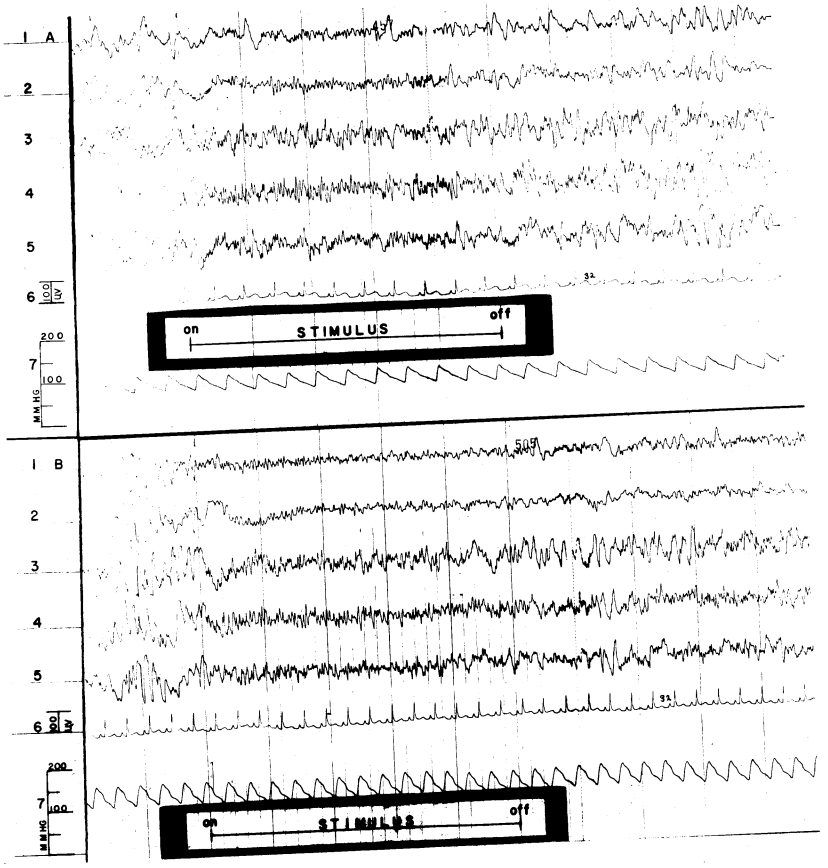

Figure 23.

Cat #32, 2.2 Kg. male. JB-835, 3 mg/Kg. I.V. followed in 15 minutes by 1 mg/Kg. 5-HTP, I.V. Posterial medial ARAS stimulation. A- Control stimulation of threshold voltage of 1.5 volts. B- 1.5 volts stimulation 20 minutes after injection of 5-HTP, No latent period is evident but there is a definite prolongation of desynchrony. (Threshold has been lowered from 1.5 to 1.0 volts.)

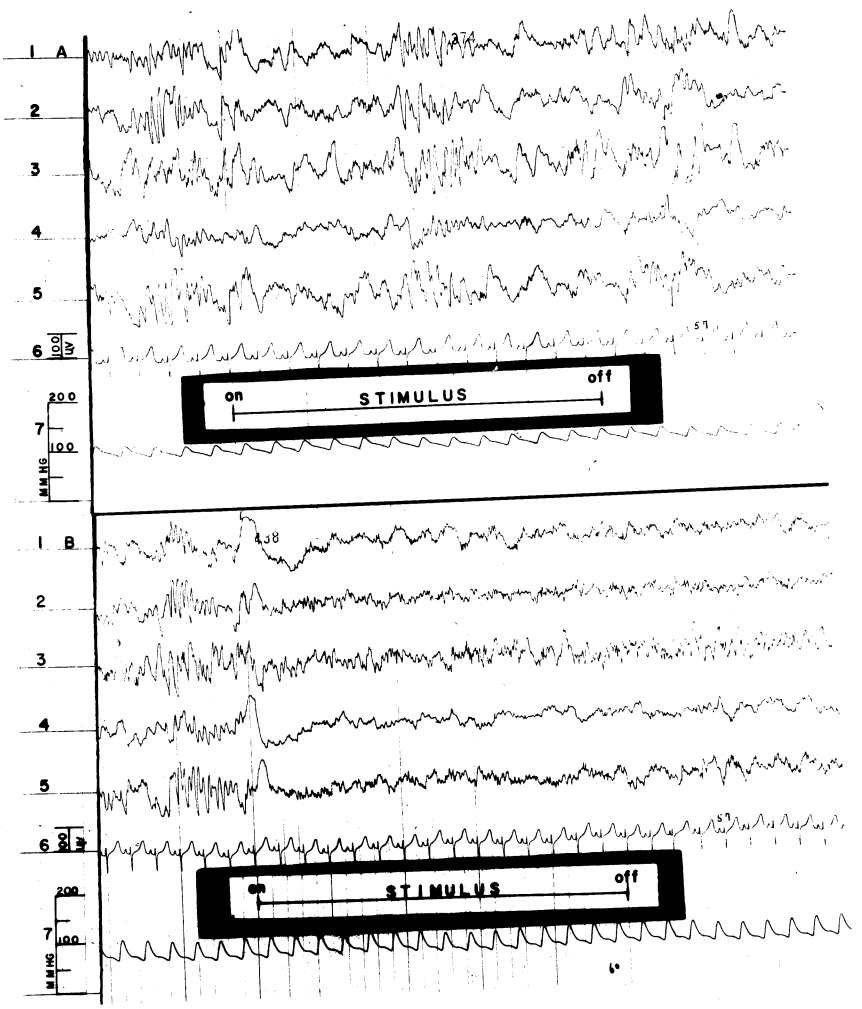

Figure 24.

Cat #57. 2.1 Kg. female. JB-835, 3 mg/Kg. I.V., 15 minutes later, 1 mg/Kg. I.V. 5-HTP. Posterior medial ARAS stimulation. A- Control stimulation of 0.75 volts is subthreshold. B- At 60 minutes 0.75 volts becomes the threshold.

A summary of the results is presented in Table I. The results indicate that both anti-ChE and MAOI decrease the threshold of ARAS stimulation and also cause an increased period of desynchronization following termination of the stimulus. With the MAOI but not the anti-ChE, a latent period was seen when the anterior lateral ARAS was stimulated but the latency was not evident on stimulation of the posterior medial ARAS.

TABLE I

SUMMARY OF THE RESULTS

COMPOUND	PRESUMED LEVEL			THRESHOLD		Prolonged Desynchrony	Latency	EEG
	ACH	5-HT	NE.	Ant.R.F.	Post.R.F.			
Anti-CHE	↑	-	-	decreased	decreased	+	-	desynchrony at high doses
MAOI (JB-835)	-	↑	-	decreased	decreased	+++	+	no change from control
MAOI and 5-HTP	-	↑	-	decreased	decreased	+++	++	no change from control

CHAPTER IV

DISCUSSION

A. ARAS Stimulation and the Desynchronization Pattern.

Under the conditions of the experiments reported here, the animals rarely exhibited a synchronized, sleep-like EEG pattern. For this reason, atropine sulfate, 0.5 mg/Kg. I.V., was given to produce a stable, synthronous EEG pattern. It was found that this dose of atropine could be easily overcome by low voltage stimulation of the ARAS. Control threshold stimulation voltage varied from animal to animal but the majority of threshold voltages were in the vicinity of 3.0 volts with some as high as 7.0 volts and a few with control threshold readings of 0.75 volts. Regardless of the strength of stimulation necessary to produce desynchronization in the control period, the effects on the threshold of the compounds tested did not vary from animal to animal.

The change in cortical EEG from the pre-stimulation, hypersynchronous atropine pattern to a low voltage, high frequency desynchronized EEG record upon threshold stimulation of both sites in the ARAS was easily observed visually. The visual method of ascertaining the threshold pattern appeared to be as dependable as the quantitative method developed by Riehl et al. (1959). A modi-

fication of the Riehl method of quantitation was used in a few experiments to determine if the visual estimation of the desynchronization would correlate with a quantitative analysis of the record.

It was found that where it was decided on visual inspection that threshold desynchronization occurred, there was a significant change in Riehl's "unit activity" when the pre-stimulation period was compared to the stimulation period. No significant change in "unit activity" was calculated when the stimulus appeared visually to effect no change in the cortical EEG (cf. Appendix).

The conditions of the experiments and the use of atropine precluded any behavioral observations in these animals. In spinal cats, Bradley and Elkes (1957) used the size of the pupil and the state of the nictitating membrane as the criteria on which they based their evaluation of the behavioral state of the animal. Because atropine was used in our experiments, the pupils were constantly dilated.

B. <u>The Effects of the Drugs</u>.

The results obtained indicate that both the anticholinesterases (anti-ChE) and the monoamine oxidase inhibitor (MAOI) used in these experiments affect the threshold of the cortical desynchrony by ARAS stimulation in the same manner, causing primarily a lowering of the threshold. Both types of enzyme inhibitors used

also increased the duration of the desynchronization, but only one, the MAOI, gave evidence of producing a latent period. This latent period, before the onset of the prolonged desynchrony, was seen only on stimulation of the anterior lateral ARAS. When the precursor of serotonin, 5-HTP, was given in conjunction with the MAOI, one of the seven cats so treated showed a short latent period upon stimulation of the posterior medial ARAS site in addition to usual latency seen in all the cats on stimulation in the anterior lateral ARAS.

1. Anticholinesterases.

Wescoe et al. (1948), Rinaldi and Himwich (1955), Desmedt and LaGrutta (1957) and others have shown that DFP will produce cortical desynchronization and that this effect can be blocked by the tertiary atropinium compounds. According to the above mentioned investigators, an LD_{50} dose of DFP was needed to produce antagonism of an atropinized cat EEG pattern.

The results of the experiments reported here indicate that, in the case of the anti-ChE agents, a dose-response relationship with regard to the cortical EEG response to ARAS stimulation can be observed. At doses of the anti-ChE agents which produce a significant inhibition of cortical ChE, there is a marked lowering of the threshold to ARAS stimulation to the point where a sufficiently large dose (approximating the LD_{50}) will lower the threshold to zero or produce a spontaneous antagonism of the atropine

EEG pattern. Lower doses of the anti-ChE agents which do not produce an appreciable amount of ChE inhibition, lowered the threshold to ARAS stimulation, as well as producing a prolongation of desynchronization.

DFP, a potent irreversible ChE inhibitor, produced a spontaneously desynchronized EEG pattern in the spinal, atropinized cat at a dose of 0.05 mg/Kg. I.V. At a lower dose of 0.02 mg/Kg. I.V., DFP did not lower the threshold to ARAS stimulation but the period of desynchrony lasted beyond the termination of the stimulation, whether the anterior lateral or the posterior medial ARAS sites were stimulated.

Physostigmine, a reversible ChE inhibitor, at a dose of 0.05 mg/Kg., I.V., did not antagonize the atropine EEG pattern nor did this dose of physostigmine lower the threshold to ARAS stimulation. Again, however, there was a definite prolongation of cortical desynchrony after termination of the stimulus in both ARAS sites at this dose.

The organic phosphorus compounds, EPN and malathion, even though they are much less potent anti-ChE compounds than either DFP or physostigmine, showed a similar action on the EEG of the spinal cats.

EPN at a dose of 10 to 15 mg/Kg., I.V., spontaneously antagonized the atropine pattern of the spinal cat so that the threshold for desynchronization was essentially zero. At a dose of

EPN of 5 mg/Kg., I.V., the threshold of cortical desynchronization by ARAS stimulation was lowered by 30 to 40% from the control readings. At 1 mg/Kg., I.V., EPN did not lower the threshold but again a prolongation of desynchrony was observed after termination of the stimulation in both ARAS sites.

Malathion caused no antagonism of the atropinized cat EEG pattern until the LD_{50} dose of 350 mg/Kg., I.V. was reached. Even at this dose, the antagonism is not as well defined as it is with the more potent anti-ChE's. However, much lower doses of 50-100 mg/Kg., I.V., lowered the threshold and increased the duration of desynchronization in the cortex upon stimulation at both ARAS sites.

The results of these experiments and of those previously mentioned (Bradley and Elkes, o.c., and many others) indicate that a dose of an anti-ChE approaching the LD_{50} of the compound is necessary to antagonize an atropine EEG pattern in the absence of ARAS stimulation. The amount of ChE inhibition (at the LD_{50} dose level) in cortical tissue varies from 60% in the case of malathion (Karczmar et al., 1961) and 65% in the case of DFP (Frawley et al., 1952) to an inhibition of 90% of the medullary ChE in the case of EPN (Karczmar et al., o.c.). Lower doses of the irreversible anti-ChE agents do not show a significant level of inhibition. EPN at a dose of 1 mg/Kg., I.V., in dogs, showed no inhibition of the cortical ChE, nor did malathion at a dose of 50 mg/Kg., I.V. (Awad, 1960). Michaelis (1954) by determining the increase in cortical

ACh after DFP, had to use a dose of 0.3 mg/Kg., I.V., to show a significant increase in ACh.

Doses of anti-ChE's which produce little, if any, manometrically detectable ChE inhibition are shown by these experiments to have a definite effect on the threshold and duration of desynchrony upon ARAS stimulation at the two sites used in these experiments. While these doses of anti-ChE's would not raise the level of endogenous ACh, it is probable that the degree of ChE inhibition is sufficient to decrease the rate of metabolism of the ACh released by stimulation of a cholinergic pathway in the brain. This decrease in metabolism of ACh could account for the prolongation of desynchrony seen with the small doses of anti-ChE.

There is also the possibility that the effect seen with the low doses of anti-ChE could be the result of the direct action of these compounds on the cholinergic receptors as suggested by Koppanyi et al., (1947), Heymans et al., (1946), and Heymans and Jacobs (1947).

The first suggestion seems more probable for the following reasons: 1. A prolongation of desynchrony and lowering of threshold would be expected from ChE inhibition rather than a direct action and 2. the time of onset and peak activity of the anti-ChE compounds used in these experiments correlate well with the time of onset of threshold alterations or antagonism of the atropine EEG pattern. As an example, DuBois (1951) has shown that EPN must be

metabolized in the liver before any potent anti-ChE action can be obtained. Accordingly, there is a latent period of 20-30 minutes before the onset of ChE inhibition (Karczmar et al., o.c.). EPN injected intraventricularly caused no ChE inhibition in cortical tissue unless it had been incubated in liver tissue before injection (Awad, o.c.). This agrees with the present data being reported here that there is a latent period of 30-60 minutes before any antagonism of the atropine EEG pattern or any effect resulting from ARAS stimulation is noted. The three other anti-ChE compounds, DFP, physostigmine and malathion, are reportedly the active compounds and do not need to be metabolized before causing ChE inhibition. Peak activity of these compounds in our experiments was observed at 15 to 30 minutes after injection which were the times of the first two threshold determinations after injection of the drugs.

The ability of the tertiary anticholinergic compounds to block the cortical desynchronization produced by DFP and physostigmine (Longo, 1956; Bradley and Elkes, o.c.; Rinaldi and Himwich, o.c.; and Holmstedt, 1959) as well as the central toxicity of the phosphonate anti-ChE's as reported by Karczmar and Long (1958) would also indicate that there is a cholinergic mechanism present in the brain.

The results presented here show that the threshold for cortical desynchronization in response to stimulation at both sites in the ARAS is altered in the direction one would expect if a chol-

inergic mechanism were involved in this pathway. The response to low doses of anti-ChE's suggests that even a slight inhibition of the ChE in the brain will alter the capability of the ARAS and its diffuse projection system to evoke a cortical response upon stimulation. It is quite likely, as suggested by Rinaldi and Himwich (o.c.) and Rothballer (1956), that at least a part of the ARAS is cholinergic in nature.

Finally, since the anti-ChE's used in these experiments had a similar effect on cortical desynchronization and threshold alterations with respect to the two divergent ARAS sites stimulated, it could be suggested that cholinergic neurons, if present in the ARAS, are fairly evenly distributed along these paths through the brain stem and its cortical projections. The effects seen with low doses of anti-ChE might also suggest that this pharmacological method could be more sensitive to anti-ChE action than the biochemical methods in use today.

2. Monoamine Oxidase Inhibitor.

On injection of 3 mg/Kg., I.V., of JB-835, there was an immediate increase in blood pressure and a tachycardia. This hypertensive phase diminished to near control levels within 15 minutes but the heart rate remained increased throughout the duration of the experiment. During the hypertensive phase there was no antagonism of the atropine pattern in the EEG.

The results seen with JB-835, at a dose which inhibits

70% of the brain monoamine oxidase (Spector et al., 1960; Brodie et al., 1959; and Biel et al., 1959), indicate that this compound mimics the action of the anti-ChE agents in two respects. First, JB-835 decreased the threshold to cortical desynchronization on stimulation of both sites in the ARAS; in the second place, it increased the duration of desynchrony beyond termination of the stimulation. On the other hand, JB-835 differed from anti-ChE agents in that there was a latent period. During this period, a high voltage, slow frequency activity similar to the prestimulation atropine pattern continued before the onset of desynchrony. When JB-835 was used alone, the latent period occurred only upon stimulation in the anterior lateral ARAS site. In one animal out of seven that was treated with the precursor of serotonin, 5-HTP, after receiving JB-835, a short latent period was observed also on stimulation in the posterior medial ARAS. The latency period upon stimulation in anterior lateral ARAS site was more pronounced in all seven animals treated with 5-HTP and JB-835, then with the JB-835 alone, but the threshold changes and prolongation of desynchrony appeared to be equivalent in both cases.

The pharmacology and biochemistry of JB-835 has been presented in a series of articles (Spector et al., o.c.; Brodie et al, o.c.; and Biel et al., o.c.). These reports indicate that JB-835 is a potent, fast acting, irreversible inhibitor of monoamine oxidase in cortical tissue without any direct amphetamine-like CNS

action. The important finding as it pertains to this report is that JB-835 raises the level of serotonin rapidly (almost 70% in 1 hour) while the level of norepinephrine remains constant in the brain stem for a period of 4 hours.

Shore et al, (1957), Brodie and Shore (1957), and Spector et al, (o.c.) have postulated that the mechanism of the sedative action of reserpine lies in the ability of reserpine to release endogenous serotonin and to block storage sites of serotonin, so that there is a continuous, small excess amount of free serotonin available to its receptors. If a large excess of unbound serotonin is present, serotonin will block itself producing the reverse behavioral activity (Brodie, 1959).

Rather than use reserpine and a MAOI, Udenfriend (1957) gave large amounts of the serotonin precursor, 5-HTP, and an MAOI, iproniazid. He found that the concommitant increase in free serotonin in the brain produced an animal whose behavior was not that of a reserpinized, sedated animal; to the contrary, the animal showed general skeletal muscle tremors, loss of placing reactions, postural incoordination, lacrimation, salivation and increased gastrointestinal activity. This type of response to excess levels of serotonin in the animal resembles the response to toxic doses of anti-ChE's. This effect may be explained, if serotonin can cause, when present in large amounts, a blockade at its normal site of action thus allowing cholinergic dominance.

Brodie's experiments (1959) showing that pretreatment with an MAOI reversed the effect of reserpine from sedation to aggressive behavior may be similarly explained. In these experiments, serotonin is released by reserpine and the MAOI prevents the normally rapid metabolism of serotonin; a high level of free serotonin results with the concurrent "self block". This would then prevent the modulating effect of serotonin in the brain from producing a behavior pattern opposite to that seen in an animal treated with reserpine alone.

Costa et al. (1960) reported similar results in rabbits. These investigators found that animals given 22 to 44 mg/Kg. of 5-HTP, I.V., showed a biphasic EEG response. The first response was that the animals' EEG "<u>became more resistant to modification by alerting stimuli</u>" and then within 30 minutes, the EEG became continuously desynchronized. Costa and his co-workers (o.c.) also measured the changes in levels of cortical serotonin and found that only when the serotonin level had increased 3 fold did the EEG pattern show continuous desynchronization. The present writer prefers the term <u>modulate</u> (to regulate, to adjust) to Costa's term <u>modify</u> because the term modulate has the added implication of frequency regulation.

In the experiments reported here, it was not possible to raise the level of endogenous catechol amines without the concurrent rise in serotonin levels. The use of dl DOPA (3,4 dihydroxy-

phenylalanine) has been reported (Weil-Malherbe and Bone, 1959 and 1961, Vogt, 1959) not to alter the normal levels of norepinephrine in the cat's brain. On the other hand, Udenfriend et al., (o.c.) and spector et al., (o.c.) have shown that the combination of an MAOI and 5-HTP will increase primarily the brain levels of serotonin in the cat. Accordingly, our experiments with 5-HTP-MAOI combinations were designed to produce a small increase in the amount of free serotonin in the cortical tissue.

The appearance of a latent period upon stimulation of the anterior lateral ARAS suggests that the abnormal level of serotonin, produced by MAO inhibition, may be acting as a modulator of the neural transmission in the ARAS. The role of serotonin would be similar to that proposed by Costa et al. (1961) for norepinephrine in the cervical sympathetic ganglia. Costa and his co-workers found that small doses of reserpine which depleted the ganglia of norepinephrine facilitated the postsynaptic potential. These investigators also found that if an MAOI was given before treatment with reserpine, thereby increasing the amount of free norepinephrine, the postsynaptic potential was markedly reduced. Costa suggested an analogy between the cervical sympathetic ganglianic transmission and transmission in the brain, postulating that either the catechol amines or serotonin or both could act as modulators of central transmission.

The latent period seen in our experiments on stimulation

of the anterior lateral ARAS after treatment with JB-835 or JB-835 in combination with 5-HTP suggest that serotonin may be considered to have a modulating influence on the transmission in this system. The addition of the serotonin precursor, 5-HTP, caused a greater latency effect which further indicates serotonin as a modulator of central transmission.

The latent period of high voltage, slow frequency waves seen in the cortex could be correlated with Costa's (1960) report of a "less responsive to stimuli" action in the presence of excess serotonin. Because of this latency effect the present worker suggests that serotonin plays a role in modifying or regulating at least the proposed adrenergic "arousal" mechanism.

Axelrod (1959) and others have shown that catechol amines are also inactivated by an amine oxidase. Consequently, using an MAOI would decrease the rate of inactivation of norepinephrine or epinephrine if they were released upon stimulation of any existing adrenergic fibers. The prolongation of desynchrony and decrease in threshold seen after treatment with JB-835 could be explained on the basis of this phenomenon, i.e., because of the decreased rate (in the presence of MAOI) of biological inactivation of the catechol amines released by stimulation of adrenergic fibers in the ARAS or its projection system. Because no further change in threshold or desynchrony occurred on combining 5-HTP with the JB-835, the possibility of this desynchronizing mechanism being directly

involved with serotonin was not felt to be valid.

Finally, it is felt that if serotonin is a transmitter in the ARAS or its projections, that it is located primarily in the anterior lateral ARAS or along the pathways from this anterior ARAS site. The results obtained with the MAOI showed no marked differences in alterations of threshold or in prolongation of desynchrony from stimulation at either ARAS site so that it is probable that an adrenergic mechanism, like the proposed cholinergic mechanism, is spread throughout the ARAS and/or its projections to the cortex.

CHAPTER V

SUMMARY

The experiments reported here were performed on C-1 sectioned, atropinized cats. The EEG was recorded from the anterior sigmoid and the suprasylvian gyri. The response of the threshold of cortical desynchronization to stimulation of the posterior medial and anterior lateral ARAS under the influence of two types of enzyme inhibitors was studied.

The two types of enzyme inhibitors under investigation were the anticholinesterases, (DFP, physostigmine, EPN and malathion) and the monoamine oxidase inhibitor, JB-835.

The results obtained with the anticholinesterases showed that these compounds markedly lowered the threshold to cortical desynchronization until the atropine EEG pattern was completely abolished. This antagonism of the atropine pattern occurred at or near the LD_{50} dose of the irreversible anti-ChE agents tested. Lower doses of the anti-ChE's which have little or no effect on the cortical ChE (as measured manometrically) did not lower the threshold but there was a definite prolongation of cortical desynchrony which persisted after the termination of the stimulus at both ARAS sites

The results with the MAOI, JB-835, at a dose (3 mg/Kg., I.V.) which has been shown to produce 70% inhibition of the cortical MAO, were similar in two respects to those obtained with the anti-ChE agents. The threshold to cortical desynchrony upon ARAS stimulation in both sites was lowered and the desynchronization was prolonged. The one difference noted between the anti-ChE compounds and JB-835 was that a latent period consisting of high voltage, low frequency waves was noted on stimulation of the anterior lateral ARAS, but not on stimulation of the posterior medial ARAS, after treatment with JB-835. In one animal out of seven treated with a combination of the serotonin precursor, 5-HTP, and JB-835, a latent period was also seen in response to stimulation in the posterior medial ARAS.

Because of the characteristics of the JB-835, and also on the basis of the observations made with the 5-HTP in combination with the JB-835, it is speculated that serotonin was responsible for the appearance of the latent period, while catechol amines were responsible for the alterations in the threshold and the prolongation of desynchronization.

The implications of serotonin being a modulator of adrenergic transmission and the possibility of a cholinergic mechanism in the CNS are discussed.

BIBLIOGRAPHY

Allen, W. F., Formatio Reticularis and the reticulospinal, their visceral functions and possible relationships to tonicity. J. Wash. Acad. Sci. 22: 490, 1932.

Amin, A.H., T.B.B. Crawford and J. H. Gaddum. The distribution of Substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol.(Lond.), 126: 596, 1954.

Arduini, A. and M. G. Arduini. Effects of drugs and metabolic alterations on the brain stem arousal mechanism. J. Pharm. and Exper. Therap., 110: 76, 1954.

Awad, O. PhD. Thesis submitted to the Graduate School, Loyola Univ. 1960.

_____, S. Ridlon and A. G. Karczmar. Central anticholinesterase and EEG actions of EPN and malathion. Fed. Proc., 18(1): 1435, 1959.

Axelrod, J. The metabolism of catechol amines in vitro and in vivo. Pharm. Rev. 2(2): 402, 1959.

_____ The fate of adrenaline and nor-adrenaline, in Adrenergic Mechanisms, ed. by J. R. Vane; Little, Brown and Co., Boston, Mass., 1960.

Beecher, H. K., F. K. McDonough and A. Forbes. Effects of blood pressure changes on cortical potentials during anesthesia. J. Neurophysiol. 1: 324,1938.

Berger, F. M. , G. L. Campbell, C. D. Hendley, B. J. Ludwig and T. E. Lynes. The action of tranquillizers on brain potentials and serotonin. Ann. N. Y. Acad. Sci. 66: 686, 1957.

Biel, J. H., P. A. Nuhfer and A. C. Conway. Structure activity relationships of monoamine oxidase inhibitors. Ann. N. Y. Acad. Sci., 80: 568, 1959.

Blaschko, H. Biological inactivation of 5-HT. in 5-Hydroxytryptamine ed. by G. P. Lewis, Pergamon Press, N. Y. 1958.

Bogdanski, D. F., H. Weissbach and S. Udenfreind. Pharmacological studies with the serotonin precursor, 5-hydroxytryptophane. J. Pharmacol. and Exper. Therap. 122: 182, 1958.

Bonvallet,M., P. Dell and A. Hugelin. Influence de l'adrenaline sur le control reticulaire des corticale et spinale. J. Physiol. (Paris) 46: 262 1954.

Bradley, P. B. Electrophysiological evidence relating to the role of adrenaline in the central nervous system. in Adrenergic Mechanisms. ed. by J. R. Vane. Little, Brown and Co. Boston, Mass. 1960.

_____, S. Cerquiglini, and J. Elkes. Some effects of di-isopropyl fluorophosphate on the electrical activity of the brain of the cat. J. Physiol.(Lond.) 121: 5Φ P, 1953.

_____, and J. Elkes. The effects of atropine, hyoscyamine, physostigmine and neostigmine on the electrical activity of the brain of the consciuos cat. J. Physiol. 120: 14 P, 1953.

_____, The effects of some drugs on the electrical activity of the brain. Brain, 80: 77, 1957.

_____, and E. J. Key. The effect of drugs on the arousal response produced by electrical stimulation of the brain. EEG Clin. Neurophysiol., 10: 97, 1958.

Bremer, F. Cerveau isole et physiolgie du sommeil. C. R. Soc. Biol. Paris. 118: 1235, 1935.

_____, The arousal of the brain. J. Nerv. Ment. Dis. 130: 467, 1960.

_____, and J. Chatonnet. Acetylcholine et cortex cerebrale. Arch. int. Physiol. 57: 106, 1949.

Brodie, B. B. Selective release of norepinephrine and serotonin by reserpine like compounds. Dis. Nerv. Syst. 21(3): 107, 1960.

_____, K. F. Finger, F. B. Orlans, G. P. Quinn and F. Sulser. Evidence for serotonin action as necessary for the sedative action of catechol amine depletion. J. Pharm. and Exper. Therap. 129(3): 250, 1960.

_____, and P. A. Shore. A concept for the role of serotonin and norepinephrine as chemical mediators in the brain. Ann. N. Y. Acad. Sci. 66: 631. 1957.

_____, S. Spector and P. A. Shore. Interaction of,drugs with norepinephrine in the brain. Pharm. Rev. 2: 548, 1959.

Carlson, A. The occurrence, distribution and physiological role of the catechol amines in the central nervous system. Pharm. Rev. 2: 440,1959.

_____, M. Lindquist and T. Magnuson. On the biochemistry and possible functions of dopamine and noradrenaline in the brain. in Adrenergic Mechanisms, ed. by J. R. Vane. Little, Brown and Co., Boston, Mass. 1960.

Chatfield, P. O. and D. P. Purpura. Augmentation of evoked potentials by topical application of prostigmine and acetylcholine after atropinization of the cortex. EEG Clin. Neurophysiol. 6: 287, 1954.

Costa, E., G. R. Pscheidt, W. G. Van Meter and H. E. Himwich. Brain concentrations of biogenic amines and EEG patterns of rabbits. J. Pharmacol and Exper. Therap. 130: 81, 1960.

_____, A. M. Revsin, R. Kuntzman, S. Spector and B. B. Brodie. Role for ganglionic norepinephrine in sympathetic synaptic transmission. Science, 133: 1822, 9 June, 1961.

Crawford, T. B. B. The distribution of 5-hydroxy tryptamine in the central nervous system of the dog. in 5-Hydroxytryptamine , ed. by G. P. Lewis, Pergamon Press, N. Y., 1958.

Curtis, D. R. and K. Kuizumi. Chemical transmitters substances in the brain stem of the cat. J. Neurophysiol. 24: 80, 1961.

Dell, P. Intervention of an adrenergic mechanism during brain stem reticular activation. in Adrenergic Mechanisms, ed. by J. R. Vane, Little, Brown and Co., Boston, Mass. 1960.

_____ and M. Bonvallet. The central action of adrenaline; pharmacological consequences. EEG Clin. Neurophysiol. 8: 701, 1956.

DeMarr, E. W. and W. R. Martin. Effects of chlorpromazine on EEG and its activation. Fed. Proc. 15: 416, 1956.

Desmedt, J. E. and G. LaGrutta. The effects of selective inhibition of pseudocholinesterase on the spontaneous and evoked activity of the cats cerebral cortex. J. Physiol.(Lond.)136: 20, 1957.

Domino, E. F. A pharmacological analysis of the functional relationships between the brain stem arousal system and the diffuse projection system. J. Pharmacol. and Exper. Therap. 115: 449, 1955.

_____, A pharmacological analysis of some reticular and spinal systems. in Reticular Formation of the Brain. ed. by H. H. Jasper. Little, Brown and Co., Boston, Mass. 1958.

DuBois, K. P., J. Doull and J. M. Coon. Studies on the toxicity and pharmacological actions of octamethyl pyrophosphate (OMPA). J. Pharmacol. and Exper. Therap. 99: 376, 1950.

Eldred, E. and B. Fujimori. Relations of the reticular formation to muscle spindle activity. in Reticular Formation of the Brain. ed. by H. H. Jasper. Little, Brown and Co., Boston, Mass. 1958.

Elliot, K.A.C., D.L.Swank and N. Henderson. Effects of anesthetics and convulsants on the acetylcholine content in the brain. Amer. J. Physiol., 162: 469, 1950.

Exley, K. A., M. C. Fleming and A. D. Espelien. Effects of drugs which depress the peripheral nervous system on the central reticular activating system of the cat. Brit. J. pharmacol. 13: 485, 1958.

Feldburg, W. The role of acetylcholine in the central nervous system. Brit. Med. Bull. 6: 312, 1950

_____, and M. Vogt. Acetylcholine synthesis in different regions of the central nervous system. J. Physiol.(Lond.) 107: 372, 1948.

Frawley, J.P., E.C.Hagenand O.G. Fitzhugh. A comparative pharmacological and toxicological study of the organic phosphate anticholinesterase compounds. J. Pharmacol. and Exper. Therap. 105: 156, 1952.

French, J. D. The reticular formation. J. Neurosurg. 15: 97, 1958.

_____, M. Verzeano and H. W. Magoun. A neural basis for the anesthetic state. AMA Arch. Neurol. and Psychiat. 69: 519, 1953.

Funderburk, H. W. and T. J. Case. The effects of atropine on cortical potentials. EEG Clin. Neurophysiol. 3: 213, 1951.

_____, E. E. King, E. F. Domino and K.R. Unna. Pharmacological propeties of the benzazoles. 11. Sites of action in the central nervous system. J. Pharmacol and Exper. Therap. 107: 356, 1953.

Giarman, N. J. Neurohumours in the brain. Yale J. of Biol. and Med. 32: 73, 1959.

Gosselin, R. E. , J. D. Gabourel, S. C. Kalser and J. H. Wills. The metabolism of C^{14}- labeled atropine and tropic acid in the mouse. J. Pharmacol. and Exper. Therap. 115: 217, 1955.

Granit, R. and B. R. Kaada. Influence of stimulation of the central nervous system structures on muscle spindle activity in the cat. Acta physiol. scandinav. 27: 130, 1952.

Hampson, J. L., C. F. Essig, A. McCauley and H.E. Himwich. Effects of di-isopropyl fluorophosphate on the electroencephalogram and cholinesterase activity. EEG Clin. Neurophysiol. 2: 41, 1950.

Hebb, C. O. and A. Silver. Choline acetylase in the central nervous system of man and some other mammals. J. Physiol. (Lond.) 142: 187, 1956.

_____, and V. P. Whittaker. Intracellular distribution of acetylcholine and choline acetylase. J. Physiol. (Lond.) 142: 187, 1958.

Henneman, E., A. Kaplan and K. R. Unna. A neuropharmacological study on the effect of myanesin (Tolserol) on motor systems. J. Pharmacol. and Exper. Therap. 97: 331, 1949.

Hess, W. R. Diencephalon, Autonomic and Extrapyramidal Functions. publ. by Grune and Stratton, N. Y., 1954.

Heymans, C. and J. Jacobs. Sur la pharmacologie du di-isopropyl flourophosphate (DFP) et la role des cholinesterases. Arch. int. Pharmacodyn. 74: 23, 1947

_____, R. Pannier and R. Verbeke. Influence des anticholinesterases prostigmine, eserine et di-isopropyl fluorophosphate, et de l'atropine sur la transmission centrale et peripherique des excitation nerveuses. Arch. internat. de Pharmacodyn. et de Therap. 72: 405, 1946.

Himwich, H. E. in Effects of Pharmacologic Agents on the Central Nervous System. Res. Publ. Ass. Nerv. Ment. Dis. XXXVlll. ed. by F. J. Braceland, 1959.

_____, and F. Rinaldi. Reticular activating system and anti-parkinson drugs. Yale J. of Biol. and Med. 28: 308, 1955-56.

_____, _____. The effects of drugs on the reticular activating system, in Brain Mechanisms and Drug Action. ed. by W. S. Fields, C. C. Thomas Springfield, Ill., 1957.

Hoagland, H. A simple method for recording electrocorticograms in animals without opening the skull. Science, 92: 537, 1940.

Holmstedt, B. Synthesis and pharmacology of dimethyl amido- ethoxy- phosphoryl cyanide (Tabun) together with a description of some allied anticholinesterase compounds containing the N-P bond. Acta physiol. scandinav. 25(suppl.90); 1, 1951.

_____, Pharmacology of organophosphorus cholinesterase inhibitors. Pharm. Rev. 11: 567, 1959.

Ingvar, D. H. Reproduction of 3/sec. spike/wave EEG pattern by subcortical electrical stimulation in cats. Acta physiol. scandinav. 33: 137, 1955.

_____, Electrical activity of isolated cortex in unanesthetized cat with intact brain stem. ibid.: 150, 1955.

_____, Extraneuronal influences upon the electrical activity of the isolated cortex following stimulation of the ARAS. ibid: 169, 1955.

Jasper, H. H. Diffuse projection systems: the integrative action of the thalamic reticular system/ EEG Clin. Neurophysiol. 1: 405, 1949.

Jasper, H. H. and C. Ajmone-Marsan. A Stereotaxic Atlas of the Diencephalon of the Cat. publ, by National Research Council of Canada. Ottawa, Can. 1957.

_____, _____, and J. Stoll. Corticofugal projections to the brain stem. AMA Arch. neurol. and Psychiat. 67: 155, 1952.

Kaada, B. R. Site of action of myanesin (mephenesin, Tolserol) in the central nervous system. J. Neurophysiol. 13: 89, 1950.

Karczmar, A. G., O. AWad and K. Blachut. J. Appl. Pharm. and Toxicol. in press, 1961.

_____, K. Blachut and S. A. Ridlon. Interaction of EPN and malathion. Fed. Proc. 18(1);1614, 1959.

_____, and J. P. Long. Relationship between peripheral cholinolytic potency and tetraethylpyrophosphate antagonism of a series of atropine substitutes. J. Pharmacol. and Exper. Therap. 123: 230, 1958.

Killam, E. K. and K. F. Killam. The influence of drugs on the central afferent pathways.in Brain Mechanisms and Drug Action. ed. by W. S. Fields C.C.Thomas, Springfield, Ill. 1957.

_____,_____. Drug action on pathways involving the reticular formation. in Reticular Formation of the Brain. ed. by H. H. Jasper. Little, Brown and Co., Boston, Mass. 1958.

_____,_____. in Effects of Pharmacologic Agents on the Nervous System. Res. Publ. Ass. Nerv. Ment. Dis. XXXVLLL ed. by F. J. Braceland.1959.

_____,_____, and T. Shaw. The effects of psychotherapeutic compounds on the central afferent and limbic pathways. Ann. N. Y. Acad. Sci.66; 784, 1957.

King, E. E. and K. R. Unna. The action of mephenesin and other interneuron depressants on the brain stem. J. Pharmacol. and Exper. Therap. 111: 293, 1954.

_____, Differential action of anesthetics and interneuronal depressants on EEG arousal and Recruitment responses. J. Pharmacol. and Exper. Therap. 116: 404, 1956.

Knott, J. R., W. R. Ingram and W. D. Childs. Effects of subcortical lesions on the cortical EEG in cats. AMA Arch. Neurol. and Psychiat. 73: 203, 1955.

Koelle, G. B. and A. Gilman. Anticholinesterase drugs. J. Pharmacol. and Exper. Therap. 95: 166, 1949.

Koizumi, K., J. Ushiyama and C. McBrooks. Reticular formation interactions. Amer. J. Physiol. 195: 1, 1958.

Koppanyi, T., A. G. Karczmar and T. O. King. Effect of tetraethyl pyrophosphate on sympathetic ganglionic activity. Science, 106: 492, 1947.

Kreindler, A., E. Zuckerman, M. Steriade and D. Chimion. The action of chlorpromazine on the central and peripheral effects induced by cortical and reticular stimulation. Arch. int. pharmacodyn. 120: 263, 1959.

Kuehn, A. and W. Schallek. Effects of drugs on resting and activated EEG of cats. Fed. Proc. 17(1); 1522, 1958.

Longo, V.G. Acetylcholine, cholinergic drugs and cortical electrical activity. Experientia 11: 76, 1955.

_____, Effects of scopolamine and atropine on electroencephalographic and behavioral reactions due to hypothalamic stimulation. J. Pharmacol. and Exper. Therap. 116: 198, 1956.

Longo, V. G., G.P. von Berger and D. Bovet. Action of nicotine and the"ganglioplegiques centraux" on the electrical activity of the brain. J. Pharmacol. and Exper. Therap. 111: 349, 1954.

Magoun, W. H. An ascending reticular activating system in the brain. AMA Arch. Neurol. and Psychiat. 67: 145, 1952.

_____, and R. Rhines. An inhibitory mechanism in the bulbar reticular formation. J. Neurophysiol. 9: 165, 1946.

Marazzi, A. S. and E. R. Hart. The relationship of the hallucinogens to cerebral adrenergic neurohumours. Science, 118: 367, 1953.

_____, _____. ibid: 121, 365, 1955.

_____, The effects of certain drugs on cerebral synapses. Ann. N. Y. Acad. Sci. 66; 496, 1957.

_____, The effects of drugs on neurons and synapses. in Brain Mechanisms and Drug Action. ed. by W. S. Fields. C.C.Thomas, Springfield, ILL. 1957.

_____, _____, M. I. Gluckman and Z. Horowitz. Cerebral synaptic inhibitory action of " in situ" serotonin. Fed. Proc. 20(1): 324d, 1961.

Marley, E. Release of an adrenaline-like substance by electrical stimulation of the brain stem. in Adrenergic Mechanisms , ed. by J. R. Vane. publ. Little, Brown and Co., Boston, Mass. 1960.

Martin, W. R., E.E. J. DeMarr and K. R. Unna. Chlorpromazine:1, The action of chlorpromazine and related phenothiazines on the EEG and its activation. J. Pharmacol. and Exper. Therap. 122:343, 1958.

Michaelis,M., J.E. Finesinger, F. deB. Verster and R.W.Erickson. The effects of intrvenous injection of DFP and atropine on the level of free acetylcholine in the cerebral cortex of the rabbit. J. Pharmacol. and Exper. Therap.111:169, 1954.

Modell, W.,S.Krop and W. F. Riker,Jr. General systemic actions of DFP in cats. J. Pharmacol. and Exper. Therap. 87:400, 1946.

Monnier, M. et R. Tissot. Action de la reserpine et de ses mediatours (5-hydroxytryptophane-serotonin et DOPA-norepinephrine) sur le comportment et le cerveau du lapin. Helv. Physiol. and Pharmac. Acta. 16: 255, 1958.

_____, _____. Suppression de l'action de la reserpine sur le cerveau par ses antagonistes; ibid: 268, 1958.

Morison,R. S. and E. W. Dempsey. A study of the thalamo-cortical relations. Amer. J. Physiol. 135: 281, 1942.

Moruzzi,G. and H.W. Magoun. Brain stem reticular formation and activation of the EEG. EEG Clin. Neurophysiol. 1: 455, 1949.

Nauta, W. J. H. and P. A. Gygax. Silver impregnation of degenerating axons in the central nervous system: A modified technique. Stain Technol. 29: 91, 1954.

_____, and H. G. J. M. Kuypers. Some ascending pathways in the brain stem reticular formation. in Reticular Formation of the Brain. ed. by H. H. Jasper. Little, Brown and Co. Boston, Mass. 1958

Olszewski, J. The cytoarchitecture of the human reticular formation. in Brain Mechanisms and Consciousness. ed. by J. F. Deladresnaye. C.C.Thomas, Springfield, Ill. 1954.

Paasonen, M. K. and N. J. Giarman. Brain levels of 5-hydroxytryptamine after various agents. Arch. int. Pharmacodyn. 114: 189, 1958.

Page, I. H. Neurochemistry and serotonin: A chemical fugue. Ann. N.Y.Acad. Sci. 66(3): 592, 1957.

Pepue,G., D.X. Freedman and N.J.Giarman. The biochemical and pharmacological action of Deanol. J. Pharmacol. and Exper. Therap. 129(3): 291, 1960.

Perry, W.L. Central and synaptic transmission (pharmacological aspects). Ann. Rev. Physiol. 18: 270,,1956.

Pfeiffer, C.C. Parasympathetic neurohumours: Possible precursors and effect on behavior. Int. Rev. Neurobiol. 1: 1959.

Pletscher, A. Significance of monoamine oxidase inhibition for the pharmacological and clinical effects of the hydrazine derivatives. Ann. N.Y.Acad. Sci. 80(3): 1039, 1959.

Purpura,D.P. A neurohumoural mechanismof the reticulo- cortical activation . Amer. J. Physiol. 186: 250, 1956.

Richter, D. and J. Crossland. Variation of acetylcholine content of the brain with the physiological state. Amer. J. Physiol. 159: 247, 1949.

Ridlon, S.A. and A.G.Karczmar. The effects of EPN and malathion on reticular stimulation. Fed. Proc. 20(1): 180, 1961.

Riehl, J.S., J.C.Paul and K.R.Unna. Quantitation of the activation response of the EEG. Fed. Proc. 18(1): 437, 1959.

Rinaldi, F. and H. E. Himwich. A comparison of effects of reserpine and some barbiturates on the electrical activity of the cortical and subcortical structures of the brain of rabbits. Ann. N. Y. Acad. Sci. 61(1): 27, 1955 a.

_____, _____. Alerting responses and the actions of atropine and cholinergic drugs. AMA Arch. Neurol. and Psychiat. 73: 387, 1955 b.

_____, _____. Cholinergic mechanisms involved in the function of the mesodiencephalic activating system. ibid: 396, 1955 c.

Rodriquez, J.M., E.R.Hart and A.S. Marazzi. Lack of carotid body effect in the cerebral synaptic inhibition by serotonin. Fed. Proc. 20(1): 333a, 1961.

Rosenburg, P. In vivo reactivation by PAM of brain ChE inhibited by paraoxon. Biochem. Pharmacol. 3: 212, June 1960.

Rothballer, A.B. Studies on the adrenaline sensitive component of the reticular activating system. EEG Clin. Neurophysiol. 8: 603, 1956.

_____, The effect of phenylephrine, methamphetamine, cocaine and serotonin on the adrenaline sensitive component of the reticular activating system. ibid, 9: 409, 1957.

_____, The effects of catechol amines on the central nervous system. Pharm, Rev. 2(2): 494, 1959.

Schallek, W. and A. Kuehn. Drug effects on the reticular stimulation and EEG activation. Arch. Int. Pharmacodyn. et de Therap. 120: 319, 1959.

Scheibel, M.E. and A.B.Scheibel. Structural substrates for integrative patterns in the brain stem reticular core. in Reticular Formation of the Brain Stem. ed. by H. H. Jasper. Little, Brown and Co., Boston, Mass. 1958.

Sharpless, S. and H. H. Jasper. Habituation of the arousal reaction. Brain, 79: 655, 1956.

Shore, P.A., A. Pletscher, E.G.Tomich, A.Carlson, R.Kuntzman and B.B.Brodie. Role of brain serotonin in reserpine action. Ann. N.Y.Acad. Sci. 66: 609, 1957.

Spector,S, P.A.Shore and B.B.Brodie. Biochemical and pharmacological effects of the monoamine oxidase inhibitors, iproniazid, JB-516 and JB-835. J. Pharmacol. and Exper. Therap.128:15,1960.

Starzl, T.E. and H.W.Magoun. Organization of the diffuse thalamic projection system. J. Neurophysiol. 14: 133, 1951

Stewart, W.C. Accumulation of the acetylcholine in the brain and blood of animals poisoned with cholinesterase inhibitors. Brit. J. Pharmacol. 7: 270, 1952.

Udenfriend,S. H.Weissbach and D.F.Bogdanski. Biochemical findings relating to the action of serotonin. Ann. N.Y. Acad. Sci. 66: 602, 19557.

Vogt,M. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol.(Lond.) 123: 451, 1954.

_____. The catechol amines in the brain. Pharm. Rev. 2: 483, 1959.

Weil-Malherbe, H. Synthesis of catechol amines in the depleted brain. in Adrenergic Mechanisms. ed. by J.R.Vane. Little, Brown and Co. Boston, Mass. 1960.

_____, The passage of catechol amines through the blood-brain barrier. ibid,

_____, and A.D.Bone. The effect of reserpine on the intracellular distribution of catechol amines in the brain stem of rabbits. J. Neurochem. 4: 251, 1959.

_____, H.S.Posner and G.R.Bowles. Changes in the concentration and intracellular distribution of brain catechol amines: The effects of reserpine, beta-phenyl isopropyl hydrazine, pyrogallol and 3,4, dihydroxyphenylalanine, alone and in combination. J.Pharmacol. and Exper. Therap. 132: 278, 1961.

Wescoe, W. Clark, R.E.Green B.P.McNamara and S. Krop. The influence of atropine and scopalamine on the central effects of DFP. J. Pharmacol. and Exper. Therap. 92: 63, 1948.

Wikler, A. Pharmacologic dissociation of behavior and EEG sleep patterns in dogs: Morphine, n-allylnormorphine and atropine. Proc Soc. Exp. Biol. and Med. 79: 261, 1952.

Wooley, D.W. and E.N.Shaw. Evidence for the participation of serotonin in mental processes. Ann. N.Y. Acad. Sci. 66(3): 649, 1957.

Zeller, E.A., L.A.Blanksma, W.P.Burkhard, W.L.Pachand and J.C. Lanzanas. Invitro and in vivo inhibition of amine oxidases. Ann. N.Y. Acad. Sci. 80(3): 583, 1959.

_____. The role of amine oxidases in the destruction of catechol amines. Pharm. Rev. 2(2): 387, 1959.

APPENDIX 1

Fig. 12 contains, in the control tracing, a subthreshold stimulation, and in tracing B, a threshold or desynchronized pattern which was obtained by stimulation of the posterior medial ARAS. This figure was employed to illustrate the comparison of a quantitative and the visual method of determina threshold desynchronization of the EEG pattern.

A modification of the method of Riehl (1959) was used to determine the quantity Ua, or unit activity. Each deflection of the pen, during a certain time period, was measured and converted to microvolts according to the calibration of the instrument. The sum of the voltages were taken and the frequency quantitated by the number of pen deflections during that time period. These quantities are substituted into the formula Ua $F \times \frac{1}{V}$; F frequency, V sum of the voltages.

The data were quantitated from channel 5 to obtain values for the two second period immediately prior to stimulation and the two second period following the onset of stimulation. In the control record A, prestimulation Ua $\frac{40}{2370}$ 0.017; stimulation Ua $\frac{32}{1685}$ 0.019. After treatment, the Ua values were as follows; prestimulation Ua $\frac{39}{2150}$ 0.018; the stimulation Ua $\frac{65}{1910}$ 0.314.

By definition, a synchronized pattern consists of of low frequency, high voltage waves while a desynchronized pattern is characterized by high frequency, low voltage waves. Therfore, one would expect, using a frequency x reciprocal voltage relationship, a greater quantity (Ua) as the voltage decreased and the frequency increased as in a desynchronized EEG pattern. Conversly, a synchronized EEG pattern of low frequency, high voltage would result in a smaller (Ua) quantity.

By comparing the Ua's calculated from figure 12, it is evident that there is no significant change in the control record A from the stimulation of the posterior medial ARAS, the Ua's being 0.017 and 0.019. Visual observation similarly suggested that there was no change in the EEG pattern. In record B, the Ua of 0.018 calculated for the prestimulation period is markedly lower than that obtained during the stimulation period(0.314). Visual inspection, made independently, led to a similar conclusion.

On the basis of this and similar analyses, the present investigator concluded that visual inspection of the EEG pattern is as reliable as the quantitative method in determining a threshold desynchronization pattern.

APPROVAL SHEET

The dissertation submitted by Seward A. Ridlon has been read and approved by five members of the faculty of the Graduate School of Loyola University.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Sept. 26, 1961
Date

Alexander C. Kazmaier
Signature of Advisor