DRUG STUDIES ON THE EPILEPTIFORM AFTERDISCHARGE IN THE CHRONICALLY NEURONALLY ISOLATED CEREBRAL CORTEX

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by

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ABSTRACT

Chronic neuronal isolation of a mass of neurons in the cerebral cortex results in an increased tendency of the deafferented region to produce an epileptiform afterdischarge (EAD). This experimentally induced epileptogenesis is associated with some degenerative structural changes of some of the cortical elements. The hypothesis that this abnormal epileptiform activity is the result of altered synaptic activity in the cerebral cortex and can be modulated by appropriate pharmacological intervention was tested.

Several types of autonomic agents and their antagonists were administered intraperitoneally into unanaesthetized and unrestrained cats with unilaterally chronically isolated slabs of cerebral cortex, bearing permanently implanted electrodes. This laboratory preparation permitted study of some of the chemical modulator and mediator systems postulated to be operative at the cortical level, without interference from afferent influences arising from various subcortical structures.

Administration of centrally active cholinergic drugs results in significant decreases in afterdischarge duration. Anti-muscarinic drugs prolong EAD duration and antagonize the action of cholinergic drugs. The action upon EAD duration of cholinergic drugs appears to be exerted on specifically muscarinic structures since neither nicotinic agonists nor antagonists modify EAD duration, the action of

cholinergic drugs, or the action of anti-muscarinic drugs. These results suggest that there is operative in the cerebral cortex a cholinergic inhibitory system, specifically muscarinic in type.

Several sympathomimetic agents were investigated and found to cause significant reductions in EAD duration. Amphetamines were utilized as the prototype agent and their action upon EAD duration in the isolated cortex is partially antagonized by α-adrenergic receptor blocking drugs, but not by β-adrenergic receptor blockers. The α and β adrenergic receptor blocking drugs do not produce any consistent significant changes in EAD duration when administered alone. Moreover, the adrenergic blockers do not modify the response to muscarinic or anti-muscarinic drugs. The action of amphetamine is also prevented and reversed by anti-muscarinic agents. These results suggest that apparently there is no adrenergic tone in the isolated cortex and that adrenoceptive sites are subtended onto cholinoreactive receptors in the cerebral cortex.

Cortical levels of serotonin were elevated indirectly by administration of its metabolic precursor 5-hydroxytryptophan (5-HTP). Injection of 5-HTP is found to be capable of reducing EAD duration significantly below control levels. This action of 5-HTP is blocked either by antiserotonin or by anticholinergic drugs, but not by adrenergic antagonists. Serotonin blocking drugs when administered alone cause significant increases in EAD duration. These antagonists were capable of blocking the action of amphetamine, but not the action of cholinergic drugs. These results suggest that there is serotonergic tone in the isolated cortex and that serotonergic structures probably subserve a cholinergic system.

More precise conclusions regarding the role of adrenergic drugs in EAD modulation are not presently possible owing to the lack of definitive knowledge pertaining to their central actions. It is possible that amphetamine might be acting through serotonin structures. or it may be acting via a parallel pathway.

The chronically deafferented (denervated) cortex seems to be an experimental model of *grand mal* epilepsy as the duration of EADs elicited in cortical slabs is reduced by diphenylhydantoin and phenobarbital, but not by pentobarbital, trimethadione or ethosuximide. Moreover, diphenylhydantoin, and to a limited extent, phenobarbital, interact to a certain degree with cholinergic and anticholinergic drugs. Therefore, these anti *grand mal* agents might limit EAD duration by acting, at least in part, through cholinergic synapses in the cerebral cortex.

Gamma-aminobutyric acid (GABA) was tested for its effects on EAD duration in the isolated cortex and found to lack any action upon EAD duration following its systemic administration. Several benzodiaepine derivatives were investigated and found to depress EAD duration significantly.

It is tentatively suggested that a defect in the availability of active acetylcholine at intracortical cholinergic pathways results in prolonged epileptiform afterdischarges. To a lesser degree, adrenergic, and to a greater degree, serotonergic structures, both also limit paroxysmal cortical activity, and their effect seems to be achieved by modulation of cholinoceptive neurons. Anti-epileptic agents capable of arresting these seizure discharges also appear to interact with these cortical cholinergic neurons.

Dedicated

to

my wife and parents

whose continued confidence and assistance

have made this achievement possible

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To

George Santayana

for Pointing Out:

"those who cannot remember the

past are condemned to repeat it."

I. HISTORICAL PERSPECTIVE

Epilepsy has one of the longest recorded histories in medical literature and, probably, can claim some of the most extensive of therapeutic trials with traditional materia medica and primitive rituals. The diverse preparations suggested at different times for treating the <u>falling sickness</u> or the <u>sacred disease</u>, as epilepsy came to be known, include almost every substance in the world capable of being introduced into the oral cavity of man. Temkin (1945) has published a fascinating account of the history of this disease from 2,000 B.C. to modern times. The picture that emerges from this span of 4,000 years is one of sheer desperation. A typical approach to therapy at the height of the Roman Empire was for the unfortunate epileptic to rush into the arena and lap up the warm blood of slain gladiators.

Some other remedies tried were, for example, ashes of asses hooves and stork's dung. One direct orthopedic approach consisted of tying a ligature around a limb to "postpone the attack which announced itself in a limb". Trephination of the skull to allow the entrapped evil spirits an opportunity for escape became a routine procedure for some time. These bizarre attempts at therapy more often increased the patient's suffering rather than effectively countering the frequency of seizures. Many years had to pass before more precise concepts and measures were formulated and applied.

Throughout history, epilepsy has been regarded as a manifestation of the presence of an evil spirit in the afflicted individual. Dominating the existing therapeutic measures were procedures believed to exorcise these demons, in the hope that they would abandon

the body during the seizure. Theologians and physicians were preoccupied with dogma and the occult. Treatment during the middle ages consisted of peony root, frog's liver, human urine which was preferably collected from the first human witness to the seizure, and drinking the blood of beheaded criminals.

The term epilepsy is derived from the Greek epilambanein, which means "to seize" or "to attack". Hippocrates in his monograph "On the Sacred Disease" (ca. 400 B.C.) coined the term epilepsy and recognized that the primary origin of convulsions was in the brain. Galen (130-200 A.D.) introduced the term idiopathic epilepsy. In the New Testament (Mark, IX:17) appears a good description of a major seizure. As advances in medicine became real, the typical clinical features of major seizures, and also the occurrence of minor forms of epilepsy, were noted. In the late nineteenth century, Hughlings Jackson (see Merritt, 1967) differentiated focal epilepsy from grand mal (major seizures) and petit mal (minor seizures). From this point on, dogma yielded increasingly to scientific reason.

In essence, Jackson commented extensively on concepts concerning the epileptogenic focus, the local seizures and the spread of the seizure to the rest of the brain involving the "compelled" excessive discharge of stable cells. Jackson expressed the opinion that cells which have "become very highly explosive continue to be elements of the same nervous arrangements and that these nervous arrangements are still connected in the very same way with the remainder of the nervous system". Consonant with this reasoning would be the ability of epileptogenic elements, upon maximal discharge, to produce exces-

sive discharge of the immediate, normally stable, nervous elements. On this basis, he proceeded to outline the concept of spread of seizure discharge from point to point in the cerebral cortex.

The cause of the abnormal paroxysmal discharge is still today imperfectly understood. An etiological distinction is frequently made between symptomatic or organic epilepsy in which an underlying traumatic, infectious, neoplastic or metabolic cause is known or suspected, and idiopathic or essential epilepsy in which there is no demonstrable physiological basis for the affliction. In idiopathic epilepsy an heriditary causality is suspected but, it cannot be said unequivocally that epilepsy is inherited.

The nature of the neuronal abnormality that permits the seizure discharge to occur is also uncertain. Grey Walter (1953) has conjectured that in all probability the safety margin between normality and epileptogenic abnormality is relatively small, since an epileptiform encephalogram can be elicited in about 4 per cent of non-epileptics by a flickering light. The concept of a relatively slight cerebral abnormality is further supported by the normality of patients between convulsive episodes.

Although the presence of a cerebral abnormality such as an organic lesion is a common factor responsible for the development of a seizure, it is difficult in many cases to correlate the epileptic attacks to the presence of abnormal tissue. Many factors are involved in the generation and spread of seizure discharges. Very likely the normal or nearly normal cerebral tissue adjacent to an epileptic focus is intimately involved in the origination and spread of seizures.

A large number of seizures involve sensory, visceral, emotional or behavioural disturbances and in many cases are non-convulsive. Some clinicians claim that epilepsy is associated with personality disorders (Merritt, 1967). This relationship is common in frontal lobe, temporal and hypothalamic epilepsy, but when these areas are not involved, there is apparently no visible disorder of personality (Schmidt and Wilder, 1968).

Epilepsy is not a simple neurological disease, but rather a symptom-complex having a variety of causes and with a multiplicity of forms. Therefore, it is not to be expected that any single treatment will suffice for its continued therapeutic management. A variety of drugs are often required for effective control.

Broadly defined, epilepsy is a paroxysmal disorder of the nervous system, characterized by recurrent attacks of loss of consciousness with or without convulsive movements. Penfield (1958) regards epilepsy as a tendency to periodic involuntary neuronal explosions.

Epileptic taxonomy is a popular diversion and many classifications for the epilepsies are present in the literature. Very few, if any, of the proposed schemes are comprehensive or completely satisfactory, but they do provide the basis for rational therapy. Due to our limited knowledge of the underlying pathological processes all attempts at classification of the epilepsies, are of necessity, based on the clinical features of the seizures. The International League against Epilepsy has proposed a universal classification (Gastaut, 1969) in the hope that it will facilitate communication between workers in the field. However, the classification most

commonly used at present is the one of Gibbs and Stamp (1958). The outline and definitions of seizures that follow are based on this system. This classification is useful therapeutically since one can more readily associate with each type, effective anticonvulsant drugs possessing some degree of specificity.

The more common epilepsies are:

i) Grand mal (generalized, bilateral seizures): This is the most common single form of epilepsy and is characterized by the occurrence of generalized tonic-clonic convulsions, loss of consciousness and autonomic hyperactivity. The electroencephalogram (EEG) during the attack is characterized by a dominant frequency of 10 cycles/second or more, decreasing in frequency and increasing in amplitude during the tonic phase, and with slow waves during the clonic phase. The essential feature in grand mal is the abnormal ease of generalized spread of an initial discharge of variable location (Toman and Taylor, 1952).

ii) Petit mal (minor, bilateral seizures): Formerly this term was used to describe any mild seizure. Today the term is restricted to describe brief (few seconds), but frequent (5-100 per day) attacks of impaired consciousness associated with fixed staring and eye movements (absences). Very rarely, petit mal is associated with loss of posture or with jerking movements. The essential electroencephalographic feature is a rhythmic 3 cycle/second spike and wave discharge.

iii) Psychomotor seizures (automatisms): A form of epilepsy characterized by mental confusion and elaborate, but usually poorly co-ordinated automatic movements. This form of epilepsy is frequently complicated by psychiatric illness, and in some cases, violent behaviour. The EEG displays high voltage 6/second and low voltage 4/second waves in widely separated areas of the brain.

iv) Myoclonic epilepsy: This seizure type is characterized clinically by sudden attacks of jerking movements of the head, limbs or trunk; lasting approximately 1 second, and occurring in bursts of four or five at 3-6 second intervals. The EEG abnormality when present, is a disorganized pattern of slow waves mixed with multiple high voltage spikes.

v) Jacksonian epilepsy (partial, focal seizures): These are focal seizures originating in relatively restricted populations of neurons and characterized by an orderly sensory or motor progression. Often, an initial twitch in one peripheral muscle group spreads centrally, following the sequence of motor localization of the precentral cortex, involving other muscle groups. Though this march may suggest a cerebral cortical focus, the actual point of origin may be temporal or occipital. Most Jacksonian seizures are localized and often are not accompanied with loss of consciousness.

There are many other less common types of seizures with known and unknown etiology. Other convulsive attacks tend to be associated with acute illness, e.g. fever, acute encephalopathy, or other processes involving the brain in a different manner. Most any acute insult to the brain can result in some convulsions.

Recently, McIlwain (1957) has reviewed the origins of the scientific approach to the use of effective anti-epileptic drugs. This term is reserved for drugs actually employed in the therapeutic treatment of epilepsy. Anticonvulsant agents are defined as compounds having potential value in the treatment of seizures on the basis of experimental testing.

The first effective remedy was bromide, introduced by Locock in 1857 (see review by Temkin, 1945). This author believed that epilepsy was the result of excessive masturbation. In 1856 he

noticed a report by Sieveking in 1857 stating that bromides caused a decrease in libido in one man. On this basis he administered bromides with beneficial results to a case of epilepsy, "confirming" his theory about the etiology of this disease. Locock obtained excellent results with potassium bromide in all but one of another fourteen cases of epilepsy associated with "sexual colouring", and he recommended that this drug be employed for the control of epileptic seizures.

In 1868, Clouston conducted well-designed clinical trials, confirming the anti-epileptic action of bromides by measuring the reduction in number and severity of "fits" during several months of treatment (see review by Temkin, 1945). In the following years bromides were used on an enormous scale as this was truly the first dependable anti-epileptic drug. Unhappily, toxic effects made this remedy far from ideal, and the search for better medication continued.

During the 19th century atropine was administered widely for the treatment of epilepsy (Gowers, 1885). The dosages tended to be low and it can be questioned whether this treatment was of any benefit to patients. The introduction of bromides in 1868 severely limited the use of atropine in epilepsy and, accordingly, its use as an anticonvulsant drug was soon discontinued.

The next important development in the therapy of epilepsy came in 1912 when Hauptmann (see review by Temkin, 1945) tried in several epileptics, as a result of an incidental clinical observation, the relatively new sedative drug phenobarbital. Hauptman reasoned that as epileptics were excitable, they might benefit from sedation.

Almost immediately it was recognized as an outstanding anti-epileptic drug, as marked decreases in the number and severity of epileptic attacks were observed. Phenobarbital still remains a potent anti-*grand mal* agent, which is well tolerated by patients taking the drug on a chronic basis.

It should be noted that up to this point, the development of anti-epileptic medication was carried out in a clinical setting, largely on the basis of empirical observations and trials on patients, usually against a background of failure. The immediate success of phenobarbital set the stage for an intensive search for other pharmacological agents useful in the management of epilepsy. Not surprisingly, a large proportion of the more successful anti-*grand mal* agents that were developed subsequently, incorporate certain aspects of the chemical structure of phenobarbital. Up to this point, any compound known to possess sedative properties was usually tried in epileptics.

II. EXPERIMENTAL EPILEPSY

A. Historical Review

In 1760 Lorry concluded from experiments involving medullary compression in cats that only the medulla oblongata could give rise to convulsions. This theory was contrary to previous concepts which held that the entire brain was epileptogenic. Flourens (1824), on the basis of ablation experiments in animals, concluded that the cerebral hemispheres were not concerned with motor phenomena, but with perception and higher mental functions. Even Todd (1855) who investigated the causes of epilepsy in rabbits, ignored his finding of discrete movements in facial muscles upon stimulation of the cerebral hemispheres, since he felt that his findings were artefactual in view of the "authority" of earlier studies on epilepsy.

Early animal experiments conducted in the 19th century led some authors to the conclusion that convulsions could be provoked by irritating the brain below its surface (Kussmaul and Tenner, 1859). Other authors (Nothnagel, 1868; Schroeder van der Kolk, 1859) believed that seizures had their origin in the medulla or the pons or both structures, since generalized convulsions were observed in animals which were left with only the medulla and pons, but not in spinal animals. From these studies it was postulated that there was an anatomical structure, believed to be the common motor centre, for the transmission of convulsant impulses.

Albertoni (1882) studied the location of the convulsant action of camphor in mammals and demonstrated that sections below the optic thalami abolish or greatly reduce the intensity of the clonic convulsions provoked by large doses of camphor. Hildebrandt (1901)

reported that the volatile oil absinthe was similar to camphor, since following intravenous administration of small doses into cats and rabbits it produced marked excitation of the autonomic nervous system, followed immediately by unconsciousness and general epileptiform convulsions, first clonic, then tonic.

The first relatively precise study of the electrical excitability of the cerebral cortex was the classical work of Fritsch and Hitzig (1870), which made known the fact that electrical surface stimulation of certain "strips" on the exposed brain, induced motor responses and epileptiform convulsions. Removal of the cortex established that this motor function was localized to the cerebral cortex. Ferrier (1873) showed that not only contralateral limb movements and generalized muscular movements, but also relatively localized convulsive seizures could be produced by local electrical stimulation of certain cortical areas in experimental animals, which confirmed the existence of a motor centre in the brain of the dog and the monkey.

It was soon realized that epileptiform convulsions could easily be induced merely by increasing the intensity of the electrical stimulus (Albertoni, 1876; Bubnoff and Heidenhain, 1881; Francois-Franck and Pitres, 1883; Luciani and Tamburini, 1879). Horsley (1889) was interested in epilepsy and concerned himself with the detailed mapping of the movements elicited by electrical stimulation of the cortex in primates. In his early experiments on cortical stimulation, he observed that it was possible to obtain certain movements in the periphery from more than one cortical area, but that they were elicited preferentially from a particular point. This author stirred up much

discussion by holding firmly that tonic-clonic spasms could originate only from the cerebral cortex. Batelli (1903) induced "artificial" epileptic attacks by means of electric current, but without exposing the brain.

The spontaneous electrical activity of the brain was first described by Caton (1875), who detected "feeble currents of varying direction" using non-polarizable electrodes on the skull or exposed brain of cats, rabbits and monkeys and an ingenious system of amplification using a mirror attached to a galvanometer needle. Since sensory stimulation altered this activity, he assumed that these electrical phenomena were related to cerebral function. Further progress was slow, as the necessary improvements in detection, amplification and recording were slow in coming. Hans Berger (see review by Walker, 1957) apparently began in 1902 to verify Caton's original observation.

Even after these findings it was not possible to localize an epileptogenic centre in the motor cortex, and investigators began to postulate that the epileptiform activity propagated from the stimulated point to the whole of the cerebral cortex, or to subcortical structures believed to be involved in the sustainment of seizures. Unverricht (1889) postulated that the seizure propagated "superficially" by intracortical conduction to the whole of the cortex. Karplus (1914) suggested that cortical discharges spread by subcortical pathways. Conduction to spinal neurons was believed to proceed by way of the pyramidal tracts. This was an erroneous assumption, as much later work by Mettler and Mettler (1940) on animals with bilateral destruction of pyramidal structures, showed that extra-pyramidal structures

and pathways are a predominant part of the mechanism for secondarily or partially generalized seizures. In the early part of the 20th century the cortex was considered by many to be the locus of origin for focal as well as non-focal seizure attacks (Oppenheim, 1908).

In 1882 Albertoni published the first experiments using chemically and electrically-induced convulsions in monkeys and dogs for the screening of potential anti-epileptic drugs. This author showed that adequate doses of potassium bromide raised the electro-convulsive threshold and lowered the excitability of cortex, apparently confining epileptiform activity to the area directly stimulated. This line of work was further elaborated and confirmed by Bikeles and Zbyszewski (1914) and by Schilf (1922). Albertoni (1882) found that the seizure responses to electrical stimulation were increased in severity and duration by large doses of atropine sulfate (10 mg/kg). On the basis of his results, Albertoni concluded that the widespread administration of atropine to epileptics, in vogue at this time, was not beneficial and might even be harmful.

In 1883 Openchowski observed that the local application of cold to the surface of the cerebral cortex in experimental animals usually resulted in the production of epileptic-like seizures. This cooling did not induce chronic recurrent seizures, and Openchowski reasoned that direct trauma to the brain was somehow involved in epilepsy.

At about the same time, the classical clinical observations of Gowers (1885) and Hughlings Jackson (1886) on the epilepsies began to appear. These two pioneers studied the neurological aspects of

epilepsy from different viewpoints. Gowers believed that there were clear clinical distinctions between convulsions which result from an identifiable lesion in the brain, and convulsions in which there was not "any visible alteration" of the brain associated with the seizure.

Hughlings Jackson believed that all forms of epilepsy were due to local "discharging lesions" in the gray matter of the central nervous system. He categorized generalized major convulsions as genuine or idiopathic, and focal seizures in which parts of the body are involved one after the other as "deliberate spasms". Hughlings Jackson (see Taylor, 1931) assumed that epilepsy associated with an initial loss of consciousness would originate in the highest centres of the brain controlling consciousness. Hughlings Jackson's early description of epilepsy as "occasional, sudden, rapid, and local discharges of gray matter" was based on many clinical and post-mortem studies and on investigations dealing with the electrophysiology of peripheral nerve.

B. Modern Studies

Useful knowledge of the normal electrical activity of the human brain and of the characteristic and dramatic way in which it is altered in epilepsy did not come until the 1930's. Pioneering studies by Berger (1929), Adrian and Mathews (1934) and Rosenblueth and Cannon (1942) served as a foundation for a new conceptualization of epileptic mechanisms.

Hans Berger can be truly considered the father of electroencephalography. Using very primitive but sensitive instrumentation, including a double galvanometer, he was able to demonstrate clearly

the electrical activity of the brain, recorded from the surface of the scalp through an intact skull. The advent of this revolutionary technique laid the groundwork which made it feasible to use electroencephalography to detect changes in the electrical activity of the brain, and which also serves as a useful diagnostic tool for detecting the electrical signs of epilepsy, even in the absence of overt seizures.

Adrian and his colleagues (1934) verified Berger's important finding. This group conducted many investigations in experimental preparations studying the nature of the oscillating potentials, apparently originating from the electrical activity of the brain. Rosenblueth and Cannon (1942) extended the early experimental studies in an attempt to discover the characteristic responses of the cerebral cortex. They hoped that these studies would help to explain the function of the brain in the electrophysiological sense.

Penfield (1958, 1969), after a life-long involvement with neurological and neurophysiological studies on the cerebral cortex, concluded that the effect of any epileptogenic lesion can be reproduced by direct electrical stimulation, if the parameters of the stimulus are carefully modulated. For the experimental investigator consideration of the seizure discharge as an electrical phenomenon, provides wide scope for laboratory studies of epilepsy.

Penfield and Jasper (1954) proposed a "centrecephalic hypothesis" to explain the bilateral, symmetrical and synchronous spike and wave discharges observed in the inter-ictal EEG pattern of patients with clinically non-focal <u>grand mal</u> seizures and the ictal pattern of patients with <u>petit mal</u> seizures. These authors assumed that the

origin of the epileptic discharge and the clinical seizure was in the centrecephalic system, which they conceived as consisting of the reticular formation and non-specific nuclei of the thalamus.

However, Ingvar (1955b) has shown in the isolated cortex of the cat that the spike and wave complex can be obtained from the cortex without requiring a diffuse projection system. Other authors Gastaut, 1969a; Marcus et al., 1968) have attempted to re-evaluate the basis of the centrecephalic theory. Rodin and his associates (1971) have recorded high-frequency EEG activity during Metrazol-or Megimide-induced grand mal seizures in cats and shown that clinical myoclonic jerking was associated with large amplitude bursts of high-frequency activity in the brain stem reticular formation. These authors were able to show that the functional state of the animal was an important consideration, since in paralyzed and unanaesthetized animals the intensity of the seizure discharges is reduced and the maximal activity tends to be in the thalamus and cortex, rather than in the midbrain and pons, as is the case with freely moving animals.

C. Laboratory Development of Anticonvulsant Drugs

Based on the work of Spiegel (1937a), Merritt and Putnam (1937; 1938; 1938a) conducted the first investigation specifically aimed at screening and evaluating anticonsulvant compounds in a laboratory model of epilepsy. They studied the effect of diverse compounds upon the threshold current necessary to produce convulsions in the cat by means of occipito-buccal electrical stimulation. The most effective compound that emerged from these studies was diphenylhydantoin. The research and development that produced diphenylhydantoin was the first practical and highly successful consideration of pharma-

cological activity based on chemical structure. Ironically, a closely related anticonvulsant hydantoin, phenylethylhydantoin, had been introduced as a sedative in 1916, but was soon withdrawn because it caused blood dyscrasias.

A clinical trial incorporating diphenylhydantoin administration to 200 patients with severe epileptic symptoms proved to be an immediate success for this new anti-epileptic (Merritt and Putnam, 1940). A great majority of patients suffering from *grand mal* were completely relieved, and there was some relief in patients with *petit mal* and psychomotor epilepsy. In no case was the epilepsy worsened. Diphenylhydantoin itself remains one of the most fully studied and most frequently used anticonvulsant drugs.

The years following the introduction of diphenylhydantoin brought considerable refinement into the methods used for detecting anticonvulsant activity. All forms of epilepsy did not respond equally well to the available drugs. Thus, in the hope of developing more effective agents, a search was undertaken for suitable animal models exhibiting the specific convulsive patterns of the different clinical forms of epilepsy.

Many different approaches have been utilized to discover the protective actions of drugs against different epileptic manifestations; from single cell studies, isolated nerve or nerve-muscle preparations, to studies in intact animals. The most fruitful tests, however, have been the electroshock studies in mice, as demonstrated by the work leading to the discovery and introduction of trimethadione (Everett and Richards, 1944), a useful anti-*petit mal* agent (Goodman *et al.*, 1946). Here was another demonstration that effective anti-epileptic

drugs could result from a planned laboratory search for candidate anticonvulsant drugs.

Since not all forms of epilepsy responded satisfactorily to the available anti-epileptic drugs, there was a stimulus to develop additional compounds by means of more specific assay techniques in experimental animals, which had now become the basic investigational tools in the search for more effective anticonvulsants.

Without a doubt, the most significant contributions to this rapidly expanding area of research were made by Goodman, Toman and Swinyard, 1946, 1949) and their associates in Salt Lake City (Goodman _et al._, 1953; Swinyard _et al._, 1946, 1952; Tedeschi _et al._, 1956; Toman _et al._, 1946, 1947, 1948, 1952). In spite of the great volume of work conducted it was apparent to Goodman and his associates (1949) that "there has existed an unfortunate assumption that, in order to be of clinical value, an anti-epileptic drug must raise the threshold for seizures however induced". The lack of regard for considering the effect of drugs on other parameters such as the severity of extensor seizures, the electroencephalographic pattern and extensor seizure latency probably led to the rejection of some potentially useful anti-epileptic drugs.

The prolific and pertinent work of this group of investigators was deliberately aimed at discovering agents specific to different forms of epilepsy. Other groups of researchers also provided notable contributions to the methodology for screeing and evaluating potential anticonvulsant compounds (Barany, 1947; Chen _et al._, 1954; Jenney and Pfeiffer, 1956; Tainter _et al._, 1943).

Different methods of inducing seizures have been explored extensively, essentially in rats and mice, in the hope that they would serve to demonstrate the effectiveness of different drugs in counteracting experimental convulsions and that more specific assays would result. One approach was the chemoshock technique (Everett and Richards, 1944), employing a standard dose of pentamethylenetetrazole (Metrazol). Another consisted of experimentally lowering the threshold to electroshock by increasing the state of hydration of the test animals (Swinyard et al., 1946). Electroshock procedures (AC or DC) have been applied in various ways, e.g. by corneal electrodes or by electrodes clipped to the ears of experimental rodents. The intensity of the stimulus in some cases has been measured by the amount of current flowing for a fixed period of time (Spiegel, 1937), and in other cases by the time required for a fixed amount of current to elicit a convulsion (Kozelka et al., 1942). The relationships between the response induced and the intensity of stimulation have been explored widely. In some assay methods, the effect of drugs was observed not on total suppression of seizure activity, but on modifying their pattern and/or progress. In other procedures the parameters of the electrical stimulus required to elicit convulsion were varied.

In their review on anticonvulsants, Toman and Goodman (1948) mention that limited use of electroconvulsive therapy in man was at one time made for the express purpose of examining possible anticonvulsant compounds.

Quantitative indices of the efficacy of the anticonvulsant drugs have usually been given by the dosage necessary to eliminate or modify the experimentally induced seizures. These values are dependent

on the criteria of toxicity and the method utilized for assessing it. Since these data are also relative to a particular experimental and convulsive procedure, it becomes difficult to compare results of different investigators in this respect. Needless to say, much judgement is needed in interpreting the results of anticonvulsant assays. Nevertheless, it is tempting to suggest, on the basis of the structural similarities found in so many anticonvulsants, that a common site of action is responsible for anti-epileptic drug action and for seizure activity.

D. Useful Experimental Methods

The ideal situation would be for a single universal test to suffice, but currently a battery of tests is utilized for the pharmacological identification of potential anticonvulsants. Some of the crucial tests developed in this respect can be summarized as follows:

i) The Maximal Electroshock Seizure Test (MES) in mice (Goodman *et al.*, 1953; Swinyard *et al.*, 1952) measures the latency of the hindlimb-tonic extensor seizure component resulting from maximal electroshock delivered via corneal electrodes. An AC current of 50 milliamperes in repetitive pulses of 1 msec duration, a value 5-10 times higher than the convulsive threshold, is applied for 1 sec, all untreated animals show a preliminary tonic limb flexion, and then tonic extensor seizure, followed by a short clonic phase and coma, or automatic movements. Drugs effective in *grand mal* prolong the latency and decrease or eliminate the duration of the tonic extensor seizure phase.

ii) The Minimal Electroshock Seizure Threshold Test (EST) (Swinyard, 1949) is employed to study the ability of anticonvulsant drugs to prevent all seizure activity following stimulation with a current 120 per cent of the minimal electroshock current necessary to elicit convulsions. Animals are shocked at intervals of at least one hour with

AC pulses, increasing the current strength until clonic-type convulsive phenomena appear. In rats the convulsive threshold is characterized by a facial clonus of several seconds, bizarre positions and pawing the air with their forelimbs, i.e. submaximal seizures. A modification of this test consists of pretreatment of the animals with intraperitoneal administration of hypertonic glucose in order to dilute the concentration of extracellular electrolytes, a procedure which sensitizes the nerve cells to subsequent stimulation (Swinyard _et al._, 1946), i.e. the Hyponatremic Electroshock Seizure Threshold Test (HET).

iii) _The Psychomotor Seizure Test (PsM)_ and the low-frequency Electroshock Seizure Threshold Test (l.f. EST, Toman, 1951) provide an index of the ability of a drug to prevent all seizure activity following a stimulus train consisting of 0.2-1.0 msec rectangular pulses at a frequency of 6/sec delivered for 3 seconds. This procedure results in seizures which resemble the clinical psychomotor type. The animals appear stunned, assume upright unorthodox positions with concomittant motor automatisms and catatonia. Effective compounds, such as phenacemide and primidone, abolish all seizure activity after stimulation with currents 100 per cent above the psychomotor threshold.

iv) _The Maximal Metrazol Seizures Test (MMS)_ (Goodman _et al._, 1953) studies the ability of drugs to prevent the hindlimb-tonic extensor component of maximal seizures following the systemic administration of pentamethylenetetrazole (Metrazol). The methods employing chemical convulsants to induce seizures result in a general stimulation of the intact animals to produce, either threshold (clonic) seizures, or maximal flexor-extensor seizures. In most cases insufficient evidence is available concerning the locus and mechanism of action of proconvulsant compounds.

v) _The Metrazol Seizure Threshold Test (s.c. MST)_ measures the ability of anticonvulsant drugs to provide complete protection against seizures elicited by subcutaneous injection of maximal doses of Metrazol in mice (85 mg/kg) or in rats (70 mg/kg), (Goodman _et al._, 1953).

With very few exceptions, all anti-epileptic agents elevate threshold and/or modify the pattern of electrically or chemically-induced seizures in the above laboratory methods. On the basis of protective indices anti-*grand mal* agents appear to be better able to modify electrically-(maximal) or chemically-induced seizures, and to increase low-frequency electroshock threshold. A similar spectrum of efficacy is observed with drugs used in the treatment of psychomotor epilepsy. Clinically effective anti-*petit mal* agents are more effective in elevating the threshold for Metrazol seizures and modifying their pattern (Toman, 1959). This latter class of drugs also elevate the threshold for seizures elicited electrically. Swinyard (1969) expresses concern that the present tests may serve only if the agents under investigation are chemically related to compounds possessing presently known anti-epileptic properties.

A novel approach for the production of experimental seizures is the use of a strain of mice susceptible to sensory audiogenic seizures. Audio-stimuli are administered to initiate a convulsive seizure, and the effect of anti-convulsant drugs is then determined (Fink and Swinyard, 1959). Photic sensitive epileptics have been discovered and accordingly, photically-elicited seizures in a special strain of baboons have received some attention recently (Killam *et al.*, 1967). Epileptogenic lesions have also been produced by immunological and chemical procedures (Kopeloff *et al.*, 1954). Another method consists in irradiating mice with microwaves, to elevate body temperatures (Millichap *et al.*, 1960). These authors have investigated the ability

of drugs to prevent or modify the febrile seizures induced by this procedure.

These methods are preliminary screening techniques and are sometimes useful in suggesting new compounds for clinical trials. Thus, they have not helped to elucidate the mechanisms of action of anticonvulsants, nor for that matter, convulsant drugs. Some interesting and unique observations toward this end have been obtained from experiments conducted on relatively simple neural mechanisms. These facts have emerged from studies of the effects of anticonvulsant drugs on the action potential in peripheral nerve, on monosynaptic and polysynaptic reflexes in the spinal cord, and on the electrical activity of isolated units in higher centres.

Toman (1952) has found that phenobarbital protects isolated peripheral nerve against hyperexcitability induced by repetitive electrical stimulation. When isolated sciatic nerve is stimulated repetitively the threshold stimulus current for elicitation of an action potential is reduced to half, but returns to normal values within a minute of cessation of the stimulus. During this period in which threshold is reduced, repetitive discharges of fibres are produced by single shock stimulation. The protective action of phenobarbital against the response to single shock stimulation during this period of experimental hyperexcitability is achieved at doses which have negligible effects on the normal properties of nerve. Toman (1952) suggests that this action of phenobarbital may conceivably be associated with the selective anticonvulsant effects of this drug. Various

other phenyl-substituted barbiturates also produce block of conduction in isolated nerves, but at concentrations that are at least 20 times higher than that required with phenobarbital.

In a similar set of experiments in which hyperexcitability was induced chemically by reduction of extracellular calcium, diphenylhydantoin had a similar effect. No other effects with regard to impulse conduction were noted. Toman (1952) has reported that trimethadione is virtually ineffective in preventing the repetitive firing in these hyperexcitable peripheral nerves and in the modification of conduction in sciatic nerve.

The similar actions of phenobarbital and diphenylhydantoin suggest that reduction of induced hyperexcitability rather than a general depression of neural conduction processes underlies their anticonvulsant actions. The action of other barbiturates is more in line with the concept of an increase in threshold necessary for excitation, since the margin of safety between protective actions against induced hyperexcitability and depression of normal parameters of nerve excitability appears to be much lower than with clinically useful anticonvulsants.

In general, the systemic administration of convulsant agents such as strychnine, picrotoxin, thiosemicarbizide and methionine sulfoximine has little utility in the screening of anticonvulsant drugs. These experimental substances have enjoyed wider application in the study of the neurophysiological, neurochemical and neurohistological mechanisms believed to be involved in seizure mechanisms.

Another approach, which has also found little usefullness, consists in the induction of seizure by stimulation of discrete areas

of the brain with topically applied convulsant drugs or irritant substances. Penicillin applied to the cerebral cortex or injected into various brain structures results in an acute hyper-irritable focus (Matsumoto and Ajmone-Marsam, 1964). This penicillin focus displays interictal epileptiform discharges, and this activity may spread to involve other areas of the brain and produce generalized convulsions.

It would appear more appropriate to consider experimental methods which produce chronic epileptogenic lesions in animals, in the hope that these will relate better to the chronic nature of epilepsy. Accordingly, it was found that the injection of alumina cream into the brain of monkeys results in a reliable chronic model which bears some relationship to epilepsy as seen in the clinic (Kopeloff *et al.*, 1954; Ward, 1961). Morrell and Baker (1961) have utilized this method to study the epileptogenic lesions which develop secondarily, in homotopic cortex contralateral to the primary focus, and have elaborated their concept of a mirror-focus. To them, this secondary lesion represents a spread of epileptogenecity from the primary zone to a naive area of the cortex, which can now be defined by the presence of paroxysmal discharges. These authors assumed that this secondary lesion results from the abnormal activity of synaptic mechanisms in complex neural pathways connecting with the primary injured zone. Other investigators have found that implantation of various metals results in the establishment of a chronic focal epileptic process at that site (Chusid and Kopeloff, 1962). Although these preparations are probably useful for drug screening, they have been mostly used for investigating the electrophysiological and histomorphopathological correlates of epileptiform activity (Morrell, 1969; Ward, 1969).

E. The Isolated Cerebral Cortex

Kristiansen and Courtois (1949) completely sectioned the neuronal connections of a small area of cerebral cortex in cats. In acute experiments they recorded bursts of very low voltage rhythmic electrical activity, which appeared to resemble the normal alpha rhythm. Independently, Burns reported that he was unable to record any spontaneous electrical potentials from acutely-isolated cortex in cats anaesthetized with chloralose (Burns, 1949; 1950), or in decerebrate preparations (Burns, 1951). This author did observe occasional paroxysmal bursting responses in these isolated slabs, but he believed this was due to trauma. Furthermore, Burns (1950) attributed bursting responses to small neuronal bridges acting as a connection at various points, between isolated and non-isolated cortex. Burns (1950; 1958) reported that he could elicit various burst responses by direct electrical stimulation of isolated cortex.

Ingvar (1955) using a similar preparation was able to demonstrate that spontaneous patterns of activity in isolated cortex could be influenced by direct stimulation of the reticular system in the brain stem. Partial isolation of human cortex acutely and chronically by means of undercutting or lobotomy has been reported (Henry and Scoville, 1952). No histological proof of isolation was provided and it is, accordingly, difficult to decide on the origin of the periodic bursting that these workers observed in the EEG. Subsequently, evidence was provided that hypersynchronous spontaneous electrical activity is an inherent property of chronically neuronally isolated cerebral cortex in human and animal cortex (Echlin *et al.*, 1952;

Grafstein and Sastry, 1957).

The isolated slab of cat's cerebral cortex has been the subject of many electrophysiological studies in the past, in an effort to determine the origin of spontaneous and elicited electrical activity in the brain (Burns, 1958). One property of this unique preparation is that following prolonged deafferentation the cortical neurons become hyperexcitable and respond to electrical stimulation with considerably prolonged epileptiform afterdischarges (EADs) (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962). Ward (1969) has suggested on the basis of studies performed on chronic alumina foci in monkey cortex that one mechanism involved in epileptogenesis is partial deafferentation of neurons. It would seem reasonable to consider the isolated cortex preparation as a relatively useful and discrete preparation for the purpose of studying physiological and pharmacological aspects of the epileptiform hyperactivity in cerebral cortical neurons.

The afterdischarge response has been defined by Kreindler (1965) as a local process representing the self-sustained repetitive discharge of a group of neurons. Isolation of a portion of cortex favours this response (Burns, 1954), and in chronic preparations the duration of the afterdischarge is much increased (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962). The epileptiform afterdischarge (EAD) results from the direct local electrical stimulation of an area of the cerebral cortex for a few seconds (Jasper, 1955). Jasper (1955) has described several successive stages in an EAD: asynchronous activity, rhythmic synchronous discharge, interrupted clonic discharge, exhaustion, and return to a normal rhythm. Penfield and Jasper (1954)

have reported that intense repetitive stimulation gives rise to long duration EAD's that form the basis of a focal epileptic seizure which may become generalized.

Afterdischarge responses are not an exclusive electrophysiological property of the cerebral cortex and can be observed, in response to the proper stimulus, in other cerebral regions (Kreindler, 1965).

Thus, on many counts it would seem that the chronically neuronally-isolated cerebral cortex provides a reasonable experimental model for the study of epileptiform activity in the cerebral cortex. The isolated cortical slab may only provide experimental information on some aspects of the pathophysiology of epilepsy, as it apparently relates solely to the role of the cortical elements. Since there is little or no information concerning the postulated involvement of neuropharmacological mechanisms in the initiation, termination and spread of EADs, this preparation would seem to provide a suitable approach for investigating aspects of the problem in the cerebral cortex.

It still remains to be shown clearly whether metabolic disturbances are the primary causal factors of seizures in cerebral tissue. It is reasonably clear that partially-damaged areas of the cerebral cortex can be activated by various internal or external stimuli to produce paroxysmal discharges that spread, generating a generalized seizure. These factors are beyond the scope of this investigation and only pertinent studies will be mentioned as they relate to various considerations. It is perhaps also important to consider what elements of normal cortex appear to be associated with the pathophysiology of epilepsy.

F. Relative Clinical Value of Anticonvulsant Testing

Although a battery of well-standardized tests is useful for the pharmacological identification of anticonvulsants, it is still not possible to make a clear prognosis as to the clinical value of drugs for specific types of epilepsy, in spite of the great amount of work directed towards this end. Thus, more original and rewarding experimental models are required if better analogies with seizures in the clinical situation are to be achieved. Epilepsy is a chronic condition, whereas the screening methods invariably involve acute seizures. Moreover, epileptic seizures usually originate as a local discharge and it should be apparent that the popular testing methods are none too elaborate in terms of localization of the stimulus.

Although no one experimental testing method is sufficient to delineate clearly the anticonvulsant potential of a candidate drug, it is apparent that the MES test is of great value in detecting these properties in new compounds. Unfortunately, many substances with no known anti-epileptic activity also inhibit tonic extensions of the hind limbs. In most screening methods involving anti-extensor agents against peripheral motor effects, little or no evidence regarding drug effects on the electroencephalographic abnormality was obtained, which may help to explain their failure to show activity in the clinic. Therefore, a better line of approach to the whole problem might be to consider drugs in terms of their ability to inhibit cortical discharges in addition to inhibition of motor convulsions.

Some points regarding the question of the site of integration of tonic extensor seizures in the central nervous system (CNS)

can be considered. Esplin and Freston (1960) were able to induce a tonic extensor seizure at the spinal cord level in animals with the brain stem transected. Others (Bergmann *et al.*, 1963) localized centres in the bulbopontomesencephalic reticular formation, which on stimulation gave rise to a tonic extensor seizure without the accompaniment of convulsive potentials in rostral central structures. Thus, measuring the motor seizures alone, we might be studying only the effect of compounds on the pontomesencephalic reticular formation involved with muscle tone.

It must be emphasized that the nature of epilepsy is dynamic and not static, in terms of progression of the disease process and interference with on-going physiological processes. Thus, in retrospect, the classical electroshock and chemoshock models for testing the action of potential anticonvulsant drugs appear to represent significant compromises to human epilepsy as a result of their simplicity, authenticity, sophistication, and speed of assay.

It appears sometimes that little fundamental progress has been made since Toman (1948) and Goodman (1949) and their colleagues reviewed the field. Murphy and Schwab (1956) have reported on the ability of diphenylhydantoin administered parenterally to control status epilepticus. Some indication of the desperate procedures utilized in recent times (up to 1956) to combat seizures in children can be obtained from a number of alternative procedures that these authors (Murphy and Schwab, 1956) discussed, e.g. lumbar puncture, dilatation of the anus, inhalation of ether, inhalation of 5% CO_2 in oxygen, gastric lavage, and intravenous administration of 50% dextrose. The

diffuse spread of these therapeutic measures provides a good index of the complexity and present uncertainty inherent in seizure control. Perhaps experimental models that better approximate the clinical forms of epilepsy will provide more meaningful data and specific drugs. In any case, the final proof of the effectiveness of anti-epileptic drugs can be obtained only in human patients.

Some relatively new compounds belonging to the benzodiazepine group of tranquillizers have demonstrated usefulness in controlling seizures in experimental animals and man (Toman, 1970). Nitrazepam appears to be indicated in hypsarhythmia and related infantile spasma, and myoclonic seizures (Peterson, 1967). Another derivative, diazepam, has been demonstrated to be a useful intravenous treatment in the control of status epilepticus, since it reduces seizures in 50% of cases with apparently no relation to etiology or seizure type (Nicol *et al.*, 1969). It appears likely that other congeners of this series will exert even more specific anticonvulsant effects (Toman, 1970).

Many of the reports on the effects of drugs on the electrical activity of the brain are contradictory. They are, on the whole, based on experiments carried out in anaesthetized preparations, or in acute postanaesthetized preparations immobilized by spinal section, or by curare. Very little information on the possible central effects of cerebral vasoactive substances (polypeptides), undoubtedly released by acute surgical-trauma and stress, is available. Behaviour could not be readily studied in these experiments. Since, however, many drugs, especially autonomic drugs, do affect behaviour,

it would seem that this end-point may have an important relation to the observed electrical activity, or at best act as an index of central activity. Thus, it would seem important to develop techniques for the study of the central effects of drugs which provide for the recording of electrical activity simultaneously with behaviour in the conscious, unrestrained and unanaesthetized experimental animal. Rodin and his associates (1971) have commented on the differences in seizure activity observed between paralyzed and freely-moving animals.

An additional advantage of such a technique is that it enables the operator to become familiar with the normal behaviour and EEG patterns of each animal. Variability is better controlled, and the slight effects produced by some drugs can thus be more readily detected, and the individual differences between individual animals may be given their due weight.

III. NEURONAL ELEMENTS AND ARCHITECTURE

Since epilepsy represents one of the oldest experiments of nature, it has not escaped the attention of early cellular pathologists and neuropathologists (Scholz, 1959). It has become apparent that from the histological point of view, focal epilepsies represent a whole host of complex changes resulting from diverse pathological processes. In general, it appears that seizure discharges result from slowly progressive destruction of neurons due to a variety of insults to the cerebrum. Attempts to identify the pathological processes and structural correlation that confer epileptogenicity to otherwise normal cerebral tissue have not yet succeeded.

A. Normal Histology and Cortical Connections

In the cerebral cortex six distinctly separate parallel layers of cells can be identified (Sholl, 1956). According to the predominant cell type these layers can be briefly described as:

i) Layer I or the molecular layer is found immediately under the pial surface and is relatively free of neurons but is richly supplied with interconnecting axons and dendrites. The horizontal cells of Cajal which resemble small fusiform cells are found in this layer. Their dendrites and long axons run horizontally parallel to the surface and arborize in this region.

ii) In layers II and III the predominant cells have conically shaped cell bodies. These are the pyramidal cells which are found in great numbers throughout the cortex. The cell bodies in layer III are considerably larger than similar cell bodies in layer II.

iii) Cells in layer IV have a polygonal cell-shape and are referred to as stellate cells.

iv) Layer V contains many giant pyramidal or Betz cells and some smaller ones.

v) Layer VI is situated just above the white matter and contains spindle-shaped cells called <u>fusiform cells</u>.

a. <u>Common cortical cell types</u>

There is a great deal of overlap between the various layers, and many sub-types of neurons can be found. Sholl (1956) has greatly simplified cytological descriptions of the cortex by adhering to a few basic cell types. The most prominent neurons of the cerebral cortex are the pyramidal and stellate cells. The relatively larger pyramidal cells have long apical shafts or dendrites which ascend from their conical cell body, or perikaryon, and extend toward the pia and branch profusely in layer I. These terminal dendritic arborizations extend parallel to the pial surface for short distances. The apical dendritic shaft may be devoid of branches for the first 10-40 microns after exiting from the soma, after which it gives off many side branches throughout the remainder of its length (Szentagothai, 1969). All along the membrane of the apical dendrites are numerous, minute, bud-like extrusions or dendritic "spines" ranging in size from 1-2 microns. These spines are the sites of many axo-dendritic synapses (Colonnier, 1966). The base of these cell bodies shows many basilar dendrites spreading outwards and sending numerous branches into the same and other cortical layers.

Interspersed with the upright pyramidal cells in the deeper layers are the sparsely distributed Martinotti cells. Their cell bodies also have a pyramidal shape, but the conical part is directed away from the pial surface. An axon emerges from the apical portion of the perikaryon and ramifies in layer I.

The stellate cells are distributed throughout the different layers, but are concentrated in layer IV (Sholl, 1956). Their small spherical cell bodies usually show six or more thin dendrites that emerge directly from the perikaryon, divide extensively and pass in all directions (Ramon-Moliner, 1962). A relatively short axon terminates near the cell body. The majority of stellate cells appear to have only intracortical axons distributed within their own dendritic fields and are in synaptic contact only with adjacent neurons (Sholl, 1956). These axons may enter parts of the underlying white matter. Other stellate cells appear to send axons up to the superficial layer where they make synaptic junctions with apical dendrites. It has been suggested that the role of stellate cells in the cortex is as a relay station or interneuron (Szentagothai, 1969).

Fusiform cells have a spindle-shaped cell body with two dendrites which emerge from opposite poles of the perikaryon. One dendrite usually ascends to the subpial zone, while the other dendrite arborizes extensively near the cell body. One axon emerges from the centre of the perikaryon to form, with other axons, a fibre tract in the underlying white matter.

Other less common and less studied cells have also been described (Sholl, 1956; Szentagothai, 1969). There are e.g. star-pyramidal neurons found in layer II. As a rule, categorization into sub-types proceeds according to the observed dendritic arborization pattern or surface structure and the degree of transgression of dendrites into the various cortical layers. To a lesser extent, ramification of axons permits a further classification of cortical neurons.

b. Connecting elements

It should be increasingly apparent that this extensive pattern of branching and distribution of axons and dendrites provides for complex systems of intra- and intercortical connections (Sholl, 1956). In addition, large numbers of afferent and efferent fibres enter and leave any given part of the cerebral cortex. Some efferent fibres pass to other cortical regions via the white matter, under layer VI, while others pass to various subcortical and lower centres of the brain. Axons of some pyramidal cells project only to the white matter without giving off any collateral branches. Other pyramidal cells are seen to possess horizontal or recurrent, or both types of collateral fibres (Sholl, 1956).

Afferent fibres to the cerebral cortex can be subdivided as follows (Szentagothai, 1969): (1) Specific afferents-arising from subcortical regions into primary sensory regions; (2) nonspecific afferents-arising from nonspecific subcortical structures; (3) callosal afferents; (4) association afferents from a cortical source; and (5) association afferents from subcortical sources. There is still insufficient evidence regarding the precise manner in which the specific fibres terminate in the cortex (Nauta, 1954). They might have synaptic contact with any parts of neurons or dendrites in the middle layers. Szentagothai (1969) has also described the cortical neuropil as consisting of numerous and arborizing axons arranged in parallel with the general course of dendrites. In layer I of the cortex this delicate network is tangentially oriented; in layer II it has an obliquely oriented meshwork; while in layer III it is predominantly perpendicular.

In layer IV it reassumes a tangential orientation, but it is difficult to discern the orientation of this apparently synaptically constituted network in deeper layers of the cortex.

Colonnier (1966), based on the earlier findings of Cajal, has discussed in detail the termination of specific afferent fibres in a primary sensory area of neocortex. Fibres ascend obliquely through deeper cortical layers spreading tangentially for 650 microns in layer IV where they apparently terminate. Some fine collaterals leave the tangential branches in layer IV and enter layer III where they establish contact with smaller pyramidal cells. Nauta (1954) and Szentagothai (1965) on the basis of degeneration experiments have confirmed this cortical pattern of specific afferent fibres. Association fibres from other cortical areas have been shown to ascend into all cortical layers except layer I (Nauta, 1954) and spread tangentially up to 150 microns (Sholl, 1956).

Fink and Heimer (1967) using improved staining techniques have shown that a major portion of the layer I fibres derive from callosal and association afferents. Thus, the validity of earlier work suggesting that most of the tangential fibres in this layer are of intracortical origin (Colonnier, 1966; Szentagothai, 1965) is open to question. More recently, Colonnier and Rossignol (1969) found that the degree of fibre degeneration throughout all cerebral cortical layers is greatest after undercutting a gyrus between layer V and layer IV, or after a geniculate nucleus lesion. Based on these results these authors suggest that terminals in all layers of the cortex are of intracortical origin. Szentagothai (1965a) supports his con-

tention that the dendritic shafts of pyramidal cells are contacted by axons of local neurons based on his observations that the axosmatic synaptic knobs of pyramidal neurons persist in small chronically isolated cortical slabs.

c. Physiological importance of neuronal elements

Colonnier (1966) has suggested that the majority of connections between cortical neurons occur in a vertical plane and that fibre spread is more restricted tangentially. Such a columnar organization of apparently basic cortical units has been proposed as an important aspect of neurophysiological function in the cerebral cortex (Mountcastle, 1957).

Stellate cells in layers II to IV have two "bouquets" of beaded dendrites sprouting from the upper and lower ends of the soma (Cajal in 1911, see Colonnier, 1966). These dendritic "bouquets" may extend throughout all the cortical layers. Often, the axonal arborizations of several such cells close together appear to form a long vertical cartridge or bundle, an arrangement which several authors (Colonnier, 1966; Szentagothai, 1969) suggest as favouring the establishment of multiple synaptic contacts with the spines on the apical dendrites, i.e. axon-spine synapses, on large and medium-sized pyramidal cells coursing through this cartridge structure and forming the axis of the bundle. Similar, though less well organized, cartridges may exist around pyramidal basilar dendrites. Other stellate cells form similar arrangements with their terminal arborizations, to envelope the cell bodies of adjacent pyramidal cells and their main dendritic segments (Colonnier, 1966).

There are many other varieties of neurons with ascending,

descending or horizontal branches (Colonnier, 1966). Both Colonnier (1966) and Szentagothai (1969) consider that the synaptic cartridge arrangement as provided by stellate interneurons, which are themselves in synaptic contact with specific afferents, provides for multiple and powerful excitatory synaptic connections on pyramidal cell apical dendrites. Whether this excitation is reflected in excitatory electrical phenomena recorded at the surface of the cortex is another problem.

Colonnier (1966) and Szentagothai (1969) also propose that inhibitory synapses would most likely be provided by stellate interneurons whose axon terminals arborize a short distance from their perikaryon to form a basket-like arrangement about the soma of adjacent pyramidal cells. In layer II this tangential spread is very short. In layer III and layer IV the spread is 500 microns, and may even be larger in the remaining layers (V and VI). Colonnier (1966) and Szentagothai (1969) argue that since a similar basket-like arrangement of pericellular terminals has been shown to be an essential part of powerful neuronal inhibitory mechanisms in the cerebellar and hippocampal cortex, it is reasonable to propose similar inhibitory mechanisms on pyramidal cells in the cerebral cortex.

B. Cerebral Synaptic Morphology

Within the cerebral cortex only two main types of synapses can be distinguished on the basis of synaptic vesicle morphology and synaptic membrane thickness (Colonnier, 1968; Gray, 1969). These findings have led these authors to propose the presence in the cortex of two different transmitter substances with opposite actions.

The first synaptic type (type I) has asymmetrical membrane differentiations with a spheroidal vesicle population in the presynaptic terminal, and is associated with a dense compact opacity in the cytoplasm immediately underlying the postsynaptic membrane. This synapse has been found mainly on pyramidal cell dendritic spines but never on pyramidal cell bodies. It was suggested that this axodendritic synapse possesses an excitatory function and that it is present in the terminals of specific afferents to the cortex.

The second type (type II) contains flattened types of vesicles in the presynaptic terminal enclosed in a symmetrical membrane, and is not associated with a specialized postsynaptic opacity. These axosomatic synapses are present on pyramidal and stellate cell bodies and the main shaft of their dendritic branches. They are rarely found at dendritic spines, in which case they are coupled to a synapse of the first type. It was suggested that this type of synapse is inhibitory (Uchizono, 1965). It must be pointed out that most of the work in this area has been carried out exclusively in layer IV of the cortex. Szentagothai (1965a) has observed that the second type of axosomatic contacts are least affected by undercutting the cortex.

C Structural Changes In The Cerebral Cortex Following Deafferentation

a. Connecting elements

Colonnier (1966) and Szentagothai (1965) have described in some detail the changes in degenerating terminals when a slab of cerebral cortex is chronically isolated by undercutting. Szentagothai (1965a) emphasizes that he did not observe any evidence of abnormal collateral sprouting of neuronal elements. If the cut is above layer V, following degeneration, only type II synapses remain intact. As mentioned

previously, these observations suggest that the specific afferents to the cortex reach mainly the pyramidal cells in layer V, and in layer III by means of stellate interneurons, which in turn envelope the pyramidal apical dendrite with their synaptic cartridge. This arrangement is believed to represent a major mechanism for excitation in the cerebral cortex, which would persist when the six layers of the cortex are completely isolated from their neuronal connections. Pyramidal cell axon collaterals upon adjacent pyramidal cell basal dendrites are also believed to form excitatory synapses. (Colonnier, 1968; Szentagothai, 1965a).

Some axons climbing parallel to the apical dendrites have also been observed in chronically isolated cortex (Colonnier, 1966). The elements suggested to be involved in this system are the fusiform stellate cells in layer III and layer IV. These interneurons would be capable of activating pyramidal neurons above and below this central portion of the cerebral cortex. There would be some radial spread of activity by short tangential axons to other adjacent fusiform interneurons, also with vertically-oriented axons.

The inhibitory-type II (axosomatic) synapses would persist mostly intact in chronically isolated cortex due to their inherent intracortical origin (Colonnier, 1966; Szentagothai, 1965). As mentioned earlier their arrangement about pyramidal cell bodies and major dendritic shafts provides for a powerful but short range collateral inhibitory mechanism within a given cortical layer. Presumably, the numerous inhibitory synapses present in nest-like synaptic structures are capable of preventing cell firing. It is not clear whether other inhibitory synapses are present along dendritic trees to effect a more selective

inhibition. It is also possible that the excitatory endings of pyramidal cell axon collaterals upon stellate cells may set up a pathway for recurrent inhibition. Chang (1952) has commented on the necessity for multiple impulses converging on a neuron, since the effects at one synapse would be relatively inefficient.

b. Neuronal elements

Many neurons in chronically isolated cortical slabs show chromatolytic changes (Weisman, 1969; Krnjevic, Reiffenstein and Silver, 1970; 1970a). Pyramidal cells undergo retrograde chromatolytic changes (Duncan *et al.*, 1968). Neuroglia cells are particularly abundant throughout the slab. The slabs shrink and there appears to be a considerable loss of large cells from layer V, although there is an overall increase in the density of neurons. In apparent agreement with this histological picture is the finding by Krnjevic and Schwartz (1967) that large numbers of unresponsive cells are encountered in microelectrode studies. It is somewhat difficult to rationalize the criteria for responsiveness in this context. The fact that there is no response at particular positions of a microelectrode tip seems to be a rather indirect approach. The active cells could still be present, but it might be that as a result of enhanced inhibitory synaptic efficiency (of intracortical origin), these cells require different parameters of stimulation before firing. Other possibilities should not be overlooked. Duncan, Rutledge and Domino (1968) have observed extensive fibre degeneration in isolated regions of cortex. This can be expected to result in extensive rearrangement of synaptic endings at cell body and dendritic membranes.

Utilizing the Golgi impregnation method, Rutledge *et al.*, (1969) have conducted a quantitative evaluation of cortical pyramidal

cell axon collaterals in intact, chronically-isolated undercut, and electrically-stimulated chronically-isolated undercut cortex in adult cats. Undercut tissue had cells with significantly fewer axon collaterals and fewer branches than did intact. On the basis of these two parameters there were no differences between intact, and long-term isolated electrically-stimulated undercut cortex. This treatment of chronically, partially deafferented cortex preserves, somehow, axonal components and dendritic spines (Rutledge, 1969), and prevents the degenerative changes seen in chronically isolated cortex (Cajal, 1959).

Benhamida et al. (1970) have studied the distribution of spines on the dendrites of cells in layers II - VI of intact and chronically-isolated suprasylvian gyrus in the cat. The overall distributions of spines on dendrites are similar, but isolation does diminish the number of spines on basilar, apical and collateral dendrites. Greatest losses occurred in layer IV, whereas most minimal changes were in layer V. The authors comment on the fact that large numbers of spines remain and there is evidence of synaptic connections.

Rutledge (1969) has studied extensively the apical dendrites of layer II pyramidal cells of undercut, undercut and stimulated, and intact cortex prepared by the Golgi-Cox method. This author cautiously draws the following conclusions about differences in basic dendritic processes. In undercut cortex, long term stimulation prevented decrease of apical dendritic spines and some of the degenerative changes found in unstimulated undercut cortex (Rutledge et al., 1967). The preservation appears most evident at axodendritic synapses although it must be recognized that the limitations of the Golgi method do not rule out the possibility of unseen, but significant changes elsewhere.

Attempts to explain the lack of marked histological changes in the stimulated undercut cortex are difficult. It is possible that the regular daily stimulation spreading across the cerebral synapses would somehow preserve presynaptic and postsynaptic structures, as well as the necessary supporting cellular elements. Whether the continuous daily dendritic activity of the pyramidal neurons prevents the development of an epileptogenic cortical lesion is not clear. Rutledge (1969) proposes that when synaptic units are used and maintained actively, they are preserved as morphological and functional units.

In unstimulated undercut cortex Rutledge (1969) reports that there is marked beading or varicose-like formations visible on the dendritic shafts. Weisman (1969) reports similar findings as well as similarity in dendrite density between the upper and lower portions of chronically-isolated cortex. In intact or acutely-isolated cortex the greatest dendritic densities are found in the upper cortical zones.

Weisman (1969) suggested that the varicosities observed on terminal dendrites are in fact artefacts of processing. This author was unaware of the findings of Rutledge and his associates (1967) who hold the opinion that this is a genuine feature of chronically-isolated cortex. Rutledge (1969) reports that although intermittent stimulation preserves dendritic spines in undercut cortex, there is still a very highly significant reduction in their number when compared with intact cortex.

Based on the greater degree of staining for acetylcholinesterase in undercut stimulated cortex, Rutledge (1969) considered that axodendritic synapses are preserved and in some manner some inhibitory

influence upon pyramidal cells is retained. This author argues that the inhibitory role of stellate cells is most affected by undercutting owing to the loss of afferent terminations in layer IV, which presumably are responsible for driving these interneurons. It is argued further that in some other manner the excitability of pyramidal cells will become persistent. This argument is difficult to reconcile with the significant loss of large cells reported in lower zones of the cortex (Reiffenstein, 1964; Weisman, 1969). This would tend to remove an integral part of the elements concerned with excitatory activity. Nevertheless, the most consistent finding in undercut and stimulated cortex is that there is significant preservation of axodendritic synapses of the excitatory type (Rutledge, 1969).

D. Deafferentation as a Factor in Epileptogenesis

The relevance of the previous discussion to epileptic phenomena is not entirely clear. Westrum, White and Ward (1965) have reported that there is a disappearance of spines from pyramidal cells near chronic alumina foci. There is much evidence in the literature to indicate that dendritic spines disappear after section and degeneration of their afferents (Globus and Scheibel, 1967; Jones and Thomas, 1962; Mathieu and Colonnier, 1968). Scheibel and Scheibel (1968) have also reported that the number of spines on pyramidal cells of human epileptic cortex is diminished. Thus, there seems to be some basis for assuming that dendritic spine loss is involved in the development of epileptogenicity in cortical tissue. However, this is not necessarily causal, and could just be a reflection of partial deafferentation.

Histological studies carried out in an experimental alumina epileptogenic focus demonstrate an overall decrease in neuronal elements

and an apparent increase in glia (Westrum _et al._, 1965). In contrast to normal cortex, the superficial layers do not have the intricate pattern of terminal arborizations and the neurons remaining appear to be of intermediate or small size. There is less dendritic branching and the course of the apical shaft and its branches is frequently distorted in different planes. Similar findings in the chronically isolated cortex of the cat have been reported by Rutledge _et al._ 1967; and Weisman (1969). Dendrites are marked by irregular varicosities on the large shafts and finer branches. The dendritic membranes are almost completely divested of their normal dendritic spines. The apical shafts appear to be the most affected. The above findings are most prominent within the area of the electrically-active primary focus where many neurons are completely devoid of spines. Sypert and Ward (1969) have reported a progressive transition of anatomical changes that correlates well with a similar transition of altered neuronal firing patterns to normal unit activity as observations are made at increasing distances from a chronic epileptogenic focus.

Although many assumptions have been put forward regarding the significance of the alterations in the histological conformation of cortical lesions, the exact pathophysiology of the epilepsies still awaits elucidation. The task of correlating neurochemical neuropathological, neurophysiological and neuropharmacological factors, at present, appears to be formidable. The finding of Holubar _et al._ (1967) with microelectrodes, that small round or stellate, normally inhibitory neurons appeared to lose their spontaneous intracellular activity following topical application of penicillin, while the sustained spontaneous intracellular activity of pyramidal cells increased, suggests

that the conclusions of Colonnier (1966) and Szentagothai (1969) about two types of cortical synapses may be valid.

IV. ELECTROPHYSIOLOGICAL BASIS FOR NORMAL AND ABNORMAL ELECTRICAL ACTIVITY IN THE BRAIN

The brain of a normal adult, physically relaxed, with eyes closed, produces rhythmical potentials oscillations at a dominant frequency of 8-12 Hz and with an average amplitude of some 50 μV (Wyke, 1969). This type of cerebral electrical activity recorded at the scalp surface is normally maximal over the occipital lobes and is referred to as the alpha rhythm. In the same adult under stable conditions the characteristics of this rhythm are constant at different times. Other oscillations of potentials are maximal over the frontal lobes (13-32 Hz) and comprise the beta rhythm (Wyke, 1969). A third type of activity, called theta rhythm, displays slower frequencies (4-7 Hz), usually maximal over the anterior portion of the temporal lobes and often mixed with the alpha rhythm. The theta form is prominent in children up to six years of age, after which it gradually blends with alpha waves or disappears, and is usually never found beyond the age of twenty-five. A delta rhythm slower than 5 Hz (usually < 3 Hz) is recorded on infant babies (Wyke, 1969).

Soon after its discovery, the electrical activity recorded from the surface of the cerebral cortex, electrocorticogram (ECoG), or from the surface of the skull, electroencephalogram (EEG), was suggested to be the result of the summation of the potential transients of excitable neuronal elements lying just below the cortical surface (Adrian, 1936). The classical interpretation of the generation of the EEG has emphasized the rhythmic and spontaneous slow membrane potential oscillations that are an intrinsic property of central neurons (Renshaw et al., 1940). An alternative explanation has been to consider the synaptic potentials of superficially located dendrites as the major substrate

of the EEG (Lorente de No, 1938; Purpura and Grundfest, 1956; Jasper and Stefanis, 1965; Creutzfeldt et al., 1966; 1966a). Bishop (1958) has attributed it to "dendritic activity". Fibre activity is believed to contribute to surface potentials only if it is highly synchronized (Purpura, 1959), but this is probably a negligible factor.

More likely, the EEG, represents the composite and synchronized activity of many neuronal elements, i.e. somato-dendritic decremental potantials, all-or-none spikes from axons or axon hillocks, and the electrical activity of smaller neurons (Eccles, 1951; Bremer, 1958; Purpura, 1959). The actual contributions of the various cortical cellular elements to the different potentials recorded from the brain remains to be determined definitively. It is still not clear whether certain EEG phenomena represent the summation of excitatory or inhibitory phenomena or both. The manner in which these potentials are distributed along the soma-dendritic membrane is, likewise, not readily apparent. The contribution and role of the vertically arranged neuronal elements within the different horizontal layers of the cortex as sources and sinks for electrical current is generally discussed in very speculative terms.

In patients with epilepsy, the EEG usually varies considerably from the normal pattern. Massive and exaggerated dysrhythmic, but hypersynchronous discharges are recorded. This observation not only suggests that individual neurons are firing excessively, but also that many neurons discharge in unison to generate the augmented paroxysmal spikes which obliterate, temporarily, the organized patterns of the normal EEG. Undoubtedly, this altered activity results from many unstable cells and otherwise normal neurons temporarily paced by the

epileptic neurons.

The single most distinctive feature that can be recorded routinely by means of gross electrodes at an epileptogenic focus, is the epileptic spike. There is a variety of bizarre patterns of abnormal neuronal activity observed in the EEG or ECoG recorded over epileptogenic tissue. In contrast, there is a low-frequency and well-ordered neuronal firing pattern in normal cortical tissue. There may or may not be a relation between these augmented epileptic responses and unit firing (Schmidt _et al._, 1959; Ward, 1966). The synchronized firing of many neurons invariably results in a convulsion. Firing in unison begins at the focus and propagates to secondary sites which discharge in time with the primary focus. As other areas join in the synchronous discharge, the pacemaker can shift from the original focus to these secondary points, where it continues to regulate spike activity until termination of the seizure. This shifting inter-relationship of variously located foci appears to be of particular relevance to the more general control of cerebral electrical activity by the reticular system (Penfield, 1969).

Jasper (1950) has observed that high voltage spike activity and paroxysmal discharges may or may not arise from localized superficial regions on the cortex. Whether the generating source of these slowly changing electrical potentials can be related to dipole structures is an unsettled question, but no doubt the depolarization and hyperpolarization of neuronal membranes is somehow involved.

A. Excitability of Intact Cerebral Cortex

a. Investigation with gross electrodes

Stimulation of nerve tracts leading to the cortex, usually evokes, at the cortical surface, a positive wave lasting 10-20 msec or a positive-negative sequence of briefer duration (Chang, 1959; Ochs, 1965). These "primary sensory responses" exhibit variability in their latency and form as a result of interactions with various sensory inputs and the non-specific activity of the reticular formation.

Direct cortical responses (DCRs) are responses which are elicited by stimulation of the cerebral cortex directly. A single weak stimulus delivered for 10 msec to a region of the cortex will cause that "stimulated" point to become negative with respect to a point several mm distant. The voltage of the response is greatest, and its latency minimal, in the immediate vicinity of the stimulating electrodes. It spreads in a decremental fashion, and this spread is accompanied by a progressive increase in the latency of the response. Adrian (1947) suggested that this negative response or surface negative wave is generated by neurons which lie in the superficial layers of the cerebral cortex, and which are directly excited by the stimulus. Chang (1951) has proposed that this response is a long action potential of apical dendrites, i.e. a "dendritic potential". Depending on the parameters of the stimulus, a variety of different types of DCRs may be elicited by direct stimulation of the cerebral cortex. Eccles (1951), and Purpura and Grundfest (1956) have suggested that this DCR is an excitatory postsynaptic potential (EPSP) of the apical dendrites, excited by tangential axons in layer I. Burns and Grafstein (1952)

considered that tangential axons in layer I generate these surface responses. Ochs (1965) in his monograph concludes that DCRs result from postsynaptic potentials in apical dendrites.

Creutzfeldt and his associates (1969) have reported that surface negative potentials coincide with depolarization and activation of the majority of the cortical pyramidal cells. In other cortical cells this group of workers observed a reverse relationship, i.e. surface negative potentials coincided with inhibitory effects. Their results indicate that stimulation leads to different temporal excitation-inhibition patterns of the cortical cell population. This pattern could be attributed to the involvement of different synapses and of different cells aggregates in the same cortical volume resulting in different surface evoked potentials and in different relations of surface polarities to cellular events (Creutzfeldt _et al._, 1969).

Increasing the stimulus intensity changes the form of the response. The stimulated zone then becomes positive with respect to distant points, and the responses become larger as the stimulation proceeds. Adrian (1936) called this the _deep response_, since it has a longer latency and propagates in an all-or-nothing manner at much slower velocities than the surface negative response. Moreover, this surface positive wave is also observed when the cerebral cortex is stimulated below the surface (Adrian, 1936).

Semi-microelectrode studies have revealed that the lowest threshold for eliciting the surface positive response is found 1.4-2 mm below the cortical surface (Burns _et al._, 1957). The neurons in this region have been termed "B" neurons by these authors and it is

postulated that they are also the site of synaptic connections for the lateral spread of cortical excitation. Suzuki and Ochs (1964) reported that the negative wave at the surface becomes, as the recording electrode penetrates into the mid-cortical layers, a positive-negative sequence of waves. This sequence is not present in chronically deafferented cortex as Suzuki and Ochs (1964) observed only negative DCRs when recording 1-1.2 mm below the surface in isolated cortex. No responses were recorded in deeper regions. These authors noted further that slow negative fluctuations of potential in the middle layers of the cortex were in phase with surface positive waves. They interpreted this finding in terms of neuronal activity in deeper layers giving rise to surface positive slow waves by flow of electrical currents down to the initial segment of the dendrite.

Eidelberg _et al_. (1959) have recorded DCRs of greater amplitude than at normal cortical sites at cortical epileptogenic foci. On this basis they suggested that abnormal dendritic depolarization, and the occurrence of epileptiform afterdischarges are related events. Brazier (1955), based on the observation of a close relationship between dendritic depolarization and epileptic discharges, suggested that abnormal spikes might be generated by apical dendrites of the cerebral cortex. Abdullah and Magoun (1957) noticed that induced seizure activity interfered with DCRs, which suggested to them that both responses had a common anatomical site of origin. As these authors are of the opinion that the DCR is the result of dendritic activity they concluded that epileptiform activity also stems from dendritic structures. The results of Chang (1950), and Morrell and Torres (1958) suggest also that experimental seizure discharges are confined to superficial dendritic

layers. The interparoxysmal afterdischarges also appear to be restricted to superficial cortical somatic layers. Thus, the dendritic network of the cortex may have a fundamental role in the generation of epileptic activity.

b. Epileptiform afterdischarge activity in the cortex

The cerebral cortex responds to strong, direct, repetitive stimulation with afterdischarges (Adrian, 1936). The epileptiform afterdischarge (EAD) is a repetitive response consisting of a train of increasing high voltage, fast frequency, spiking waves capable of persisting for many seconds after termination of the local evoking stimulus (Jasper, 1955). Insufficient data are available concerning the mechanism(s) responsible for this response. Perhaps it is the result of the synchronized activity of the same units involved in the other direct cortical responses. The manner in which epileptiform activity spreads through the cortex suggests a network of deep neurons with their rich plexi of transverse intracortical connections, e.g. the "B" neurons described by Burns and his co-workers (1957). Adrian (1936) assumed that the initial waves of an EAD resemble a DCR and originate from the same stimulus zone. Self-sustained afterdischarges following prolonged surface stimulation were also studied by Rosenblueth and Cannon (1942). Sub-threshold electrical stimulation, at best, leads only to a very minor response consisting only of a few bursts.

Bremer (1958) and Purpura (1959) have reviewed the literature subsequent to Adrian's early findings and support this proposed mechanism for propagation of DCRs as proposed earlier by Adrian and others. The augmented amplitude of the afterdischarge response is

similar to that found in spontaneous paroxysmal seizure discharges in the same region (Chatrian and Petersen, 1960). The self-sustained EAD can be readily elicited in many areas of the brain (Gangloff and Monnier, 1957).

Jasper (1955) has described five successive stages in an EAD response: asynchronous activity resembling cortical activation which precedes the seizure discharge, rhythmic synchronous and repetitive epileptiform discharges of high amplitude, interrupted clonic discharges, exhaustion, and return to normal activity. Fully developed seizures after direct electrical stimulation of the cortex are characterized by an initial decrease in amplitude of the EEG, immediately following the withdrawal of the stimulus (Sugaya _et al._, 1964; Creutzfeldt _et al._, 1966a). This is soon followed by a phase of regular oscillating waves (tonic phase), which is succeeded by bursts of high voltage spikes interrupted by increasingly longer pauses (clonic phase). This sequence of seizure discharges is usually succeeded by a period of comparative EEG silence (post-ictal depression).

According to Chang (1959) afterdischarges fall into one of three types: 1) repetitive firing of single elements which are self-maintained without the participation of other elements in their production; 2) persistent local afterdischarges involving the activity of closely situated intrinsic neurons which form short neuronal circuits; and 3) periodic discharges involving reverberating activities of a closed neuronal circuit formed by long chains of neurons connecting remotely separated structures.

Whenever a local discharge occurring in the cortex is severe

enough it spreads along neuronal pathways to distant parts of gray matter (Penfield and Jasper, 1954). In this manner a secondary discharging state is produced. If this secondary discharge takes place in gray matter of the higher brain stem, it produces a loss of consciousness and automatic movements during the ensuing generalized convulsions. How hypersynchrony and recruitment of hitherto normal neurons into the epileptic pool of neurons occurs is obscure. It is not known whether the focal epileptogenic discharge and the electrically-induced EAD are caused by repetitive self-propagating discharges of individual neurons or by restimulation of neurons through reverberating circuits (Poggio *et al.*, 1956; Ajmone-Marsan, 1961; Li *et al.*, 1961). Most likely both factors are involved. Excessive stimulation may lead to the repetitive discharge of individual neurons, the rebound spike of the peripheral nerve being a simpler example (Toman, 1959; Morrell *et al.*, 1958). Repeated bombardment of normal neurons at a focus distant from the discharging primary focus may lead to continuance of the epileptic discharge in the neurons of the secondary (or mirror) focus after the original focus ceases discharging (Morrell and Baker, 1961).

It is generally assumed that the EAD is a useful experimental model which accurately simulates the EEG manifestations of clinical epilepsy (Penfield and Jasper, 1954). Thus, it seems feasible to utilize one or another aspect of the EAD response elicited in the cerebral cortex as a means of elucidating and defining the role of drugs and neurohumoural agents in seizure phenomena.

There is controversy regarding the stability of cortical EAD durations. Straw and Mitchell (1966) investigated EADs on intact ecto-

sylvian cortex of acute, immobilized cats, stimulating at 25 or 50 Hz at intervals of 5 or 30 min. These investigators reported considerable variability in EAD duration, with durations increasing significantly over a test period of 5 hours. In contrast, Schallek and Kuehn (1963) reported no significant differences in EAD duration under similar conditions. Berry (1965) has studied EAD duration in rabbits with chronically-implanted cortical electrodes, and reported finding no relationship between the intensity of stimulation and the duration of afterdischarges, which differs from that in acute experiments. Berry (1965) suggested a "quantal" or all-or-none property for EADs in "chronic" animals. Straw and Mitchell (1966a), reported that EAD durations elicited in cats with chronically-implanted cortical electrodes are relatively stable after a few trials. Subsequently (Straw and Mitchell, 1966b), it was concluded that using paralyzed or anaesthetized animals is of limited value for studying cortical EADs, especially their pharmacological aspects.

Locally elicited EADs can produce generalized convulsions. Straw and Mitchell (1966a) routinely observed overt seizures accompanying EADs distributed bilaterally. There are two distinct patterns of peripheral seizures observed in similar numbers of cats. Severe seizures begin with clonic movements of the fact, spread to the contralateral forelimb (Jacksonian), becoming generalized. Another group of cats showed longer EAD durations, and less severe motor seizures which are generalized from the onset. Thus, generalized motor seizures accompany sustained EADs elicited in the cortex by electrical stimulation. Maximal overt seizures vary, but all cats exhibit at least generalized

clonus (Straw and Mitchell, 1966b).

c. Microelectrode studies of epileptic neurons

In experimental epileptic preparations a wide variety of patterns of cortical neuronal hyperactivity can be observed. Very few cells appear to be inactive in epileptiform areas of cerebral cortex, as evidenced by the significantly greater number of spontaneous and repetitive burst discharges at all levels. Adrian and Moruzzi (1939) were the first to observe the result of the electrical activity of small groups of neurons during epileptiform afterdischarges (EADs) by recording with fine wire electrodes in the pyramidal tract. The activity induced in single units by convulsant drugs was investigated in acute preparations by Li and Jasper (1953). Similar experiments, but in chronic preparations, were conducted by Schmidt *et al*. (1959) and by Morrell (1961).

The results of the above studies show that the epileptic neurons have, when contrasted to normal neurons, greater amplitude and duration of potentials in the soma and dendrites, and a much higher frequency of potentials recorded along the axon (up to 1,000 Hz). In some instances, there are trains of rapid repetitive spikes, waxing and waning in frequency, accompanied by abrupt cessation of firing at random intervals. In other cases units may fire rhythmically or there may be interspersed episodes of repetitive, hypersynchronized firing, as well as mixtures of various types of hyperactivity. These high-frequency discharges can be sustained for long periods of time, up to hours.

Microelectrode analysis of afterdischarges in the suprasylvian gyrus of the cat (Gerin, 1960) shows that while the stimulus train is

being applied, cortical neurons and apical dendrites depolarize. Following withdrawal of the stimulus train, membrane potential slowly returns to its normal value, passing through a phase of regular synchronous spiking, which approximates the surface potentials of the tonic phase of the seizure. During the first phase of these oscillations, spike generation may still be blocked, and fully developed spikes appear only after recovery of the membrane potentials (Gerin, 1960).

Gloor et al. (1961) have investigated, in cats, the mechanisms of epileptic discharges induced in the hippocampus by repetitive cortical surface stimulation. This stimulation leads to a progressive negative DC shift in the apical dendritic layer and a positive DC shift in the cellular layer of the hippocampus. Progressively, the DC shift reverses its direction and the potentials in the above layers are now opposite. At this moment (i.e. positive potential in the apical dendrites) the hippocampus is capable of self-sustained seizure discharges during which the DC shifts are exaggerated. These authors believe that the DC shifts are a result of the residual depolarization of postsynaptic structures.

Ajmone-Marsan (1961) has analysed, using macro and microelectrode techniques, the processes responsible for paroxysmal cyclical firing (inter-ictal) and for organized, self-sustained (ictal) activity in epileptic neuronal aggregates, as well as the transition from one type to the other. He attributes paroxysmal firing to an isolated neuron, but seizure discharges could only result from the synchronization of cellular bursts spreading in a neuronal aggregate. In an

epileptic cell population he found a close correlation between spike amplitude and the level of membrane polarization.

Jasper (1961) has discussed the importance of sustained membrane depolarization in the arrest of epileptic activity. This author mentions that excessive depolarization may arrest unit firing. He adds that recovery of polarization to a level favouring firing may be slow, as after a seizure. He also considered the possibility of discrete inhibitory mechanisms, i.e. a homeostatic hyperpolarizing or repolarizing mechanism, which could assist in the termination of the seizure.

Single cell studies in a chronic epileptic focus, produced by intracerebral injection of alumina cream, reveal a wide spectrum of neuronal discharge patterns (Schmidt et al., 1959; Ajmone-Marsan, 1961; Ward, 1961). During the onset of the seizure, spontaneous bursts of high-frequency discharges appear along with slow fluctuations in membrane potential. The frequency of cell discharge waxes and wanes, with periods of intense discharge during an active or "tonic" phase, followed by intermittent bursts during the "clonic" phase. The slowly fluctuating potentials increase during the tonic phase and merge into the clonic phase. Ajmone-Marsan (1961) reports that in his microelectrode studies almost all neurons sampled were firing during convulsive activity, a finding which most other authors have not observed.

One prominent feature of focal epileptic tissue at a cortical cellular level is the development of paroxysmal depolarization shifts in neurons, coincident with each epileptiform discharge occurring during the inter-ictal period between sustained bursts of repetitive epileptiform spiking (Li, 1959; Prince and Futamachi, 1968). The precise mech-

anisms responsible for this electrophysiological phenomenon, or whether these mechanisms are similar in various foci is presently unknown. Similar potentials may be evoked in normal cortical neurons during intense synaptic activation (Prince and Wilder, 1967). There are differences between cellular responses in acute and chronic epileptogenic foci (Prince and Futamachi, 1968). These authors suggest that the smaller amplitudes observed in responses recorded from chronic epileptic foci are due to a more diffuse area of cortical abnormality.

Burns (1958) and Schmidt _et al_. (1959), and later Pinsky and Burns (1962), postulated that the autonomous activity which characterizes the epileptic neuron is due to a relatively prolonged dendritic polarization with a resultant difference in potential between the cell body and its dendrites. Under these circumstances, the membrane potential of the soma would recover rapidly, acting as a source, with re-resultant current flow from the soma to the depolarized dendritic tree, which acts as a continuing sink. If this flow of current continues until it reaches threshold, a high-frequency discharge can be expected to result. The precise mechanism for the depolarization of epileptic cells is not presently known, but it is not unlikely that it is due to some altered neurochemical function in adjacent actively-metabolic cortical elements such as astrocytic glia. As the dendrites are progressively involved during the repetitive phase of rapid cell firing, increased synchronized slow wave activity slowly replaces the earlier phase of rapid neuronal discharges. Thus, it would appear that maximum invasion of the dendrites has taken place at the height of the clonic phase.

Ward (1961) has proposed that the critical step leading to epileptiform spiking is a persistent depolarization of apical dendrites which would result in current flows on the rest of the neuron, inducing repetitive discharges. This is in accord with the intracellular studies of Li (1959) who demonstrated that slow changes in polarization level are associated with rhythmic bursts when depolarization reaches a critical level.

Other evidence suggests that the depolarization shift is a giant synaptic potential reflecting the altered response of some neurons to orthodromic activation (Prince, 1968). The amplitude of the response may be increased by intracellular polarizing currents, and decreased or inverted when evoked during an intense depolarizing current pulse, a pattern similar to that observed with EPSPs. Further, this shift would be disassociated from spike electrogenesis. When not involved in epileptiform activity these neurons generate synaptic potentials indistinguishable from those of neurons in normal cortex (Prince, 1968a). Spike generating properties appear to be normal in epileptic neurons (Prince, 1969). In any case, the depolarizing shift generated in penicillin foci is not an unique response and does not necessarily distinguish primarily involved cells from those which are passively driven by the excitatory synaptic barrage.

Because neurons predisposed to bursting responses also generate normally-appearing spikes and synaptic potentials, Prince and Futamachi (1968) argued that epileptic neurons in chronic foci are not necessarily hyperexcitable as proposed by Sypert and Ward (1967), and that they might be synaptically driven.

Sypert and Ward (1967) have recorded in unanaesthetized monkeys, with microelectrodes, regular, recurrent, high-frequency bursts of action potentials. Groups of neurons appeared to fire recurrently at intervals of 100-130 msec, the number of unit discharges and the interval between bursts remaining relatively constant. Firing rates in a burst ranged from 200-900 Hz. In some instances when synchronous deflections in the ECoG bore a temporal relation to unit bursts, a correlation could be made. More often, it was difficult to correlate local EEG changes with the patterns of repetitive unit firing.

Sawa and his group (1963) have studied intracellular potentials during epileptiform activity induced electrically by single and repetitive shocks delivered to the cerebral cortex and hippocampus. In response to a single pulse at the surface, most cortical and hippocampal neurons showed a depolarizing wave followed by a hyperpolarizing wave. Prolonged high frequency repetitive stimulation at suprathreshold intensity resulted in a progressive depolarization of increasing duration, accompanied by temporal summation of the depolarizing wave, until the neuron remained in a state of sustained depolarization. After cessation of the repetitive stimulation, the sustained depolarization remained, and large long-lasting depolarization waves occurred periodically superimposed on the slower waves in the ECoG. In the final stages of the seizure the depolarization disappeared and slowly became hyperpolarization. During the period in which this hyperpolarization is evident the ECoG remains relatively silent. It was tentatively suggested (Sawa et al., 1963) that the initial progressive depolarization following repetitive stimulation is probably due to a decrease in the

available quantity of some inhibitory synaptic transmitter. These experiments suggest also that excitatory synaptic transmission remains fully operative after inhibitory transmission has been blocked, or reduced.

It is difficult to correlate the repetitive activity of epileptic neurons to the electrical potentials recorded at the pial surface with macroelectrodes. Perhaps the hyperactivity of these neurons generates discharges at the soma of distant cells. Eccles (1957) has suggested that excitation of epileptic neurons is somehow facilitated by amplification of excitatory postsynaptic potentials (EPSPs) and the decline of inhibitory postsynaptic potentials (IPSPs). Ward (1969) suggests that the follower neurons in a seizure discharge are synaptically related to the focus and could account for the summed EPSPs presumably recorded in the EEG. Unfortunately, it is difficult to obtain consistent results from most single cell studies, which no doubt reflects the magnitude of the sampling problems. Schmidt _et al_. (1959) found it virtually impossible to find units that respond with epileptiform firing patterns to repetitive afferent stimulation.

Thus, it appears that synaptic depolarizations during normal synaptic activity of the cortex result in surface negative waves. Highly synchronized synaptic depolarizations following electrical stimulation or arising in epileptogenic regions lead, as a rule, to surface positivity. Highly synchronized IPSPs elicited by various means may result in negativity or positivity at the surface (Kuno and Miyahara, 1968). Slow DC potential shifts of the cortical surface are in phase with the intracellular potential change, i.e. a negative DC shift is accompanied by cellular depolarization. Abrupt DC changes during sei-

zure activity exhibit an inverse relationship. The simplest explanation for these differences is that different synapses are involved, possibly different cell populations, the temporal sequence varying during different types of activity (Humphrey, 1968; Creutzfeldt, 1969). The degree of synchronization of the cortical cell population also affects the waveform and polarity of the surface potential. The surface positive potentials observed during afterdischarge and other types of seizure activity indicate that the apical dendrites serve mainly as sources for such cellular phenomena. These dendrites do not appear to constitute a primary direct mechanism of seizure activity. Experiments carried out on penicillin foci suggest that apical dendritic structures are involved in surface negative paroxysmal discharges (Prince, 1969). This would help to support the hypothesis presented by Ward (1969) that neurons in epileptic foci show significant morphological alterations on their apical dendrites.

It is perhaps understandable what prompted Toman (1959) to remark that "convulsibility is the price of nervous complexity".

B. Studies on the Excitability of Isolated Cortex

According to Jasper (1969), when attempting to draw conclusions upon the role of the cerebral cortex in epileptic phenomena from studies in the whole brain, the many functional relations between cortical and subcortical structures influence the response and make interpretation uncertain. On the other hand, experimental preparations should not be so localized or discrete so as not to be manifested in the EEG, i.e. there should be some resemblance to the clinical manifestations of epilepsy.

The isolated cerebral cortical slab provides an experimental model for the study of cortical excitability (Grafstein and Sastry, 1957) that fulfills the requirements proposed by Jasper (1969). Burns (1949), and Kristiansen and Curtois (1949) independently developed this technique to study the problem of the origin of the rhythmic changes in electrical potentials recorded from the cortex.

The first studies on populations of isolated neurons were by Adrian and his colleagues (1931) who demonstrated spontaneous activity from isolated aggregates of nerve cells in the goldfish. Following this, Libet and Gerard (1939) also demonstrated spontaneous activity in the isolated olfactory bulb of the frog. Bremer (1938) was able to demonstrate bursts of electrical activity recorded from cerebral cortex of mammals after mesencephalic sections of the brain stem. Spiegel (1937) and Swank (1949) have reported similar observations after cutting thalamorcortical projections to a given cortical area. Other investigators (Dempsey and Morison, 1941; Kennard, 1943; Obrador, 1943) have suggested on the basis of their findings on isolated aggregates

of neurons that cortical activity is dependent on subcortical and reticular mechanisms.

Kristiansen and Curtois (1949) recorded bursts of very low voltage rhythmic activity from the neuronally isolated suprasylvian gyri in the cat. They also discovered that local application of a high concentration of acetylcholine converted the rhythmic activity of the isolated slab into a series of rapid high voltage spikes. These authors distinguished these from strychnine spikes, and described them as being similar to the spikes recorded in the vicinity of a cortical epileptogenic focus. Simultaneously, Burns (1949; 1951) using a slightly different preparation of acutely-isolated cortex excluding sulci, but also retaining pial vasculature, observed that there was usually no spontaneous activity present in the isolated slabs. One possible explanation for the apparent electrical silence in Burns' preparation is that the bilateral carotid clamping during the isolation procedure may have resulted in an undue degree of cerebral hypoxia and neuronal damage. Without doubt, his habit of discarding preparations in which there was electrical activity present, claiming that they were incompletely isolated, explains the silence of the slabs used. In spite of objections to these early studies, this technique did achieve some popularity and has been modified slightly to overcome some of the difficulties encountered earlier.

It would seem that the chronic preparation of a neuronally isolated slab of cerebral cortex with indwelling surface stimulation and recording electrodes (Sharpless and Halpern, 1962) would overcome some of the undesirable aspects of acutely isolated cortex, e.g. recent

surgical trauma and disturbed pial circulation, conditions which are undoubtedly capable of releasing cerebro-active substances, as well as the residual effects of the post-anaesthetic state.

a. Acute studies with macroelectrodes

Isolated cortex, particularly the acutely-isolated cortical slab, is capable, of responding to direct stimulation with electrical phenomena similar to those evoked on intact cortex (Grafstein and Sastry, 1957; Burns, 1958; Pinsky and Burns, 1962; Sanders and Pinsky, 1967).

i) the direct cortical response (DCR), or surface negative response, is a graded response to a single weak stimulus. The response lasts about 20 msec, and the amplitude decreases rapidly from the point of origin.

ii) the positive burst response (PBR) is elicited by a slightly stronger electrical stimulus. This rectangular-shaped response lasts from 0.5 to 5 sec and travels rapidly across the slab without attenuation. It displays all-or-nothing characteristics, and the positive peak has superimposed on it bursts of high-frequency potentials, suggesting that several neuronal chains may be involved.

iii) the epileptiform afterdischarge (EAD), also a paroxysmal response, follows a short period (2-5 sec) of intense repetitive stimulation (30-60 Hz). The pattern of electrical activity is, as suggested, typical of that recorded during an epileptic seizure. The EAD consists of a series of large (0.2-1 mv), multiphasic spike discharges occurring at a frequency between 10-20 Hz. These spikes are conducted rapidly across the isolated slab. This discharge pattern persists for 15 sec or more, and often becomes replaced by intermittent rhythmic activity of 2-5 Hz resembling suppression burst patterns. This activity usually ends abruptly with a short period of electrical silence.

iv) a variant of the EAD, the epileptiform sustaining response (ESR) (Sanders and Pinsky, 1967), is induced by a weak stimulus applied during an ongoing EAD which causes that EAD to be considerably prolonged in duration.

v) a response resembling spreading cortical depression of Leao (1951) may arise with very strong repetitive stimulation. It is accompanied by a slow negative shift of the cortical surface potential.

The PBR response is deafferented cortex as described by Burns (1954) displays some interesting characteristics. The after-bursts which originate with the surface positivity induced by single shocks, are capable of outlasting the surface positive response (Burns, 1954). Another intriguing aspect is that several stimuli separated by intervals of a few seconds are capable of starting a series of periodic discrete burst responses which continue to recur for many seconds after the stimulus has been withdrawn.

In the central nervous system as in most excitable biological structures, the consequence of reduction or complete removal of synaptic input is inevitably followed by hyperactivity of the neuronal elements (Stavraky, 1961). It has been well shown that reduction of afferent input as achieved in a neuronally isolated cortical slab is associated with a lower threshold and a higher amplitude of direct cortical responses evoked by electrical stimulation of the isolated cortex (Goldring et al., 1961; Suzuki and Ochs, 1964). Furthermore, as Eccles et al., (1962) have shown, partial deafferentation of a pool of neurons in the lumbar cord induces striking augmentation of the monosynaptic reflex. The mechanisms underlying such hyperactivity are, however, poorly understood. Such increased responses do not appear to be a consequence of collateral sprouting of remaining input to the neurons since Eccles et al. (1962) have shown that the EPSPs in motoneurons in partially denervated segments are appreciably reduced when evoked by dorsal root Ia volleys.

The question as to the cause and existence of spontaneous activity in isolated cortex has not been resolved to date. Burns (1958) has taken the position that the carefully isolated slab of cerebral

cortex is electrically silent unless injury, acting as a stimulus, sets up electrical responses. Preston (1955) found that no spontaneous activity could be detected from the isolated cortex for a period of 20 to 60 minutes following surgery. Short (1-3 sec) high-frequency "afterdischarges" could be elicited during this period by single shocks administered to the isolated slab. Unlike Burns, Preston found that electrical stimulation is not a prerequisite for the appearance of spontaneous activity. Preston decided that these "afterdischarges" and the spontaneous activity which appeared subsequently in isolated cortex were very similar in pattern. Preston (1955) discovered that the spontaneous cortical activity was most sensitive to anoxia, and that apnea markedly suppressed this activity for prolonged periods. Ether at high concentrations also was found to be deleterious. Pentobarbital, on the other hand, suppressed this activity completely and eliminated electrically-elicited discharges.

Ingvar (1955; 1955a) prepared large isolated slabs of parietal cortex in cats to study its electrical activity and to determine the origin of the 3/sec spike and wave complex observed on this experimental preparations. These animals were unanaesthetized by discontinuing ether and thiopentobarbital following surgery, and were curarized and ventilated mechanically. The isolated area became spontaneously active soon after surgery, producing episodic bursts having a dominant or regular component of 2.5-3.5/sec slow waves, or spike and wave patterns. The latter pattern was present spontaneously, or was provoked by local application of metrazol (10%) or strychnine (3%). Such treatment also resulted in variable, paroxysmal, tonic-clonic EADs like those described

by Henry and Scoville (1952) for apparently-isolated cortex in patients with cerebral tumours. Ingvar (1955) found it difficult to observe spontaneous and evoked activity on cortical slabs in animals with their brain stem transected.

Ingvar (1955) suggested that isolated cortex is somehow subject to extraneuronal factors (circulating and humoral) related to subcortical structures in the brain stem. Ingvar (1955a) also demonstrated that high-frequency electrical stimulation of the brain stem reticular formation (tegmental and diencephalic sites) is associated with increased cortical circulation and excitability (activation), even in completely isolated slabs. He postulated that epileptic discharges are correlated with a decrease in cortical circulation, while post-ictal atypical activity and electrical silence are due to other factors.

Ingvar (1955a) showed that in some previously "silent" slabs, reticular stimulation initiates rhythmic activity after a latency of 40 sec. In some slabs with spontaneous bursting patterns, reticular stimulation prolongs bursts. Isolated slabs showing continuous activity react differently. There is, after a latency of 1-5 sec, complete suppression of bursts for up to 30 sec, followed by a short phase of activation. Ingvar (1955a) emphasizes the long latency preceding these inhibitory effects and that adjacent intact cortex was concomittantly activated. He attributes these effects to the passage into the slab of neurohumours liberated by the non-isolated cortex.

Torres and his associates (1958) have reported that in certain experimental circumstances the afterdischarge has special characteristics of propagation in cerebral cortex. These workers recorded simultaneously the electrical activity from isolated cortex,

intact adjacent cortex, and the white matter underlying the isolated slab. In essence, they found that afterdischarges elicited within the isolated slab did not stray into non-isolated cortex, whereas afterdischarges induced in normal cortex did in fact often invade the isolated cortical slab. These results suggest that there is a possibility for some transmission to take place between surgically isolated areas of cortex, perhaps as a result of the release of some chemical mediator, that, after diffusing into the slab, is capable of initiating electrical activity within it. No spread was detected into the underlying white matter. Similar results have been reported for the isolated cortex of the dog (Maiti and Domino, 1961).

Domino (1957) has investigated the action of barbiturates in partially isolated cortex. In the process he has reported on extensive studies about the influence of volume conduction and the possible presence of intact microscopic neuronal bridges on the spread of electrical activity into the slab. Domino (1957), Rech and Domino (1960) and Maiti and Domino (1961) have concluded that since the electrical activity and the responses of isolated cortex displayed patterns and phase relations different to those of adjacent intact cortex, the electrical activity recorded from partially isolated cortex is innate and any remaining microscopic bridges do not play an important role.

The medial suprasylvian cortex in cats has been investigated by Dilts and Berry (1968) on intact, undercut or circumscribed preparations of the gyrus using the DCR elicited as an index of excitability. Both undercutting and circumscribing depress the resultant excitability curve. These authors (Dilts and Berry, 1968) reported that transection

of the brain stem elevates this curve for intact medial suprasylvian gyri. On the other hand, bilateral electrolytic lesions of anterior thalamic regions depress the excitability of suprasylvian cortex. Dilts and Berry (1968) concluded that diencephalic structures (thalamic subcortical projections) maintain area-specific cortical interaction patterns.

Sherwin (1965) has studied the relationship between sub-cortical structures and cortical epileptic discharges during hyperven-tilation. Unfortunately, this investigator used electrical silence in the isolated slab as a criterion before proceeding with his experi-mentation. Hypocapnia did not affect EAD duration or threshold in isolated cortex, but it did lower the threshold and significantly prolong EAD duration in intact cortex. Thus, subcortical influences, non-specific thalamo-cortical connections as suggested by Sherwin, seem to exert considerable influence on afterdischarge duration in intact cortex.

As indicated above, a portion of cortex devoid of connections with extracortical structures is capable of generating various local responses. The EAD once elicited will spread and progressively to apparently involve all neurons within the island of chronically iso-lated cortex (Brock, 1967) which is not in agreement with the limited spread (10 mm) proposed by Sanders and Pinsky (1967). Echlin and Battista (1963) have demonstrated, using partially isolated cortex, that paroxysmal discharges generated within the abnormal cortex can spread to non-isolated cortex and give rise to generalized seizure activity. Thus, the isolated cortex seems to possess the synaptic

structures and mechanisms necessary for self-sustained and rhythmic neuronal discharges, independent of afferent inputs. Brock (1967) considers that, owing to the "looser structural coupling resulting from degeneration, burst activity (in chronic cortical slabs) becomes independent of any single focus"(unlike the case with acute cortical slabs). This hypothesis may help to explain the ease of spread of the EAD on chronically isolated cortex and their longer durations.

Maiti and Domino (1961) found that, on isolated cortex, a suppression burst pattern re-appears following a cortical afterdischarge. This is in contrast to intact cortex where it is usual to observe only a comparative period of post-ictal isoelectric silence. Moreover, Maiti and Domino (1961) reported that the threshold for eliciting cortical afterdischarges on a given area of intact cortex, is significantly increased following the surgical deafferentation of that same area of cortical tissue. The other major difference following surgery is, understandably, the usual lack of spread of EADs in all directions, into neighboring areas of cortex. However, for some reason, it was found that, as reported earlier by Torres _et al_. (1958), there is spread, after a short delay, of epileptiform activity from intact cortex into the isolated region. In their experiments on dogs, Maiti and Domino (1961) encountered significant variability with the duration of elicited EADs in acutely-isolated cortex.

The duration of the EAD elicited in acutely-isolated cortex is short, and proportional to the stimulating voltage (Burns, 1951, 1954; Grafstein and Sastry, 1957). Burns (1954) observed that applying a current flowing in a radial direction from the surface to the depth

of the cortex burst frequency was proportional to stimulus intensity. The afterdischarge was suppressed by a current applied in the opposite direction.

Pinsky and Burns (1962) investigated the conditions necessary to elicit epileptiform afterdischarges in acutely neuronally isolated cortex. On the basis of their findings, they proposed that a critical minimum number of neurons must be excited to act as a focus for production of a sustained EAD. They found the response to be an all-or-none phenomena. A requisite of this hypothesis is that the driven cortical neurons must be in a state of exhaustion when the stimulus train is withdrawn, otherwise an EAD will not result. That is, the EAD is, in their view, a consequence of recovery by the neurons from a state of exhaustion.

Sharpless and Jaffe (1966) have questioned the above findings of Pinsky and Burns (1962) since they observed that the duration of EAD's was proportional to stimulus strength, particularly at levels close to threshold. There were differences in experimental technique, the major one was that Sharpless and Jaffe used 0.2 msec paired opposite-going pulses, while Pinsky and Burns (1962) used 2 msec monophasic pulse. Sharpless and Jaffe propose that the persistence of the excitatory process responsible for generating EAD's is partially dependent on the number of elements discharged by each pulse. Evidence obtained by intracellular recording during electrically-induced EAD's (Sugaya *et al.*, 1964) would seem to be consistent with this theory. On the other hand, the establishment of the excitatory process by repetitive stimulation might somehow be reinforced or stabilized in adjacent

neurons, e.g. by electrotonic (Grundfest, 1959) or synaptically mediated interactions between neighbouring elements.

Sanders and Pinsky (1967) studied the relation between EADs and surface PBRs in acutely-isolated cortex. They report that some neurons participating in the EAD are somehow facilitated since previously weak ineffective stimuli, continually administered during the EAD will sustain the EAD for up to 20 minutes. The neurons involved in the sustained EAD were shown to be distinct from those involved in the PBR.

b. Acute studies with microelectrodes

Sugaya *et al*. (1964) have reported that spontaneous cell firing first stops (associated with membrane hyperpolarization), but then reappears at increased frequencies (associated with excessive membrane depolarization) following an electrical surface stimulation of intact cortex that evokes afterdischarges. Transient membrane depolarization corresponds well with surface negative paroxysmal waves.

Frost (1968) has shown using computer methods that EEG and unit activity in acutely-isolated cortex are related. It was stipulated that the particular neuron under observation would have to behave synchronously with a larger population of adjacent neurons to allow a grossly detectable event in the EEG. Silent periods were accompanied by a sustained relatively high polarization of the membrane without fluctuations. The typical finding was for intracellular depolarizing waves to be associated with negative waves at the surface, indicating some accord with the results of studies in intact cortex (Sugaya *et al*., 1964). In an earlier study, Frost *et al*. (1966), had noted that in spite of a correlation between EEG active or silent

periods and single units, the majority of cells had no particular recognizable to the EEG phase or amplitude. Thus, if the EEG does represent summated postsynaptic potentials it is unfortunate that more highly synchronized unit populations of the EEG were not found by these authors.

Reiffenstein (1964) studying radial potentials with microelectrodes in both acute and chronic slabs found no differences in this parameter. This suggested to him that the differences in repolarization, proposed earlier by Pinsky and Burns (1962) as a mechanism for initiating and maintaining EADs, result from epileptiform activity, rather than causing them. Reiffenstein (1964) used large microelectrodes (7 μ) which might explain the lack of differences in potentials.

Brock (1967) has investigated with microelectrodes (2-2.5 μ) individual burst-spike episodes of neurones in acutely and chronically isolated cortex following induction of EADs by repetitive electrical stimulation at the surface. This author found that the surface stimulation apparently establishes a shell of electrical activity, with the stimulated point at its centre, which behaves as a focus for the EAD. It is from this focus that the burst spreads over a wide region and the associated spikes were suggested to result from activity in excitable cells immediately adjacent to the recording microelectrode. These observations appear to provide support for the differential repolarization hypothesis of EAD generation (Pinsky and Burns, 1962).

Jasper (1969) in a microelectrode study of acutely-isolated slabs found that each EAD is associated with an abrupt negative shift in DC potential of the cortical surface that apparently extended to

the large pyramidal cells of layer V. Rhythmic oscillations of potential (8-12 Hz) are always superimposed upon long-lasting negative DC shifts. This sudden DC shift is associated with rapid, continuous repetitive discharges, apparently from deep pyramidal cells, which are interrupted by periodic inhibitory waves.

According to Jasper (1969) there are two forms of self-sustained activity in the EAD. Occasionally at the start of the EAD 40-60 Hz spiking precedes the 8-12 Hz activity. Whenever positive DC changes are observed at the surface, rhythmic or abrupt, the pyramidal unit firing is interrupted. This superimposed interruption of the paroxysm was suggested to be due to periodic waves of inhibition controlling the otherwise continuous excitation of the pyramidal cell. Jasper (1969) proposes that it is the pattern of the inhibitory phenomena that determines the frequency of discharge observed at the surface, i.e. units would fire at high frequency, all other factors being equal, but the periodic inhibitory influence (recurrent inhibition) periodically arrests units and imparts a characteristic frequency to the EAD, 8-12 Hz.

These studies of Jasper (1969) are in accord with the hypothesis of Pinsky and Burns (1962) that the EAD results from differential repolarization rates between dendrites and the soma. Burns and Grafstein (1952) on the basis of microlaminar studies showed that cuts in isolated slabs 1 mm below and parallel to the surface prevented the appearance of DCRs, which argues in favour of the hypothesis of Pinsky and Burns (1962). Pinsky (1963) found that the maximum amplitude of the radial potential gradient is recorded 0.8-1 mm below the cortical surface.

c. Studies on chronically-isolated cortex

Chronically-isolated cortex is capable, after a few weeks of isolation, of sustaining EADs of approximately 60 seconds. EAD duration increases gradually over the period of isolation and is not due simply to recovery from the traumatic effects of the isolation procedure. Re-isolation and subjecting the isolated area to all the trauma of the original operation, does not prevent the appearance of prolonged afterdischarges (Sharpless and Jaffe, 1966). Echlin and his associates (1954) hypothesized that the increased susceptibility of chronically isolated cortex to paroxysmal activity may be due to supersensitivity of denervated cortical neurons to ACh. There is a marked fall in acetylcholinesterase (AChE) content of isolated cortex (Hebb et al., 1963; Duncan et al., 1968; Rosenberg and Echlin, 1968). The time course of this decrease in AChE activity parallels the increase in susceptibility to epileptiform activity.

In contrast, acutely isolated cortex (unanaesthetized, unparalyzed animals) will rarely sustain EADs longer than 20 sec (Grafstein and Sastry, 1957; Pinsky and Burns, 1962; Sharpless and Halpern, 1962). Maiti and Domino (1961), reported that they could elicit significantly longer EADs on adjacent intact cortex than was the case on acutely-isolated cortex of the dog.

Purpura and Housepain (1961) found that on chronically-isolated cortex of immature kittens, pyramidal neurons develop numerous axon collaterals with extensive intracortical ramifications. They propose that in chronically-isolated cortex (immature) the sprouting of axon collaterals of pyramidal cells is a major factor responsible for

the increase in excitatory synaptic (axo-dendritic) linkages. There is increased excitability in the chronic preparation as reflected in the complex 8-14 Hz bursts in response to weak stimulation, there being no response in the acute preparation. However, it is important to bear in mind that the immature cortex is in a phase of active growth and differentiation, whereas mature cortex lacks this capability.

d. Microelectrode studies in chronically-deafferented cortex

Creutzfeldt and Struck (1962) have observed in chronically-isolated cortical slabs that surface potentials are related to deep negative potentials accompanied by spike discharges. Thus, rapid transient shifts of the membrane potential are related to the surface EEG potential in such a way that depolarization coincides with surface positivity. Enomoto and Ajmone-Marsan (1959) have considered that the EAD in the cerebral cortex always results from depolarization of a large number of units producing high-frequency bursts of variable duration. Jasper (1969) reports that in acutely isolated cortex a negative DC shift of 1-5 mv always results in high-frequency spiking. Halpern and Ward (1969) have reported great difficulty in obtaining unit activity to correspond to surface electrical phenomena.

Watanabe and Creutzfeldt (1966) were unable to detect spontaneous EPSPs and IPSPs in chronically-isolated cortex, however epicortical stimulation did elicit these potentials. These authors concluded that spontaneous postsynaptic potentials require afferent or collateral fibre activity. This suggests that the spontaneous liberation of transmitter in isolated cortex is so low that it does not elicit miniature potentials.

Halpern et al., (Black et al., 1967; Cole and Halpern, 1969) have carried out extensive studies in chronically-isolated cortex with microelectrodes. These authors conclude that unit firing was not uniformly present, even during sustained afterdischarge activity. This could be due to the fact that they were recording 1.8 mm below the surface of the cortex, which according to Weisman (1969) would be beneath the grey matter. These authors also failed to obtain evidence of spontaneous neuronal firing. Spontaneous activity was detected up to two weeks following isolation, but disappeared soon after this period. These observations raise the question of the origin of the high voltage EAD activity and what is the role of cortical elements in cortical excitability and epileptiform activity. Perhaps the somatic elements are so altered that increased dendritic activity accounts for the observed EAD activity in chronic slabs.

Conditions under which Halpern and co-workers detected transient bursts of unit activity accompanying surface negative bursts included topical application of strychnine, induction of light barbiturate anaesthesia or temporary cortical anoxia. However, I.V. metrazol or direct stimulation failed to produce unit discharges accompanying the surface EAD response. Glutamate did evoke unit firing in intact and chronically-isolated cortex, the latter preparation requiring twice the ejection current for activation, suggesting that the potential for unit activity is present in chronic slabs.

A group of French workers (Hirsch et al., 1969) have studied the ECoG and unitary activity in chronically-isolated cat suprasylvian gyrus using implanted cortical electrodes. These authors showed that

the paroxysmal ECoG of isolated cortex had a systematic relationship to unit discharges. These workers did not study the EAD response but did stimulate with single pulses to produce PBRs which were noted to be very similar to waveforms occurring spontaneously in the ECoG.

Krnjevic _et al_. (1969, 1970, 1970a) have reported various studies that attempted to determine whether loss of inhibition and the development of chemical supersensitivity are related to the generation of the prolonged EAD that can be elicited in chronic slabs of cortex. It was found that cell sensitivity to glutamate was either unchanged or increased. The units thus exicted displayed spikes of smaller amplitude and bursts were poorly maintained. Ejection of _gamma_-aminobutyric acid (GABA) readily blocked glutamate induced-firing, occasionally at lower doses than those required for intact cortex.

On the whole these authors encountered few spontaneously active units. A curious finding was the inhibitory effect upon unit firing (glutamate-induced) of strong surface shocks. This inhibition seemed to be related to the generation of IPSPs evoked by the surface stimulation. These results suggested to Krnjevic _et al_., (1970a) that there is substantial preservation of normal inhibitory mechanisms in chronic slabs. These authors found that neuroglia cannot be implicated in the seizure activity, since they have a high resting potential and, did not respond to low frequency stimulation. During general seizure activity in the slab, ejection of glutamate was without effect. It was also shown that GABA reduces unit firing but not bursts. Moreover, unit spike activity present during the interictal period, rapidly subsided immediately after the onset of paroxysmal surface activity.

A most puzzling finding reported by Krnjevic _et al_., (1970) was that intravenous GABA was effective in depressing glutamate evoked unit activity just as effectively as microiontophoretically administered GABA in isolated cortex. Intravenous GABA had no effect on intact cortex. According to these authors intravenous administration of GABA is capable of terminating an EAD elicited in a chronic slab.

Analysis of the spike structure of an epileptic burst reveals that there is only high frequency firing within the burst, and a sudden end to spiking activity (Ward, 1969). The interval between the first spike and the rest of the burst can be variable. Calvin _et al_., (1968) noticed that with long intervals between the first spike and the rest of the discharge bursts are more common in the central regions of an epileptogenic focus. This suggested to them that the first spike was a separate process from the rest of the burst, and determined the beginning of the rest of the burst and the timing of the spike.

Ward and his colleagues (Ward, 1961; Ward and Schmidt, 1961; Westrum _et al_., 1965) have extended the concept of the chronic epileptogenic focus to include an aggregate of partially deafferented neurons in cortex. They suggest that a population of partially denervated hypersensitive neurons in the focus brings about excessive neuronal discharges, in large part, by antidromically stimulating axon terminals of relatively distant normal cell bodies. This concept is based both on their observation that the intracellular potentials of neurons in an alumina focus show properties suggestive of axons (Sypert and Ward, 1967) and on the pattern of firing of single neurons observed extracellularly.

Consistent with this partial denervation hypothesis is the

observation that epileptic neurons in sensory cortex are difficult to evoke by afferent volleys arising from peripheral nerves (Ward, 1969). However, not all synaptic input to the cortex is lost, because powerful stimulation of the thalamus can evoke responses in epileptic neurons. The sequence of neuronal firing in epileptogenic cortex is characterized by a longer latency between the first and second discharges of the train than between the second and subsequent discharges.

Ward (1969) proposed that the bursting behaviour could be a result of axonal conduction. The first spike would be conducted antidromically from the focus to a distant cell body. The remainder of the burst after a fixed interval would then return from the relatively distant soma along the axon. The relatively longer interval between the first and subsequent discharges is accounted for by the time elapsed between the antidromic firing and a subsequent orthodromic firing which is initiated in the distant healthy cell body by the antidromic impulse (Ward, 1969).

The crux of this argument is the mode of induction and the subsequent antidromic conduction of the spike to a normal cell body with only an abnormal input. A variety of synaptic mechanisms could account for the generation of the observed repetitive burst spiking. Perhaps, it is possible for a presynaptic terminal to be strongly depolarized by a presynaptic synapse normally involved in inhibition at this level. In an epileptogenic situation the suprathreshold depolarization would initiate a spike which would be conducted antidromically back up the axon. Such a mechanism has been considered by Wall (1962) to explain hyperactivity in the dorsal-root reflex.

It is tempting to consider that epileptiform bursting activity may be due to a sequential mechanism involving EPSPs and IPSPs as has been suggested (Biedenbach and Stevens, 1966). Kandel and Spencer (1961) have considered that bursts in hippocampal pyramidal cells could be due to a self-limiting regenerative mechanism by means of summating depolarizing afterpotentials. It is still too early to relate these findings to isolated cortex.

In attempting to confirm these results, Westrum _et al_., (1965) conducted histological studies on chronic epileptic foci produced by the alumina cream method. The primary focus was found to be immediately adjacent to the area of the alumina injection, and was surrounded by glia and zones apparently devoid of neurons. In contrast to normal cortex, the cortical surface in this epileptogenic area did not exhibit the characteristic dendritic plume or terminal dendritic arborization. The neurons stained appeared to be smaller in overall size, with less dendritic branching, and with the course of the apical shaft and its branches distorted in different planes. Close inspection of the dendrites revealed that they were almost devoid of their dendritic spines, especially on the apical shafts within the area found to display the greatest electrical activity. Weisman (1969) and Weisman and Pinsky (1970) have reported similar results in studies on the chronically neuronally isolated cerebral cortex of the cat. Within 4 mm from the focus, the histological picture gradually blends into that of normal cortex (Westrum _et al_., 1965). Dendritic branching becomes denser and there is an increase in the number of larger neurons. Sypert and Ward (1967) showed that a similar transition occurs with the discharge pattern of neurons, i.e. hyperactive synchronous discharges giving

way to spontaneous normal EEG rhythms.

One possible explanation for the increased tendency of isolated cortical slabs to respond with very prolonged afterdischarges to repetitive stimulation, is that this preparation might be deprived of inhibitory influences normally operative. On the basis of the histological studies discussed earlier it would seem that, if anything, inhibitory synapses are well preserved in chronically-isolated cortex (Colonnier, 1966). Of course, this does not rule out the possibility that there is a deficit of some inhibitory mediator, although the inhibitory synapse remains present.

Another possibility is that there might be enhanced efficiency of excitatory mechanisms in the isolated cortex. The recent studies by Halpern *et al.*, (1969) and Krnjevic *et al.*, (1970, 1970a) are not in accord with this view as they report lower sensitivity of isolated cortical neurons to microiontophoretically applied glutamate. In addition, very few cells are excited by iontophoretically applied ACh, and it was reported that with most cells there was depression (Krnjevic *et al.*, 1970) on administration of physostigmine.

C. Deafferentation as a Factor Responsible for Epileptiform Activity

Several authors have proposed that partially denervated neurons eventually become supersensitive to chemical stimulation and that this phenomenom is one explanation for the convulsive tendency observed in chronically isolated slabs of cerebral cortex (Cannon and Rosenblueth, 1949; Echlin and Battista, 1963; Duncan *et al.*, 1968). Li (1960) has remarked that it is conceivable for epileptiform discharges in epileptic cortex to be based on the same mechanisms which

occurs in denervated muscle. However, enthusiasm for invoking phenomena operant at peripheral excitable structures should be tempered with a consideration of the differing histomorphological factors. That the epileptogenic neuron possesses abnormal electrophysiological properties is obvious. What is puzzling is the site of this altered behaviour and the precise manner in which it arises.

Echlin and his collaborators (Rosenberg and Echlin, 1965) have implicated acetylcholine (ACh) as the mediator involved in the denervation hypersensitivity of epileptic neurons in chronically partially isolated cortex. Reiffenstein (1964) found that chronically-isolated cortex develops an increased sensitivity to ACh, but since atropine did not alter EAD activity in his experiments, this author concluded that there was no basis to implicate ACh in enhanced EAD activity.

Speigel _et_ _al_.,(1955), and Stavraky in his monograph (1961) have also presented evidence to support the postulate that denervation hypersensitivity to ACh may occur as a result of pathological conditions in the brain. However, since the precise mechanism of this phenomena is far from being solved at peripheral excitable tissues, and there is a limited number of studies in this area dealing with central structures, it is not possible yet to arrive at firm conclusions about its role in epileptogenic cortex.

Echlin and Battista (1963) have conducted many experiments to explore this hypothesis. These authors have found that epileptiform discharges can be precipitated in chronic partially-isolated cortex in the monkey by the topical application of ACh. This paroxysmal activity spreads out of the partially-isolated slab of cortex and causes

classical generalized or focal epileptiform electrical and clinical seizures. The majority of preparations tested required rather high concentrations of ACh (4-10%). The cortical area homotopic to a chronic partially-isolated cortical slab was found to be approximately twice as sensitive to ACh as the same area in acutely-isolated cortex, i.e. the mirror focus was also hyperexcitable (Echlin and Battista, 1963). The chronically-isolated cortex was also more sensitive to topically applied strychnine, indicating a somewhat more generalized mechanism than Echlin and Battista propose. Apparently these authors never attempted to block these effects with anticholinergics.

The role of denervation in the augmented activity of epileptogenic tissue has also been tested using the isolated cortical slab (Echlin, 1959; Sharpless and Halpern, 1962; Reiffenstein, 1964). It is reasonable to assume that if deafferented cortical neurons become hypersensitive to a neurotransmitter, e.g. ACh, this phenomenon might conceivably play some role in the hyperactivity displayed by epileptigenic cortex. Inferential data concerned with this point has been produced. Burns (1954) and Grafstein and Sastry (1957) have shown that neuronally isolated cortex responds with bursts and EADs more easily and that this activity propagates widely throughout this cortical preparation. Echlin (1959) has shown that in partially-isolated cortex it is relatively easy to elicit prolonged epileptiform activity by topical ACh in concentrations which have no effect on adjacent normal cortex. Kritiansen and Courtois (1949) have also reported similar observations on isolated and intact segments of cat cerebral cortex.

It has been reported that total AChE in chronically-isolated cortical slabs is reduced considerably (Hebb *et al.*, 1963; Duncan *et al.*, 1968; Krnjevic *et al.*, 1970). Moreover, Duncan *et al.*, (1968) and Rutledge (1969) reported that intermittent daily subconvulsive stimulation of chronic slabs prevents the development of prolonged EADs and also the decrease in AChE. There might thus be grounds to implicate or even correlate the level of hyperexcitability with the level of ACh, using AChE as an indirect index of level of cholinergic mediator.

D. Post-Tetanic Potentiation as a Mechanism in Epileptiform Activity

Immediately after high-frequency stimulation axons of peripheral nerves respond to single pulses with brief trains of repetitive action potentials (Raines and Standaert, 1966). This repetitive activity has been shown to be associated with hyperpolarization of the terminal portions of the axon (Morrell, 1959). This phenomenon is also observed in other synaptic networks, e.g. nerve-muscle preparations and has been called post-tetanic potentiation (PTP). Gage and Hubbard (1966) have ruled out hyperpolarization as its sole causal factor. These authors speculated that increased amounts of transmitter released following a train of repetitive pulses might be responsible for this phenomenon in synaptic networks.

Toman (1959) considers that this mechanism (PTP) contributes to the spread and maintenance of seizures discharges once they have been initiated. This author suggested that there is an accompanying progressive, but transient, enhancement of central synaptic transmitter release during rapid repetitive stimulation. The discharge would ter-

minate as a result of fatigue or increased inhibitory activity. Indirect evidence cited in favour of this hypothesis is the report of Esplin and Freston (1960) that spinal cord convulsions are antagonized by diphenylhydantoin, an anti-epileptic known to block PTP (Esplin, 1957; Rand _et al._, 1966).

Toman and his associates (1946) have suggested that "the efficiency of clinical antiepileptic drugs may be better correlated with a reduction in the ability of the brain to support self-sustaining discharges than with a simple increase in the electrical or chemical threshold for initiation of such discharges". The main body of this investigation is concerned with the susceptibility of the EAD, elicited in isolated masses of cerebral cortex, to pharmacological manipulations. Since neurohumoural transmitter substances can be assumed to be operative at this level of cerebral activity, an attempt has been made to implicate certain mediator or modulator systems in this form of abnormal electrical activity.

V. THE CHEMICAL BASIS OF CENTRAL NERVOUS FUNCTION

It has been postulated that the sustained discharge pattern observed in central neurons is a result of prolonged transmitter action, due in turn to continued presynaptic impulses (Eccles, 1964). There is general agreement that synaptic transmission at mammalian central neurons is chemically mediated and that there is an increase in conductance of the postsynaptic membrane as long as release of a mediator is maintained by presynaptic impulses.

As the central nervous system does not appear to have the localized synaptic regions characteristic of peripheral sites, it is not surprising that in spite of many studies, the evidence supporting the presence of specific mediators and receptors active at central neurons is still inadequate to clearly involve the popular transmitter candidates acetylcholine, serotonin, catecholamines, and other agents. The dendritic and somatic structures as well as the axons of central neurons are profusely, intimately, and intricately interconnected with each other by means of many synapses. The great anatomical complexity of cortical elements provides a formidable obstacle for attempts to investigate the specific role of neurotransmitters postulated to be operative in the CNS. Not only may there be the possibility of more than one transmitter operating on a neuron, but there may be more than one type of receptor at this basic central element (Curtis and Ryall, 1966). Nevertheless, it is apparent that interneuronal communication is achieved via the terminal branches of axons not connected to other nerve cells, by the process of synaptic transmission. In this way information is transmitted across central synaptic junctions by means

of a complex series of interrelated chemical and electrical events, whose precise mechanisms are far from being understood.

The problems associated with successful administration and collection of pharmacological substances at central nervous system cells present unique technical difficulties, which invariably involve uncertainties as to the exact cerebral penetration of these substances. Superimposed on this factor is the uncertain degree of variable interactions between the test agents and cerebral structures, prior to penetration onto the desired aggregates of neurons. Abnormalities of transmitter function culminating in excessive excitation or diminished inhibition may be responsible for the initiation of abnormal activity in neurons within an epileptogenic focus. It is reasonable to assume that the subsequent development and spread of paroxysmal epileptiform activity involves, in some manner, synaptic processes and events.

The criteria developed for the identification of central neurotransmitters have been based entirely on those invoked for the peripheral nervous system (Eccles, 1964; McLennan, 1970). Perhaps it is unrealistic to apply strictly these classical criteria to studies on the CNS, owing to the dissimilar structural and functional relationships. Nevertheless, most central neurons appear to have some properties in common with peripheral neuroeffector junctions. Therefore, fulfillment of the criteria that follow usually is sufficient to demonstrate that a candidate neurotransmitter can be identified and has a functional role at many central synapses.

These criteria can be summarized as follows:

i. The substance must be highly localized in the terminals of those neurons for which it functions as a transmitter substance.

ii. The appropriate enzyme system for the biosynthesis of the substance must be present in these localized structures, along with storage sites.

iii. The substance should be detectable in extracellular fluid collected from the localized region following activation of the neuron.

iv. A system for rapid inactivation of the substance must be present and also localized at or near the synapse.

v. The local application of the suspected transmitter, or very active derivatives, at the postsynaptic cell should elicit effects similar to those caused by nerve stimulation.

The first two criteria usually are the most difficult to fulfill. These criteria for identification can be strengthened by pharmacologically interfering with the response with specific blocking drugs, and by showing that deafferentation results in loss of the substance and activity. More recently, metabolic inhibition of key enzymes has provided a means of specifically altering levels of the transmitter or precursors. Impairment of CNS function by interfering with production or liberation, by depletion, and by pharmacological blockade of the transmitter are usually consistent with a presumed transmitter function. However, even in discrete areas of the brain such clear-cut relationships are rarely found, especially for substances other than ACh.

It is usually suggested that neurohumoural substances to which no transmitter action can be ascribed are modulators of trans-

mission (Everett, 1961). These neuromodulators are believed to operate by modifying function and output of transmitters in a variety of ways. Unfortunately, there is little experimental evidence, especially in the CNS, for these poorly-defined substances, and it is not yet possible to provide adequate criteria for their recognition.

The changes produced in the subsynaptic membrane by transmitter substances are now well characterized (Eccles, 1964; 1969). Excitatory transmission involves a local depolarization of the affected neuron and an increase in the permeability of its membrane to all ions. Inhibition provides a more complex situation, for two types of inhibitory process have been described (Eccles, 1969a). In postsynaptic inhibition, the inhibitory transmitter causes an increased permeability of the neuronal membrane to potassium or chloride ions. This results in an increase in the resting potential of the cell (hyperpolarization) which thereby becomes stabilized and unable to discharge impulses as a result of depressed excitability. In presynaptic inhibition, impulses in the inhibitory fibres interrupt the arrival of impulses in the fine terminals of excitatory fibres and reduce the excitatory drive of a neuron. These two mechanisms can be differentiated pharmacologically; postsynaptic inhibition is blocked by strychnine, whereas presynaptic inhibition is blocked by picrotoxin (Eccles, 1964).

It appears that whether neurons produce excitation or inhibition at any particular synapses is perhaps better determined by the precise nature of the chemical phenomena and events at the presynaptic fibres and the postsynaptic cell. Otherwise, more complex arrange-

ments of nerve impulses requiring more highly structured systems would have to be assembled to achieve similar results.

Whereas peripheral neuroeffectors, like the neuromuscular junction, operate in a pulsatile manner, the usual activity in the CNS consists of frequency-coded patterns of activity integrated over long periods of time. Thus, the requirements and disposal of transmitters in the central milieu may require different mechanisms and criteria. Werman (1966) has discussed the established criteria and proposes many pertinent arguments for consideration in this context. Grundfest (1964) has also reviewed the field and warns of the many possible sites of action for transmitter agents. Extracellular diffusion could provide an adequate mechanism for termination of transmitter activity in the CNS (Eccles and Jaeger, 1958). The recent histomorphological studies of De Robertis *et al.*, (1969) raise the chance that more than one type of transmitter substance may be released from a presynaptic terminal. Thus, the separation of the individual actions for the identification of neurotransmitters would become dependent on very small differences.

The CNS as a result of its highly developed circuitry could terminate transmitter activity by a time-locked sequence of recurrent collateral inhibitory pathways. The initial effects of the excitatory transmitter are mediated to an inhibitory cell such that it in turn inhibits the originally excited cell after a fixed latency, i.e. a negative feedback loop as described for the motoneuron-Renshaw cell complex in the spinal cord (Eccles *et al.*, 1954).

The necessity for employing well-defined and restricted

techniques has resulted in increased popularity of the microiontophoretic technique (Curtis, 1964; Krnjevic, 1964; Salmoiraghi and Bloom, 1964). The local application of minute amounts of charged substances from very small glass capillaries or microelectrodes onto central neurons has become a popular pursuit. Although it is now feasible to apply some excitatory or inhibitory agents extracellularly there are still many uncertainties concerning the dosage and precise location of the microelectrode tip (Werman, 1966). Extracellular recording only measures firing rate, and does not discriminate between synaptic and non-synaptic events. Excitatory substances in sufficient concentration may produce apparent inhibition by depolarization blockade, which may be preceded by increased firing before blockade. Thus, the concomitant recording of intracellular potentials helps to distinquish these effects on the basis of membrane potential and resistance changes.

Perhaps the greatest obstacle to microelectrode studies are geometry and sampling. This raises important questions as to the extent and volume of iontophoresed agent, and at what concentration it reaches active sites. It is virtually impossible to get accurate estimates of the distance to the synaptic area from the recording electrodes. Eccles (1964) has warned of the possible existence of synaptic barriers which could prevent the accessibility of locally applied substances.

Regardless of the techniques used to study the pharmacology of central neurons there are many problems encountered when attempting to resolve whether a substance acts directly on the post-synaptic membrane. It is possible that the substance may initiate events in

presynaptic elements (axons or terminals), leading to subsequent release of a dissimilar transmitter substance. In the central mesh the application of substances could result in a given sphere of activity, rather than being restricted to a single axon. This could result in the release of several types of transmitters.

On the basis of their iontophoretic studies, Bradley and Wolstencroft (1965) believe that there is sufficient circumstantial evidence for suggesting that acetylcholine, noradrenaline and serotonin are central transmitters. Their findings suggest that if neural transmission is related to chemical sensitivity, then it is likely that both cholinergic and adrenergic fibres may terminate on the same neuron. This arrangement is in agreement with that proposed earlier by Feldberg and Vogt (1948). To these authors it appeared that at certain levels of the CNS the cholinergic and adrenergic systems maintain a physiological balance by individually exerting mutually antagonistic effects. Bradley and Wolstencroft (1965) discovered that it was possible for either type of ending to produce either excitatory or inhibitory effects in response to specific agonists.

A. Acetylcholine

Many authors (Florey, 1961; Hebb, 1963; Koelle, 1963) have postulated acetylcholine (ACh) as a central neurotransmitter, even though only a fraction of the total cerebral neurons can be thought of as cholinergic (Feldberg and Vogt, 1948). There is good correlation between the levels of ACh, its synthesizing enzyme choline acetyltransferase (ChAc) and its catabolic enzyme acetylcholinesterase (AChE)

within the cerebral cortex, hippocampus, caudate nucleus, thalamus, spinal cord and cerebellum (Hebb, 1963). When nerve endings are isolated from brain tissue (De Robertis, 1967; Michaelson, 1967) ACh is found as a component of synaptic vesicles, ChAc as a component of cytoplasm and AChE bound to membranes. It is not yet clear whether ACh is definitely present in the nerve terminals of a particular central pathway. Shute and Lewis (1967) have reported evidence that some brain stem reticular and tegmental nuclei give rise to AChE-containing fibres which project extensively to cortical and subcortical structures. Undercutting of suprasylvian or pericruciate cortex reduces markedly the levels of ChAc and AChE (Hebb *et al.*, 1963).

Acetylcholine appears to fulfill a transmitter function at the collaterals of motor axons and the Renshaw cells of the anterior horn of the spinal cord, as well as in certain cortical, thalamic, hippocampal areas, and possibly in the caudate nucleus (Eccles, 1964; McLennan, 1970). Crossland in his review (1967) estimates that 15% of the cells excited by ACh in the cerebral cortex, are predominantly muscarinic in type. This author noted that some cortical cells are depressed by ACh. Powerful cholinergic neurons are also believed to be present in the medullary reticular formation (Kanai and Szerb, 1965).

Armitage and Hall (1968) have reported, based on experiments with gross electrodes, that there does not appear to be a significant component of nicotinic receptors in cerebral cortex. This is in accord with the report of Randic *et al.* (1964), who studied the depression of cortical neurons by ACh. This inhibitory action was prevented by atropine.

a. Central effects following systemic administration of cholinergics

There is no lack of data concerning the effects of drugs on the electrical activity of the brain as indicated in a review by Toman and Davis (1949). Particular attention has centred on the possible role of acetylcholine as a central neurohumoural transmitter. Early workers (Bremer and Chatonnet, 1949) injected acetylcholine and physostigmine into post-anaesthetized animals and found that the cholinergic drugs reduced the voltage and increased the frequency of electrocortical activity. These effects could be blocked by pretreatment with atropine sulfate.

Other workers (Brenner and Merritt, 1942; Chatfield and Dempsey, 1942) have applied cholinergic drugs to the surface of exposed cerebral cortex to circumvent some of the difficulties attendant with the systemic administration of these drugs. It was necessary to pretreat animals with anticholinesterases and the usual response consisted of spike discharges. Atropine administered intravenously did not antagonize this effect.

Similar results have been obtained by Cooke and Sherwood (1954) in decerebrate animals in which the cholinergic drugs were injected intraventricularly. In their experiments atropine depressed the high voltage discharges. Funderburk and Case (1951) have been able to show that such responses can be obtained from the cerebral cortex with a variety of agents having little in common, but ACh apparently has unique effects.

b. Central cholinergic pathways

The most specific histochemical method for demonstrating the distribution of cholinergic structures makes use of the presence of AChE (see review by Eranko, 1967). Shute and Lewis, (1967) have used the AChE-staining technique to extensively map cholinergic tracts in the CNS. A great number of the neurons and their processes were found to contain intermediate concentrations of AChE. Crawford *et al.* (1966) have cautioned that the presence of AChE may not necessarily indicate the existence of cholinergic neurons, since they have demonstrated that the mossy fibre-granule cell synapses of the cerebellar cortex are non-cholinergic in spite of considerable amounts of AChE in presynaptic terminals. Koelle (1969) feels that this is good evidence that ACh is involved in the release of other agents which serve as the actual excitatory or inhibitory transmitters. To reinforce this theory, Koelle mentions that ACh applied microiontophoretically to cortical neurons has a slow onset of action (Crawford and Curtis, 1966).

The above experimental observations and others (Kanai and Szerb, 1965) have led to the proposal for the presence of a reticulocortical cholinergic pathway originating from reticular and tegmental nuclei, and other forebrain structures and projecting rostrally and widely into the cortex. This pathway would be associated with arousal mechanisms. It is apparent that this system is distributed to most cortical and subcortical structures and that it might just possibly be identical to the ascending reticular activating system (Krnjevic and Silver, 1965; Shute and Lewis, 1967; Yamamoto and Domino, 1967).

A more specific thalamocortical pathway, responsible for repetitive afterdischarges has also been shown to be cholinergic (Brownlee and Mitchell, 1968; Collier and Mitchell, 1967). This latter pathway is suggested by these authors to be distinct from the direct short-latency thalamocortical fibres. This proposal is not meant to be restrictive, and does not intend to exclude intracortical cholinergic neurons from contributing to cortical ACh release and repetitive afterdischarges.

Krnjevic and Silver (1965) have demonstrated AChE-containing fibres located superficially in layer I of the cerebral cortex, but the majority occur deeply in relation to the pyramidal cells of layer V and the polymorph cells of layer VI. Some of these fibres originate from adjacent or more distant areas of the cortex; others, of subcortical origin, originate from the septal region, the striatum and the midbrain reticular formation.

c. Central release of acetylcholine

The collecting of ACh released at the surface of the cerebral cortex under various experimental conditions (MacIntosh and Oborin, 1953) gives little information regarding the actual site of release but it does provide some indication of a transmitter function for ACh in the CNS. Some evidence for a role of ACh in seizures has been suggested by Celesia and Jasper (1966) from measurements of ACh diffusing into fluid perfusing the cortical surface. These authors found that there is increased release of ACh during convulsive activity (Celesia and Jasper, 1966). The change in ACh presumably signifies increased transmission as part of the convulsive response, rather than

a triggering mechanism. ACh release is reduced by anaesthetics (Phillis, 1968) with a concomitant increase in total cortical ACh content. The release rate of ACh is higher in the presence of EEG arousal than when more synchronized rhythms are present in the EEG (Sie *et al.*, 1965; Celesia and Jasper, 1966).

The cortical release rate of ACh is enhanced by direct cortical stimulation (Hemsworth and Mitchell, 1968) and by stimulation of subcortical structures (Celesia and Jasper, 1966; Szerb, 1967). Stimulation of a specific afferent pathway increases the rate of ACh release from primary cortical receiving areas and other areas of the cortex (Phillis, 1968).

Whenever atropine or scopolamine are administered systemically, the cortical content of ACh decreases and increased amounts of ACh can be collected at the cortical surface (Giarman and Pepeu, 1964; Szerb, 1964). In atropinized animals stimulation of the mesencephalic reticular formation fails to desynchronize the EEG (Longo, 1962). Stimulation of the reticular formation enhances the rate of release of ACh from the cortex (Bartolini and Pepeu, 1967; Celesia and Jasper, 1966; Polak, 1965). Similar results have been observed after the topical application of atropine (Bartolini and Pepeu, 1967). If it is assumed that atropine blocks the cholinergic activating system, it is somewhat difficult to satisfactorily explain the enhanced release of ACh as a result of treatment with atropine. Although the competition of atropine with ACh for cholinergic receptors would account for some of the ACh collected, it would appear that atropine has other effects in these experiments, perhaps on release mechanisms.

Other experiments have attempted to show that the ACh released from the cortex originates from synaptic terminals, since reducing the calcium content in the fluid bathing the cerebral cortex results in a reduction of the release rate (Randic and Padjen, 1967; Hemsworth and Mitchell, 1968). Addition of hemicholinium-3 (an inhibitor of ACh synthesis) to normal bathing fluid decreases both the release of ACh (Szerb, 1965) and the cortical ACh content (Dren and Domino, 1968).

Undercutting the cerebral cortex but leaving the pial circulation intact virtually abolishes ACh release (Bartolini and Pepeu, 1967; Celesia and Jasper, 1966; Sie et al., 1965). However, according to Szerb (1967) the resting output is not decreased by this procedure, but the usual increase in ACh output due to reticular formation stimulation is reduced. Simply cutting around an area of cortex had no effect. The spontaneous release from undercut cortical slabs, although very low, can be enhanced to approximately one-quarter that of intact cortex when the slab is stimulated directly (Collier and Mitchell, 1966). This output is reasonable in view of the fact that undercut cortex retains only approximately 17 per cent of its normal level of ChAc (Hebb et al., 1963).

Under conditions of barbiturate anaesthesia in cats (Dial), Dudar and Szerb (1969) have reported that ACh output from acutely-undercut cortex is about 40 percent of control. Addition of low concentrations of atropine (1 μg/ml) to the collecting cylinder increases ACh output from intact and isolated cortex fourfold, whereas direct stimulation results in a twofold increase in each case. Collier and Mitchell (1967) performed similar experiments on anaesthetized rabbits

and found qualitatively similar results following direct stimulation of chronically undercut cortex, although the differences between intact and partially-isolated cortex were greater than those reported by Dudar and Szerb (1969). In his doctoral dissertation Sastry (1956) reports that the ACh content of chronically neuronally isolated cortex is reduced to 17-25 percent of control samples, which was suggested to be due to degeneration of association neurons in layers II and III of the cortex. Stimulation of chronically, but not acutely-isolated cortex results in a significant increase in ACh content, according to conclusions by Sastry (1956), but not according to his table of results.

MacIntosh and Oborin (1953), and also Collier and Mitchell (1967) report considerably reduced ACh release from chronically undercut cortex at a time when electrical activity is apparently abolished. However, recording the ECoG in these experiments may be difficult because of thick leptomeninges on the surface of the cortex as a result of the surgery. A better explanation for the lower ACh output is the diminution of ChAc (Hebb _et_ _al_., 1963) following chronic isolation of cerebral cortex.

d. _Injections directly into brain tissue_

Cortical and subcortical ACh receptors have been studied by administration of cholinomimetics and their antagonists intraventricularly (Feldberg and Fleischhauer, 1965) or by direct injection into cerebral tissue (Marczynski, 1967). Interpretations arising from these indirect experiments should be considered with extreme caution.

e. Microiontophoretic application of cholinergic drugs

A number of studies employing microelectrophoretic or systemic administration of cholinergic drugs have shown that some central neurons are excited while others are depressed by ACh (Bradley and Wolstencroft, 1965; Curtis and Crawford, 1969). Administration of specific antagonists further suggests that both nicotinic and muscarinic receptors may be present.

The cortical cells most consistently excited by the electrophoretic administration of ACh are the large deep pyramidal cells (Krnjevic and Phillis, 1963; Spehlmann, 1963; Crawford and Curtis, 1966). These authors have reported that the excitatory effect of ACh is slow in onset, and maximum rates of firing are obtained after a latent period of 10-40 sec, during which spontaneous activity is often temporarily depressed. Neuronal firing persists for approximately 30 sec after termination of ACh administration. As these cells are also excited by acetyl-β-methylcholine and muscarine, and their response is blocked by atropine, Krnjevic and Phillis (1963a) have classified this cholinergic mechanism as being of the muscarinic type found in the periphery. Similar muscarinic responses have been observed throughout the brain (Legge et al., 1966). Many neurons excited by ACh exhibit prolonged, repetitive, afterdischarges following stimulation of peripheral sensory nerves or specific thalamic nuclei (Krnjevic and Phillis, 1963). Atropine administered systemically blocks this repetitive activity (Krnjevic and Phillis, 1963a).

Acetylcholine depresses some cortical neurons, particularly those located in layers II, III and IV of the sensorimotor cortex

(Randic *et al.*, 1964; Phillis and York, 1967). Phillis and York (1967; 1968a) have observed that the depression by ACh resembles the activation of a long-lasting inhibitory synaptic mechanism which may be evoked by stimulation of axon collaterals of pyramidal tract fibres, of the lateral hypothalamus, or of the cortical surface. These depressant effects can be prevented by strychnine, atropine, or dihydro-β-erythroidine. Krnjevic *et al.*, (1966) were unable to inhibit cortical inhibitory phenomena with strychnine in their studies.

It is not clear whether the depressant effects of ACh on cortical neurons represent postsynaptic inhibition or depolarization of excitatory terminals initiating presynaptic inhibition. Probably a significant contribution results indirectly from ACh exciting an inhibitory interneuron.

The effects of atropine on spikes in the EEG induced by topically applied curare, penicillin, or ACh have been studied by Funderburk and Case (1951) in cats and monkeys. The spikes evoked by ACh differ from those evoked by topical curare or penicillin. Atropine enhances spiking by the latter agents, but abolishes or decreases that due to ACh. Curiously, physostigmine had the opposite effect, i.e. decreasing the spiking elicited by curare or penicillin. These findings suggest that apparently there are several mechanisms for producing convulsive discharges on the cortex. The observation with eserine that curare or penicillin-induced spikes are antagonized supports an earlier study by Williams (1941) that at low doses physostigmine decreases, and in large doses increases, *petit mal* activity in the EEG of epileptic patients.

f. Involvement of cholinergic mechanisms in seizure activity

It is still not possible to understand the role of ACh in the mammalian central nervous system in spite of the large amount of impressive evidence available. It seems quite reasonable to assume that ACh does have a role at both excitatory and inhibitory central synapses. It is generally observed that following the systemic administration or topical cortical application of ACh, *grand mal* seizures appear peripherally or in the EEG (Hyde *et al.*, 1949; Feldberg and Sherwood, 1954). Convulsant drugs such as metrazol increase the levels of ACh released from the brain, which results in a decrease in the residual ACh in brain tissues (Giarman and Pepeu, 1962; Beleslin *et al.*, 1965). However, there is no clear relation between ACh release and seizure discharges. Similarly, agents such as anaesthetics inhibit this release of ACh, but this does not appear to be related to anticonvulsant effects (Beleslin and Polak, 1965). Atropinic drugs have been reported to antagonize ACh-induced seizures, but they are without effect against convulsions induced by other experimental procedures (Zablocka, 1963). Atropine has been reported to have both anticonvulsant (Sie, 1968) and convulsant actions (Minvielle *et al.*, 1954; Bernard *et al.*, 1968).

B. Noradrenaline

Noradrenaline is present throughout the central nervous system in variable concentrations (Vogt, 1954). The metabolism and role of noradrenaline (NA) and adrenaline (A) in the brain have been reviewed (Bloom and Giarman, 1968) and it is apparent that there is

usually a greater content of noradrenaline in the gray matter. The highest concentrations of catecholamines (CA) are found in the hypothalamus, dorsal pons and medulla oblongata (Vogt, 1954). Low concentrations of noradrenaline are found in the hippocampus, cerebral cortex, cerebellum, and spinal cord (De Robertis, 1966). Hornykiewicz (1966) has discussed the significance and function of the dopamine found in the central nervous system. The synthesis, localization and metabolic inactivation of NA in the CNS have been discussed by Glowinski and Baldessarini (1966).

a. Central effects following systemically administered adrenergics

Noradrenaline (NA) and adrenaline (A) have been injected into experimental animals (Minz and Domino, 1953) to study their electrocortical effects. However, in view of their known inability to penetrate into cerebral tissues from the circulation, it would appear that it is more likely that the effects observed are not a result of direct adrenergic actions. To circumvent this problem, it is customary to administer sympathomimetic drugs such as amphetamine, that are supposed to have direct central actions. Bradley and Elkes (1957) have demonstrated that in the conscious animal there is a close relationship between a long-lasting adrenergic alerting or activation response in the EEG and behavioural excitment. In acute experiments these authors were able to show that amphetamine did not affect the EEG after transection of the upper region of the spinal cord, i.e. it acts predominantly on higher brain stem neurons. Longo (1962) has conducted extensive studies on experimental animals with adrenergic drugs and recorded with gross electrodes in various parts of the CNS and has commented that the

variability in the results of acute experiments is due to the variability in the level of the midbrain section.

b. Histochemical evidence for central adrenergic neurons

Strong evidence for adrenergic transmission in the CNS has been demonstrated by several groups of workers (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Hillarp *et al.*, 1966) by means of a highly sensitive formaldehyde vapour histofluorescence technique, that shows fibre tracts containing monoamines or catecholamines. These neurons are considered to constitute a phylogenetically old system, as indicated by their localization in the cell bodies of the lower brain stem, in the pons and medulla, especially within the ventrolateral portion of the medullary reticular formation. These cells in turn give rise to two sets of bulbospinal fibres and to a set of ascending fibres which distribute widely via the medial forebrain bundle. Fibres containing DA arise mainly from two groups of neurons in the ventral portion of the mesencephalon: the pars compacta of the substantia nigra, projecting to the caudate nucleus and the putamen, and a group of cells dorsal to the interpeduncular nucleus which sends some axons to the hypothalamus (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Hillarp *et al.*, 1966).

The monoamine-containing neurons, probably have important functions in the CNS, regulating and modulating functions such as spinal reflexes (Engberg and Ryall, 1966), extrapyramidal functions (Anden *et al.*, 1966), sleep and alertness (Jouvet *et al.*, 1967), aggressiveness (Fuxe and Gunne, 1964), thermoregulation (Feldberg *et al.*, 1966), sexual behaviour (Myerson, 1964) and endocrine functions (Fuxe and Hokfelt, 1967). There is indirect evidence indicating that

derangements in the metabolism of central biogenic amines might be involved in certain mental disorders (Osmond et al., 1952; Fischer et al., 1961; Schildkraut and Kety, 1967; among others).

The characteristics and the differences between catecholamine-containing (NA and DA) and serotonin (5-HT)-containing neurons in the rat have been discussed extensively by Hillarp et al.,(1966). Whereas monoamine-containing cell bodies are present in localized areas, the monoamine nerve terminals are found in all parts of the brain, from the spinal cord to the cerebral cortex (Dahlstrom and Fuxe, 1965), in varying concentrations. The majority of 5-HT terminals appear to be present in most areas receiving CA terminals, except for the cerebellum which contains very little 5-HT. The hippocampal cortex and neocortex receive scattered NA and 5-HT terminals of medium to low density in all layers (Fuxe, 1965).

There are, in addition, widely distributed systems of fibres containing NA, DA and 5-HT which originate, for the most part, from brain-stem nuclei. With the exception of the substantia nigra, these nuclei are not readily accessible to investigation by electrical stimulation. Moreover, analysis of evoked activity is complicated by slow conduction velocities and temporal dispersion of volleys (Curtis, 1969).

Fuxe and his associates (1968) have demonstrated that the dense varicose-like structures apparently located in the presynaptic elements contain comparitively high concentrations of monoamines, especially NA and 5-HT. Furthermore, these investigators were able to show that these structures are present in nearly all cortical areas. Negligible amounts of DA were detected in these same cortical areas.

These authors proposed that the majority of the monoamine terminals in the neocortex make axo-dendritic contacts in the more superficial layers.

It appears that in the brain the major metabolites of catecholamines are products of reaction with catechol-O-methyl transferase (Glowinski et al., 1965), whereas the role of monoamine oxidase is the regulation of the intra-neuronal level of catecholamine. There have been relatively few attempts to demonstrate the release of catecholamines during stimulation of nervous structures owing to the small recovery of these substances (Chase and Kopin, 1968).

In his most recent monograph, McLennan (1970) concludes that, in the CNS, biogenic amines may have a more physiological role as primary regulators of neuronal excitability by local hormone action, rather than a strictly parmacological role as synaptic transmitters. These effects are none the less powerful and functional. At the moment the available evidence is not sufficiently strong to allow formulation of a unified concept bearing on their synaptic functions.

c. Microiontophoretic application of adrenergic drugs

Microiontophoretic application of monoamine transmitter candidates to single cells resulted in both excitatory and inhibitory effects in response to NA, DA or 5-HT (Rothballer, 1959; Salmoiraghi, 1966; Roberts and Straughan, 1967; Bloom and Giarman, 1968; Curtis and Crawford, 1969).

Salmoiraghi (1966) in his review on central adrenergic synapses suggests that the inhibitory actions of NA and 5-HT on neurons in the olfactory bulb are indicative of a transmitter function for these amines in this region, since these responses can be specifically blocked

with some antagonist compounds, although it is usually difficult to specifically antagonize the depressant effects of monoamines. Strychnine may (Tebecis, 1967) or may not (Biscoe and Curtis, 1966) prevent the inhibitory effects of catecholamines on neurons.

There are comparatively few studies reporting the excitatory effects of monoamines on neurons (Roberts and Straughan, 1967; Satinsky, 1967; Yamamoto, 1967; Johnson et al., 1969). Although it was shown that beta-receptor blockade, but not alpha-receptor blockade prevented microelectrode excitation of neurons in Deiters nucleus by noradrenaline, the synaptic role of noradrenaline at central neurons is open to question. Yamamoto (1967) demonstrated that pharmacological blockade did not reduce the excitation of neurons following afferent nerve stimulation. This author also demonstrated a distinct cholinergic component involved in excitation of Deiters nucleus neurons, which was not prevented by atropine upon afferent stimulation.

Thus it should be considered that following application of these substances both excitatory and depressant effects are the result. The complex cytoarchitecture of the cortex would provide ample scope for such responses. The problem now becomes to discover which actions of the amines are exerted at subsynaptic receptive structures, and which ones are on non-synaptic structures. The fact that many neurons depressed by 5-hydroxytryptamine are affected in a similar manner by noradrenaline suggests that in all likelihood, in some structures, these amines may be acting on the same sites to produce the same response. There is no clear evidence bearing one way or the other on this point.

d. Involvement of adrenergic mechanisms in seizure activity

A large part of the released catecholamines is probably re-accumulated into the nerve terminal by an active uptake mechanism found in axons, which is believed to increase efficiency at adrenergic synapses (Fuxe and Ungerstedt, 1967). Tricyclic antidepressants seem to block this mechanism (Hamberger, 1967), and according to Lehmann (1967) these agents possess some anticonvulsant activity. Anden *et al.* (1969) have reviewed central monoamine mechanisms and pharmacology in detail.

Recent studies by Haggendal (1968) on rats exposed to hyperbaric oxygen, showed that the resultant epileptiform convulsions could be influenced by drugs acting on central monoamine neurons. Drugs suspected of interfering with monoamine transmission (e.g. reserpine or chlorpromazine) may elicit seizures in patients with latent epilepsy and aggravate the condition in epileptic patients (Hollister, 1964). Thus, it may be speculated that impaired monoamine transmission may decrease the irritability threshold, normally maintained at a higher level by the catecholamine and serotonin nerve terminals (Carlsson, 1965).

Swinyard and his associates (1964) have shown that increasing the brain level of catecholamine reduces seizure susceptibility. Intravenous adrenaline and noradrenaline in low doses elevated thresholds for Metrazol convulsions, but only adrenaline increased the threshold necessary to provoke electrically-induced seizures. On the other hand, Minz and Domino (1953) have reported that these two catecholamines, under the conditions in their experiments lower the convulsive threshold and

prolong the duration of electrically-induced seizure discharges.

Some interesting observations have been reported by White and Daigneault (1959) and Longo (1962) who mention that anticholinergics reverse the EEG activation caused by amphetamine, but do not influence the behavioural arousal. Amphetamine, has anticonvulsant properties in rats (Mennear and Rudzik, 1966) but not in mice (Alexander and Weaver, 1954). Merritt and Putnam (1938a) were not able to observe these effects of amphetamine in cats but Livingston _et al._, (1948) have reported on the anti-epileptic effectiveness of amphetamine in _petit mal_.

The results from experiments utilizing adrenergic blocking drugs are far from clear. Very little information is available concerning the central distribution of the available compounds. Since most of these drugs were developed in conjunction with studies on the peripheral sympathetic nervous system this uncertainty is understandable. Rudzik and Mennear (1966a) have shown significant increases in electroshock seizures following the administration of _alpha_ adrenergic blocking agents. Experiments with _beta_ adrenergic blocking drugs are more difficult to assess since many of the compounds possess local anaesthetic and quinidine-like effects (Goodman and Gilman, 1970).

The enzymes catecholamine-O-methyltransferase (COMT) and monoamine oxidase (MAO) which are involved in the metabolic inactivation of the catecholamines released at catecholamine nerve terminals have been the subject of manipulation in an effort to assess the influence of free catecholamines at central structures on seizure susceptibility (Lehmann, 1967; Prockop _et al._, 1959). These authors found

that increasing endogenous catecholamines by inhibition of inactivating enzymes conferred anticonvulsant effects. Monoamine depleting agents (reserpine) shortened significantly, the latency for convulsions, while MAO inhibitors significantly lengthened the time for onset of convulsions (Haggendal, 1968).

e. Dopaminergic mechanisms

Dopamine is present in highest concentration in the caudate nucleus, putamen and globus pallidus, whereas concentrations in the hypothalamus are moderate and there is little elsewhere in the central nervous system (Carlsson *et al.*, 1958). This distribution suggests that alterations in dopamine levels are involved in motor disturbances of the central nervous system other than epilepsy, although it should be kept in mind that treatments affecting the level of noradrenaline usually alter dopamine levels in the same direction.

When given alone in large doses L-DOPA does not exert any effect on seizure threshold (Prockop *et al.*, 1959). On the other hand, when monoamine oxidase is inhibited previously, small doses of L-DOPA have anticonvulsant activity (Pfeifer and Galambos, 1967). The fact that dopamine levels show a marked increase led these authors to propose that this monoamine plays a certain role in anticonvulsant activity. Of course, this dopamine may be finding its way into many other sites, e.g. serotonergic and noradrenergic terminals.

Inhibitors of DOPA-decarboxylase have been reported to increase seizure susceptibility (De Schaepdryver *et al.*, 1962; Rudzik and Mennear, 1966). However, the inhibitory agents employed, α-methyl-DOPA and α-methyl-m-tyrosine, decreased brain levels of dopamine, nor-

adrenaline and serotonin. Moreover, the picture becomes very complex when it is appreciated that the inhibitors used, enter the normal metabolic pathway as slightly modified compounds. More selective depletion of catecholamines is achieved with the tyrosine hydroxylase inhibitor α-methyltyrosine (Rudzik and Mennear, 1966a). This treatment results in increased susceptibility to experimental seizures.

C. Serotonin

Following the finding of serotonin (5-hydroxytryptamine, 5-HT) localized in discrete areas of brain (Bogdanski *et al.*, 1957) there has been discussion in the literature as to the possible central pharmacological roles of this possible synaptic transmitter (Gaddum, 1957; Costa, 1960; Garattini and Valzelli, 1965). Since 5-HT does not readily penetrate into the central nervous system from the blood (Costa and Aprison, 1958) investigators have resorted to the systemic administration of the permeable precursor 5-hydroxytryptophane (5-HTP) or related compounds (Udenfriend *et al.*, 1957).

Systemic injection of 5-HTP into experimental animals results in marked behavioural changes (Costa, 1960), usually a brief period of excitment followed by a prolonged depression (Vane *et al.*, 1961). Direct application of 5-HT into discrete regions of the CNS provokes behavioural and EEG arousal (desynchronization) (Yamaguchi *et al.*, 1964). Intraventricular injection of 5-HT results in ataxia and sleep (Feldberg and Sherwood, 1954). Koella and his collaborators (1960; 1966) have discussed the pitfalls involved in interpreting the results of conflicting experiments dealing with the central transmitter role of serotonin. Topically applied serotonin inhibits direct cortical

responses (Ochs *et al.*, 1960).

a. Systemically administered serotonin

Longo (1962) has administered 5-HTP to experimental animals and found that high doses cause a significant increase in brain levels of 5-HT within 1 hour, together with generalized diminution of voltage in the EEG. A series of uninterrupted spikes may appear in the recordings obtained from subcortical structures. There is peripheral excitation with tremors and gross body movements.

b. Histochemical identification of serotonin neurons

With the introduction of the histofluorescence method developed by Hillarp and his co-workers (1966) (see also review by Corrodi and Johnsson, 1967), it was found that 5-HT was localized within cell bodies, axons and nerve terminals of specific central neurons. The nerve terminals of these neurons had about the same appearance as those of the catecholamine (CA) containing neurons. The highest concentrations of 5-HT were found in the hypothalamus and midbrain (Udenfriend *et al.*, 1957).

The 5-HT neurons lie mainly in the lower brain stem, almost exclusively in the midline raphe complex (Dahlstrom and Fuxe, 1964). These serotonergic neurons send axons to forebrain structures via the medial forebrain bundle. The 5-HT neurons from these areas also seem to innervate the neostriatum and paleostriatum. The fibres to the neocortex ascend from the median forebrain bundle into the tractus diagonals (Dahlstrom, 1969), passing through the white matter and probably also partly within the gray matter, and sending terminals up into the cortex. Lesions in the medial forebrain bundle in the lateral hypothalamus cause a significant decrease in the 5-HT content of the

cerebral cortex and associated cerebral structures (Moore *et al.*, 1965). Considerable amounts of 5-HT are contained in the hypothalamus and caudate nucleus and since fluorescence microscopy does not demonstrate fibres in these structures, McLennan (1970) suggests the substance may be present in very fine terminals.

c. Microiontophoretic application of serotonin

Excitation and depression of cell-firing have been observed when 5-HT is administered microiontophoretically near cortical neurons (Krnjevic and Phillis, 1963b; Roberts and Straughan, 1967). Roberts and Straughan (1967) have shown that lysergic acid diethylamide (LSD), a 5-HT blocker at peripheral sites, blocks these excitant effects of 5-HT on cortical neurons. Krnjevic and Phillis (1963b) reported that 5-HT also depresses cell firing induced by glutamate and acetylcholine. In post-anaesthetized *encephale-isole* animals, Roberts and Straughan (1967) have reported that excitation was the usual response of cortical neurons to microiontophoretically applied 5-HT, whereas Crawford and Curtis (1966) made their observations on *cerveau isole* animals and reported that neuron firing was depressed. Roberts and Straughan (1967) by intermittent and prolonged administration of LSD or methysergide selectively blocked 5-HT elicited excitation of cortical neurons. Phillis and York (1967a) performed similar experiments on anaesthetized cats and reported that the predominant effect of 5-HT was depression of 5-HT neurons. Roberts and Straughan (1967) suggested that the creatine sulfate salt used, mid-brain lesions and/or anaesthetics may be responsible for the observed depressant effects of 5-HT.

d. Involvement of serotonin in seizure activity

Progress in this area is further hindered by a lack of specific antagonist drugs capable of blocking the central effects of serotonin. This is due in part to the vague nature of serotonin receptors. Prockop and his associates (1959) have observed that the 5-HT precursor, 5-HTP has inhibitory effects on experimental seizures. LSD appears to have no effects on experimental seizures (Lessin and Parkes, 1959). At an age which corresponds with the time of development of maximal seizure susceptibility, the serotonin and also noradrenaline content of brains of mice susceptible to audiogenic seizures is much lower (Schlesinger *et al.*, 1965).

Monoamine oxidase inhibitors have been shown to raise brain levels of serotonin, noradrenaline and dopamine (Brodie *et al.*, 1959), while reserpine lowers their concentration in the brain (Brodie *et al.*, 1956). Reserpine has also been shown to possess convulsant properties (Chen and Bohner, 1956). On the other hand MAO inhibitors block the tonic extensor component of electrical seizures in rats (Prockop *et al.*, 1959), though these agents are less effective against metrazol-induced seizures. The convulsant effect of reserpine is antagonized by the MAO inhibitors (Lessin and Parkes, 1959).

The close relationship of serotonin neurons to catecholamine neurons has, no doubt, prevented any marked degree of success in elucidating the role of this neurohumoural candidate in central seizure phenomena. Treatments altering catecholamine levels also alter 5-HT levels (Carlsson, 1965). Thus far, it appears that MAO is the enzyme metabolically inactivating a major portion of the released 5-HT

(Blaschko and Levine, 1966). The selective increase in cerebral 5-HT resulting from administration of 5-HTP (Bogdanski et al., 1958) resulted in slightly decreased seizure susceptibility, which can be further enhanced by the simultaneous inhibition of monoamine oxidase (Prockop et al., 1959). It remains to be shown whether the large doses of 5-HTP have any indirect effects on adrenergic neurons.

However, in spite of these circumstantial findings, it must be emphasized that it is still uncertain whether the effects of reserpine or MAO inhibitors on seizure activity are due to a modification in the level brain amines. It has been claimed earlier that serotonin itself has some effect against seizures induced in mice by high oxygen pressures (Laborit et al., 1957). However, the results of clinical trials with monoamine oxidase inhibitors in epilepsy have not proved to be too promising (Perlstein, 1959). Garattini and Valzelli (1965) in their monograph on serotonin have discussed many investigations in which 5-HT metabolism was altered to observe resultant effects on convulsant and anticonvulsant drugs. Although the results provide interesting reading, little light is thrown on the role of 5-HT in seizure mechanisms.

An interesting approach to the problem has been the use of para-chloroamphetamine and related compounds to stimulate the release of serotonin in the brain (Fuller et al., 1965). There is no appreciable concomitant modification of central levels of NA and DA, and significant reductions in the severity of audiogenic and Metrazol-induced seizures in mice have been reported (Lehmann, 1967; Pfeifer and Galambos, 1967a). It is still too early to decide whether these

effects of this unique experimental drug are direct or indirect.

Perhaps the most promising approach to elucidating the involvement of 5-HT in seizure phenomena will be provided by *para*-chlorophenylalanine, a compound which has been shown to specifically deplete serotonin in cerebral tissues (Koe and Weissman, 1967). This depleting agent apparently acts by inhibiting tryptophan hydroxylase, the enzyme responsible for the synthesis of 5-HTP *in vivo*.

In spite of the apparent relationship of monoamines to experimental seizures discussed above, any conclusions concerning their function via central synaptic mechanisms are, at best, tentative and uncertain. Definitive studies relating synaptic activity and membrane events to local electrical changes are required before the specific transmitter role of the substances postulated to function as chemical mediators at central synapses is indicated.

D. Gamma-Aminobutyric Acid (GABA)

Currently, there is much discussion concerning possible transmitter roles for cerebral amino acids (Curtis, 1963; Curtis and Johnston, 1968). Applied topically, the acidic amino acids, glutamic or aspartic, excite neurons, whereas neutral amino acids, *gamma*-aminobutyric acid (GABA) or glycine, depress neurons (Curtis and Watkins, 1965). GABA occurs in large amounts and appears to be found only in the central nervous system (Roberts and Eidelberg, 1960).

Gamma-aminobutyric acid has been detected in the brain and is widely distributed in varied concentrations. Most of the GABA present in the brain seems to be bound in synaptic vesicles (Elliott and Florey, 1956; Elliott and Jasper, 1959). GABA is synthesized

directly from L-glutamic acid, and the synthesizing enzyme L-glutamic acid decarboxylase is found only in the CNS, largely in gray matter, and highly concentrated in nerve endings (Salganicoff and De Robertis, 1965). GABA appears to be an amino acid unique to the CNS, where regional differences correspond well to the density of neurons (Roberts and Kuriyama, 1968). The highest levels of GABA are found in the outer layers of the cerebral cortex (Hirsch and Robins, 1962). GABA transaminase, the enzyme which catalyzes the reversible transamination of GABA with *alpha*-ketoglutarate, is also found in the gray matter but largely in the cytoplasmic fraction (Roberts and Kuriyama, 1968). GABA is an inhibitory transmitter on some primitive structures, like the crayfish stretch receptor (Elliott and Jasper, 1959), causing an increased membrane permeability to potassium and chloride ions.

a. *Central effects following systemic and topically applied amino acids*

Evidence for the inhibitory function of GABA in the CNS includes abolition or depression of cortical surface negative responses elicited by electrical stimulation or topically-applied strychnine (Purpura *et al.*, 1957; Rech and Domino, 1960a), sometimes with enhancement of surface-positive responses. Purpura and his associates (1957) speculated that GABA blocks the excitatory synapses of superficial dendrites.

GABA is usually ineffective following systemic administration, presumably because it does not penetrate into the brain in significant amounts (Purpura *et al.*, 1958). If the so-called "blood-brain barrier" is altered locally by freezing, marked central effects are observed following intravenous administration of GABA (Berl *et al.*, 1961).

Purpura *et al*. (1958a) found that systemically administered GABA reduced the number of paroxysmal discharges arising from cortical epileptogenic foci. In a later study (Berl *et al*. 1959) it was found that in these epileptogenic sites the concentrations of glutamic acid, glutamine and glutathione, but not GABA, were reduced. In addition, supression by GABA of paroxysmal discharges from the epileptogenic sites was not necessarily associated with changed concentrations of the above compounds. Furthermore, it was found that paroxysmal discharges were unaffected by systemic administration of GABA, despite dramatic increases in concentration of this amino acid in epileptogenic sites. Elliott and van Gelder (1960) found that the administration of commonly used anti-epileptic drugs, did not alter the concentration of GABA in the brain. Baxter and Roberts (1960) have reported further evidence dissociating seizure activity from altered GABA concentration. Brain concentrations of glutamine, glutamate and *gamma*-aminobutyrate are scarcely affected *in vivo* by administration of convulsants (Gammon *et al*., 1960).

b. *Microiontophoretic studies with GABA*

Curtis and his group (1959) have applied GABA, among other amino acids, iontophoretically to the surface of central neurons. Intracellular recordings from spinal motoneurons showed no change in resting membrane potential, but excitatory and inhibitory postsynaptic potentials were both reduced, and orthodromically or antidromically evoked spike potentials were depressed or blocked. Activation of Renshaw cells by synaptic excitation, or by iontophoretic application of acetylcholine was also blocked. These results are characteristic of a non-specific depression of neuronal activity, probably by an

increase in membrane conductance, and are not necessarily characteristic of a specific inhibitory transmitter.

More recently it has been demonstrated that GABA administered extracellularly hyperpolarizes cortical neurons and increases their membrane conductance (Krnjevic and Schwartz, 1967a). Neuroglial cells and spinal cells are apparently not hyperpolarized (Krnjevic and Schwartz, 1967a). This action of GABA on cortical neurons can be reversed by increasing the membrane potential (Werman _et al._, 1968) and the observed reversal potential is similar to that of IPSP's or inhibitory potentials evoked by direct cortical stimulation (Krnjevic and Schwartz, 1967). Thus, within the limitations of these studies, GABA appears to have similar postsynaptic actions on central neurons as do synaptically released inhibitory transmitters.

It is reasonable to expect that a modification of the extracellular amino acid concentrations will exert profound effects on nerve cell excitability. The extent to which these changes are necessary before disturbances and seizures result is not presently known. When administered microiontophoretically L-glutamic acid was shown to reversibly depolarize cortical neurons (Krnjevic, 1964). Since intracellular ejection of this amino acid has little or no effect (Coombs _et al._, 1955) it is to be assumed that the depolarization results from a change in membrane properties. The possible central transmitter role of amino acids will be better established once specific antagonist compounds become available.

Glutamate, the metabolic precursor of GABA, can depolarize neurons and exert powerful excitatory effects on neurons (Curtis and Watkins, 1965). Administration of glutamate by various routes will

result in convulsive discharges, and it has been reported that these can be blocked by GABA (Hayashi, 1958). Roberts (1963) has observed that the total cerebral concentration of glutamate changes very little following inhibition of the decarboxylase or transaminase enzyme. Perhaps it is the relative ratio of glutamate to GABA that is important in terms of neuronal excitability.

c. Relation of GABA levels in brain to seizures

Many of the enzymes concerned with cerebral amino acid metabolism are pyridoxal dependent (Roberts *et al.*, 1964). Accordingly, they are inhibited by hydrazines, hydrazides and other pyridoxal antagonists which attack the decarboxylase and transaminase. Thiosemicarbazide depresses central GABA levels, presumably by inhibition of the decarboxylase, and treated animals are more susceptible to seizures (Preston, 1955). Kuriyama, Roberts and Rubinstein (1966) have reported that amino-oxyacetic acid, an inhibitor of GABA transaminase which increases GABA levels has anticonvulsant effects. Maynert and Kaji (1962) using several experimental preparations (electroshock and chemoshock) were unable to distinguish any consistent reduction in seizure latency in the presence of significantly increased brain GABA levels. Perhaps, GABA has only metabolic functions as suggested by McKhann and Tower (1961). Rech and Domino (1960a) have shown that topically applied GABA elevates thresholds for experimentally-induced seizures in the acutely-isolated cortex of the dog.

Disturbances of cerebral amino acid levels, particularly of glutamine, glutamic acid, aspartic acid and GABA have been reported to be associated with convulsive disorders (Tower, 1960; Watkins, 1968).

It is not clear, however, whether the altered levels are responsible for, or are a result of the abnormal neuronal activity. This confusion arises from the intimate involvement of these amino acids in cerebral oxidative metabolism. Probably the altered metabolism of cerebral amino acids may be the basis for a biochemical lesion which results in epilepsy. Subcellular distribution studies have not indicated specific localization of excitant or depressant amino acids in synaptic terminals isolated from cortical tissue (Mangan and Whittaker, 1966).

It has been demonstrated that pyridoxine deprivation caused by dietary deficiency or from treatment with thiosemicarbazide induces convulsions (Preston, 1955). It was later shown that these seizures in rats (Killam, 1957) were associated with a decrease in the amount of GABA in the brain, and with reduced activity of glutamic acid decarboxylase, which is dependent on pyridoxal phosphate (Killam, 1957). Although intravenous GABA seemed to antagonize seizures, it seemed that some other unknown consequence of pyridoxal inactivation was associated with the epileptic state (Killam, 1957).

It may prove to be more correct to suspect that the inhibitory action of GABA is a general one at the level of the soma and not, as claimed by Purpura *et al.* (1960) exerted exclusively at excitatory superficial axo-dendritic synapses. Thus, it may be that GABA is a general regulator of central neuronal activity. That is, a reduction in the level of GABA may lead to states favouring convulsions, whereas an increase may favour generalized anticonvulsant effects.

The total evidence for GABA seems to justify the following conclusions. Firstly, one likely function for GABA in the brain is

as an intermediate in the shunt pathway from alpha-ketoglutarate to succinic semi-aldehyde. It seems improbable that gamma-aminobutyrate is a specific inhibitory transmitter. Secondly, it is possible but not very probable, that the level of gamma-aminobutyrate plays a non-specific part in the regulation of central nervous excitation. It is further possible, but unproved, that some seizure states may be caused by an abnormality of gamma-aminobutyrate metabolism or concentration.

Finally, there is no good evidence to show that any clinically important anticonvulsant acts by influencing the metabolism, concentration or action of gamma-aminobutyrate. Gamma-aminobutyrylcholine has been detected in brain (Kewitz, 1959) but no relationship to seizures or anticonvulsant action has so far been disclosed.

E. Interaction of Seizure Mechanisms with Pharmacological Agents

There is an extreme paucity of reports discussing the relationships between the proposed neurotransmitters and the mechanisms of action of clinically useful anti-epileptic drugs. The recent demonstration by Mennear and Rudzik (1966) that the anticonvulsant activity of acetazolamide involves an alpha-adrenergic component is the first report that central synaptic mediators may be directly involved in a pharmacological mechanism for seizure control. Although this is the first direct finding in this area, it should not be disregarded, but should provide the necessary impetus for more exhaustive investigations in this crucial area. The previous discussion was restricted to those substances for which there is more impressive evidence of a central mediator function. It is possible that other substances may be demonstrated to account for the many uncertainties concerning present-day

knowledge on definitive seizure mechanisms and anticonvulsant activity. It has been reported (Bose *et al.*, 1958) that several commonly-used anti-epileptic drugs inhibit the synthesis of ACh in the brain. Richter and Crossland (1949) have reported a relationship between convulsive activity and the ACh-content of the brain. However, it is difficult to correlate anticonvulsant activity with cholinesterase activity and anti-cholinergic drugs have not proven to be effective in seizure management.

VI. INHIBITION OF ELECTRICAL ACTIVITY IN THE BRAIN

Since epileptiform seizures could be readily elicited by electrical (Fritsch and Hitzig, 1870) or chemical (Albertoni, 1876) stimulation of normal brain tissue, it occurred to some early investigators (Bubnoff and Heidenhain, 1881) that some restraining mechanism must exist which prevents normal neuronal activity from precipitating cerebral explosions. These authors (Bubnoff and Heidenhain, 1881) proposed a process of central inhibition to provide such a control mechanism, and postulated further that there was a breakdown of inhibition in seizure initiation. Thus, disturbances of the normal balance between excitation and inhibition, by any means, would overwhelm the inhibitory mechanism. Bubnoff and Heidenhain (1881) imagined that brain excitability was regulated by gross inhibitory areas which operate upon the cerebral cortex through extracortical pathways.

More recently (Gasser, 1937) has defined central inhibition as "the arrest or prevention of nervous action through the temporary operation of a process that involves no damage to the tissue in question". Inhibition should not be viewed as only the manifestation of the withdrawal of some excitatory influence, but rather that this integral process results from an increase in some kind of activity that opposes the processes of excitation in postsynaptic cells. The overall tendency has been to identify this process by a decrease in amplitude of various potentials.

Moruzzi (1946, 1950) has discussed, on the basis of analysis of high-frequency epileptiform discharges in pyramidal cells, the concept that suppression of inhibition is a requirement for the appearance

and maintenance of seizure activity, and that an increase in inhibition contributes to the termination of the seizure and post-ictal depression. The cerebral cortex, and perhaps brain stem and cerebellar systems are likely to exert important tonic inhibitory effects upon synapses in the various afferent pathways, failure of which might permit excessive neuronal discharges associated with epilepsy.

The usual effect of inhibition is to increase the degree of cell membrane polarization (Jasper, 1961). Thus, one consequence of a state of persistent depolarization (hyperdepolarization) has been reported to be a continuous, excessive, rapid and exhaustive neuronal firing which is usually hypersynchronous (Ward, 1961). As cell membrane stability is influenced by the state of ionic balance across it, it is conceivable that, at the membrane level, metabolic derangements may give rise to epileptiform discharges.

A. Inhibitory Mechanisms

Contemporary studies (Eccles, 1964) have identified two fundamentally different varieties of inhibitory processes in the mammalian CNS, designated as presynaptic inhibition (more significant in the spinal cord) and postsynaptic inhibition (more significant in the brain). Eccles (1964) proposes that presynaptic inhibition is produced by a prolonged depolarizing blockade of primary afferent terminals. These terminals, whose activation otherwise results in an EPSP at their postsynaptic cell, remain inhibited as long as some depolarizing transmitter is released due to activity in the interneuronal chain subtended upon these terminals. Eccles (1963) considers that the presynaptic inhibitory fibre makes axo-axonic junctions that are chemically differ-

ent from those operating at axosomatic or axodendritic junctions. The extent and efficacy of presynaptic mechanisms is not clear, but since such a mechanism would be relatively remote from the effector site, a minor role can be assumed.

Postsynaptic inhibition is exerted directly upon the postsynaptic neuron through a synaptic junction which functions, upon activation, to lower the facility with which the postsynaptic neuron can respond to stimulation (Eccles, 1964). Thus, postsynaptic inhibition involves a temporary hyperpolarization of the cell, during which an IPSP is produced and excitability of the postsynaptic element is diminished. An example of this form of inhibition is the Renshaw cell inhibition of spinal motoneurons as result of impulses in recurrent motor-axon collaterals (Eccles, 1964). A reversed direction of current flow, compared to excitatory synapses, is suggested by this author to be the mechanism by which postsynaptic inhibition is effected. The electrical events are the culmination of a series of chemical events which increase membrane conductance (Pollen and Lux, 1966) and shift membrane potential as a result of increased permeability of potassium and chloride ions. The time course of IPSPs in the brain is much slower (100-200 msec) than elsewhere (Eccles, 1964). Thus, in the neurophysiological sense, inhibition implies hyperpolarization (Li, 1959). It may be that in a pathological state such as epilepsy, inhibition may be reflected in the degree of membrane stabilization by a relative increase of membrane potential. Li (1959) in studies with strychnine on cortical neurons encountered higher thresholds, longer latencies and summation, but remarks that once elicited, the action of

inhibitory neurons appears to be very powerful.

The potential mechanisms by which excitatory or inhibitory neural processes are increased or decreased respectively, and how the resultant modification may lead to convulsive activity, has been discussed in detail by Esplin and Zablocka-Esplin (1969). Another distinct possibility considered by these authors is electrogenic pump inhibition, since drugs such as the cardiac glycosides which are known to block Na - K pump activity through inhibition of an ATPase system, are also known potent convulsants. Along these lines, Woodbury and Kemp (1970) has reported an antagonism between ouabain and diphenylhydantoin. One action of diphenylhydantoin is to enhance Na - K pump activity. Whether these observations provide a link between neurometabolic processes, electrical activity in the brain, and central inhibitory actions, remains to be demonstrated.

A recurrent disinhibiting mechanism which removes background inhibition exerted upon motoneurons and is not in itself an excitatory pathway has been proposed by Wilson and Burgess (1962). These authors have shown that under some instances a facilitation of monosynaptic reflexes may occur following a conditioning antidromic volley. Since tetanus toxin or strychnine pretreatment reduces this "recurrent facilitation" Wilson and Burgess (1962a) propose that this effect is mediated via inhibitory synapses. Thus, at the level of the spinal cord, and also in the cerebellum (Ito *et al.*, 1968), there appears to be a group of tonically firing interneurons which inhibit neurons, and are themselves inhibited by antidromic stimulation of motor axons.

B. Inhibitory Pathways

As discussed earlier, it is common to find that most cortical neurons are subject to antagonistic excitatory and inhibitory synaptic contacts. Strictly excitatory neurons could exercise inhibitory actions via recurrent axon collaterals upon an efficient inhibitory interneuron. Such an arrangement has been proposed by Andersen *et al*. (1964) for thalamo-cortical relay cells. Eccles (1969) discusses another form of central inhibition on the basis that IPSPs are also generated in cortical pyramidal cells by specific and non-specific afferents (Andersen *et al*., 1964). Watanabe *et al*. (1966) have reported that stimulation of the afferent pathway from the lateral geniculate body evokes IPSPs with a mean latency 1 msec longer than that for EPSPs in neurons of the visual cortex. Eccles (1969) suggests that the later IPSP is not due to differences in conduction rate but that it represents delay as a result of relay of an axon collateral through an inhibiting interneuron(s). This latter structure is assumed to function by a synaptic mechanism on the excitatory neurons.

a. Involvement of cortical elements in inhibition

Eccles (1965), basing his conclusions on the results of studies of responses evoked by stimulation of afferent pathways to the cortex, postulated a system of postsynaptic inhibitory control of neocortical pyramidal neurons similar to the inhibitory system demonstrated for hippocampal pyramidal cells (Andersen *et al*., 1964a).

Inhibitory control is believed to be largely due to recurrent inhibition via axon collaterals and inhibitory interneurons. In this system the axon collaterals of cortical pyramidal cells activate not

only inhibitory interneurons, but also complex interneuronal pathways that ultimately excite both the inhibitory interneurons and pyramidal cells themselves. The IPSP in response to a single antidromic volley in pyramidal tract fibres (Eccles, 1965) has a long latency, whereas brief repetitive stimulation results in a reduced latency for the IPSP, which suggests that there is facilitation of direct activation of inhibitory cells by axon collaterals. Sometimes, the long IPSPs (50-200 msec) appeared to terminate in a "rebound" discharge composed of a burst of spikes.

Another system for the production of cortical inhibition by collaterals of afferent fibres to the cortex has been proposed by Eccles (1965). Single or low frequency volleys at specific thalamic nuclei produce in pyramidal cells an initial EPSP with superimposed spikes, followed (after 10 msec) by a prolonged IPSP with suppression of background discharges (Li, 1963). When the afferent stimulation (ventrolateral nucleus) is at 40 Hz, there is an initial suppression of surface and pyramidal cell discharges (Li, 1963), which, as a result of wide cortical distribution, is postulated to be generated via an afferent collateral pathway, as opposed to more localized recurrent inhibition via axon collaterals (Eccles, 1965). Lux and Klee (1963) stimulated the thalamus repetitively (13 Hz) and evoked EPSPs with superimposed bursts of spikes followed 70 msec later by IPSPs maintaining a hyperpolarization on which EPSPs were superimposed. Thus, there appears to be, at a unitary level, cortical organization that recruits inhibition following excitation, by means of complex pathways.

A unique suggestion (Rall and Shepherd, 1968) to explain

recurrent inhibition in the olfactory bulb proposes that pathways involving only dendrites and reciprocal dendro-dendritic synapses are operative during inhibition. Such an arrangement presumably provides a self-regulatory mechanism between secondary mitral cell dendrites and the dendrites of granule cells in the olfactory bulb (Rall et al., 1966).

Krnjevic et al. (1966a) have demonstrated well developed cortical inhibitory effects when an electrical stimulus is applied directly to the cortical surface. The inhibition can be recorded 1 cm away from the site of stimulation, and spreads along the plane of a gyrus. It was further reported (Krnjevic et al., 1966a) that this response was elicited more readily by stimulating deeper layers of the cortex, and spreads to neurons throughout all cortical layers. Since this inhibition is observed in chronically-isolated cortical slabs (Krnjevic et al., 1970), there is strong support for the existence of a predominantly intracortical inhibitory system.

C. Transmitters of Cortical Inhibition

Much evidence supporting an inhibitory function for various neurohumoural candidates has been reported. A most popular candidate is the ubiquitous central substance GABA, which is particularly effective in producing large conductance changes and hyperpolarization in brain cells (Dreifuss et al., 1969). Substantial amounts of GABA and glutamic decarboxylase are found in the cortex (Roberts and Kuriyama, 1968). Subfractionation techniques reveal large amounts of GABA localized in nerve terminals (De Robertis,1967). Mitchell and Srinivasan (1969) have demonstrated that GABA release from the surface of the

cortex is directly correlated with stimulation of inhibitory fibres. It has also been shown that the rate at which GABA is released from the cortical surface is inversely related to the level of electrocortical activity (Jasper and Koyama, 1969). Evidence published suggests that inactivation of GABA may be due to a process of uptake into neuroglia (Utley, 1963; Margolis et al., 1968).

Other transmitter substances have been reported to exert depressant effects on cortical neurons. Phillis and York (1968a) showed that ACh applied microiontophoretically apparently inhibits cortical neurons by acting upon muscarinic and nicotinic sites. Prolonged cortical inhibition is also induced by thalamic stimulation (Phillis and York, 1967a), stimulation of the mesencephalic reticular formation, or the pyramidal tract (Phillis and York, 1967). Since strychnine and atropine abolish this evoked inhibition and the cells are no longer sensitive to ACh a cholinergic mechanism has been suggested.

If ACh has an inhibitory role in the cortex it is puzzling that several authors (MacIntosh and Oborin, 1953; Collier and Mitchell, 1967) suggest a reduced ACh release as an explanation for the electrical silence that they find in chronically undercut cortex. This might be best explained by the fact that these investigators worked with quite small pieces of isolated cortex (5 mm^2). Other workers (Sharpless and Halpern, 1962; Echlin and Battista, 1963; Rutledge et al., 1967) reported that chronically undercut cortex (50-100 mm^2) is far from being electrically silent, and in fact they consider it to be hyperactive, as if restraint was somehow removed.

Dudar and Szerb (1969) have shown that acute isolation of a cortical slab (25 mm x 12 mm) results in reduction of ACh output to 40 percent the level measured on intact cortex, at a time when there is complete electrical silence recorded on the isolated cortical slab of anaesthetized cats. The lack of electrical activity may be due to recent trauma and residual anaesthetic. Addition of atropine to the collection cylinder results in an increased amount of ACh collected in the cylinders, as it does on intact cortex, although the absolute amounts are still 40 percent of control. The increased release caused by atropine is abolished by mesencephalic lesions or tetrodotoxin. In the presence of atropine, stimulation of the reticular formation increases release from intact cortex 400 percent, whereas direct stimulation of the cortex results in a 200 percent increase. Similar effects were observed on isolated cortical slabs following direct stimulation. Nicotinic blocking drugs (dihydro-β-erythroidine or D-tubocurarine) were without effect. These authors conclude that atropine increased ACh output by blocking cortical cholinergic synapses which are a part of a circuit inhibiting cholinergic neurons.

These investigators (Dudar and Szerb, 1969) discuss the increase in ACh output on the basis of the suggestion by MacIntosh (1963) that this increase is the result of the interruption of a negative feedback circuit. According to these authors, if cholinoceptive units have an inhibitory effect upon cholinergic neurons ascending to the neocortex already described (Shute and Lewis, 1967), it seems reasonable, therefore, to conclude that the increase in total ACh output, as a result of the interruption by atropine of this synaptically-

transmitted inhibition, is now due to increased trans-synaptic stimulation (Dudar and Szerb, 1969), i.e. getting through and making more cholinergic neurons active. In agreement with this assumption it was shown that atropine is without effect on spontaneous ACh release and affects ACh output due to reticular formation stimulation more than it does to local stimulation. The increase in ACh release following local stimulation on isolated cortex may be a result of trans-synaptic activation of cholinergic fibres with cell bodies within the cortex, or of intracortical inhibitory stellate interneurons (Colonnier, 1966). Since antidromic stimulation of Betz cells does not inhibit cortical ACh output (Dudar and Szerb, 1969) it appears that other cortical cells mediate this inhibitory effect, perhaps smaller cells.

Brooks and Asanuma (1965) have injected tetanus toxin into cats and shown that there is a reduction or abolition of cortical recurrent inhibition produced by medullary conditioning stimulation. When these changes begin, the ECoG shows small spikes and bursts which develop into seizure discharge patterns. Administration and dihydro-β-erythroidine (0.15 - 3 mg/kg) produces small and inconsistent changes. Injection of atropine (0.1 - 0.8 mg/kg) causes the appearance of small spikes and bursts, within 5 min following treatment with the higher doses. These abnormal electrographic patterns bear similarity to the early effects of tetanus intoxication, which apparently acts presynaptically (Semba and Kano, 1969). Thus, atropine appears to have removed a restraining mechanism, or it may have direct excitant actions.

The central inhibitory actions of catecholamines and serotonin has been reviewed extensively by Salmoiraghi and Bloom (1964) and

McLennan (1970). These substances have been shown by various techniques to have depressant effects on many neurons. It is not clear if these are pre-or postsynaptic actions. On the other hand, Satinsky (1967) reported that catecholamines cause excitation of lateral geniculate neurons. Yamamoto (1967) studying the sensitivity of neurons in Deiters nucleus and Johnson _et al_. (1969) studying cortical neurons in _encephale isole_ preparations also have reported that the majority of units were excited. In the spinal cord, the depressant effect of noradrenaline on Renshaw cells is not antagonized by strychnine (Biscoe and Curtis, 1966). However, Tebecis (1967) has demonstrated that strychnine blocks the depressant action of noradrenaline (and also of reticular formation stimulation) on neurons in the medial geniculate body.

Roberts and Straughan (1967) using _encephale isole_ animals, and the bimaleate salt of 5-HT, rather than the commonly used creatinine sulfate salt of 5-HT, have shown that 5-HT has excitatory effects on cortical neurons. These authors also found neurons depressed by 5-HT, but unlike the 5-HT excited neurons, the depressant effect was not antagonized by LSD and related 5-HT antagonist drugs.

Cholinergic and adrenergic inhibition has been investigated by Malcom _et al_. (1967) on the rat cerebral cortex. These investigators measured the amplitude of evoked responses on the somato-sensory cortex of anaesthetized rats. The rats had a plastic cylinder for topical application of drugs fitted on the exposed cortex. Nicotinic drugs and blockers were without effect, while atropine increased, and cholinergic drugs decreased the amplitude of the evoked responses.

Noradrenaline also decreased the amplitudes, and this action was blocked by phenoxybenzamine, but not by beta-adrenergic blocking drugs. Pretreatment with atropine blocked cholinergic drugs, but did not block the inhibitory effects of noradrenaline. Phenoxybenzamine blocked the usual responses to cholinergic or adrenergic drugs.

It is known that after the administration of atropine, in a dose sufficient to block the effects of injected ACh, ACh will have sympathomimetic actions (Burn and Rand, 1965). Further work on their cholinergic link hypothesis (Lindmar *et al.*, 1968) suggests strongly, that atropine is removing a cholinergic inhibitory mechanism, specifically muscarinic, which normally inhibits the release of noradrenaline. These studies have been carried out on peripheral structures and their import for the central milieu awaits further investigation. However, Ferry (1966) has reviewed the field and considers that there is insufficient evidence for a cholinergic link in adrenergic processes. A brief, but pertinent, discussion by McLennan (1970) on the relevance of similar studies on ganglia succeeds mainly in presenting a complex picture of pharmacological inter-relationships which appear to be difficult to set into the more complex cerebral milieu.

D. Significance of Inhibition for Paroxysmal Electrical Activity in the Brain

The cortical self-sustained afterdischarge, regarded as a counterpart of a subclinical seizure (Symonds, 1959), apparently provides some basis for consideration of central inhibitory phenomena operating during convulsive seizures. As previously described the cortical EAD consists of characteristic patterns (Jasper, 1955; Maiti

and Domino, 1961), initially spike-like waves at 10-15 Hz are continuous, but they soon increase in amplitude and become segregated into groups with increasingly longer pauses or phases of relative electrical inactivity in between the large rhythmic spikes. At some point after the appearance of this intermittent silence in the EAD the afterdischarge ceases and the cortex appears to be inactive and inexcitable for a short period (Symonds, 1959). Although it is perhaps easier to explain arrest of seizure discharges simply by total exhaustion of the participating neurons, a more academic approach is to consider whether hyper-excitation in turn evokes augmented inhibitory effects, which contribute to the ultimate termination of epileptiform activity, and post-ictal depression (c.f. collateral inhibition in Renshaw cells). Earlier work by Davies *et al*. (1944) reported a marked decrease in oxygen tension of cerebral tissues following experimental convulsions. These authors correlated post-ictal depression to the relative hypoxia of cortical neurons.

Pertinent knowledge bearing on this point could also help to explain generation of epileptiform activity, since arrest of vulnerable cellular inhibitory systems, disinhibition (Ito *et al*., 1968), may set the stage for hyperexcitability leading to hypersynchronized and excessive electrical activity in the brain. This is a very general explanation and it remains to be demonstrated what are the endogenous and extrinsic factors that do remove the restraint provided by normally present central inhibitory mechanisms. Andersen and Sears (1964) proposed a widespread recurrent inhibitory system operative on thalamocortical cells that leads to synchronization of neuronal discharges by a gating mechanism.

Since Betz cells give rise to axon collaterals ending on short interneurons, which in turn form synapses with Betz cells, this suggested to Phillips (1959) the possibility for an arrangement in the cortex of collateral axons; similar to the motoneuron-Renshaw cell system of recurrent inhibition in the spinal cord. Whether such a feedback system is important for terminating seizure activity is not entirely clear.

Dawson (1958) has suggested that there might be tonic activity descending from the cortex (and other centres) which has an inhibitory effect at synapses on afferent pathways at a lower level. Thus, in normal cortex afferent inflow would be subject to continuous inhibitory control. This may prove to be a tenable explanation for isolated cortex, since deafferentation of cortical neurons, under this proposal, would render isolated cortex hyperactive which is usually the case. Although Burns (1951, 1958) considers isolated cortex to be inherently quiescent, there are other accounts (Sastry, 1956; Creutzfeldt and Struck, 1962; Pinsky and Burns, 1962; Sharpless and Halpern, 1962; Hirsch _et al_., 1969) of considerable spontaneous activity even in chronically-isolated cortical neurons. Thus, there is a basis for extensive intracortical organization causing both excitatory and inhibitory effects within the cerebral cortex. Neuronally isolated cortex is capable of responding with various electrical rhythms (Grafstein and Sastry, 1957; Goldring _et al_., 1961; Pinsky and Burns, 1962).

According to Eccles (1965, 1969a), there is a possibility that postsynaptic inhibitory control of cortical pyramidal neurons is shared between recurrent inhibitory and afferent (onward) collateral

arrangements of synapses, which may have an important role in limiting the build-up of excitatory circuitry that would otherwise result in convulsions. Eccles _et al_. (1954) proposed a recurrent feedback inhibition to impose temporal limits on afterdischarges. Spatial spread of activity appears to be limited by a process of "surround" inhibition (Mountcastle, 1957). Thus, in the presence of a continued source of excitation, high-frequency firing of neurons may be terminated by the intervention of this mechanism, or by excessive depolarization by other means.

Levy and O'leary (1965) have reviewed briefly, electrophysiological and a variety of other intrinsic factors that appear to limit seizure discharges. Tower (1965) provides a detailed discussion of neurochemical factors, especially the regulation of basic membrane processes, presumed to play a role in the promotion of seizure arrest. The slow components of cortical potentials have been considered as restraining or braking waves by Jung (1963) since repetitive stimulation diminishes their surface negative components prior to an elicited seizure discharge. This author speculates that a presynaptic mechanism may adequately explain central inhibitory phenomena. The intense depolarization assumed to be present in the afferent terminals causes negativity which is lost upon interruption of unitary autorhythmic activity following brief excitation of cortical neurons (Fessard, 1963).

Thus, facilitation of inhibitory processes would provide a means by which effective drugs could exercise control over excessive discharges. Since most studies have been concerned with elevation of thresholds for chemically or electrically-induced seizures, little or

no information has appeared on the role of other cortical inhibitory processes in the control of epileptiform electrical activity.

In closing this part of the discussion it is perhaps pertinent to consider a fundamental concept originally formulated by Dr. J.E.P. Toman, viz "enough of anything will inhibit anything".

VII. PHARMACOLOGICAL STUDIES ON NEURONALLY ISOLATED CORTEX

During the last twenty years the isolated cortical slab has been employed by a number of investigators for studying drug action at a purely cortical level (Domino, 1957; Crepax and Infantellina, 1958; Rech and Domino, 1961; Sharpless and Halpern, 1962; Sanders and Gravlin, 1968; Frank and Jhamandas, 1970). Most of the studies on chronically-isolated cortex were conducted under acute experimental conditions. It is important to make a distinction between such experiments on recently anaesthetized, immobilized and artificially-ventilated animals, and those experiments performed on alert, unrestrained experimental animals with chronically-implanted electrodes (Sharpless and Halpern, 1962). Under the latter conditions, the physiological status and autonomic responsiveness of the animal are optimal. In addition, the behavioural state of the animal is an important biological endpoint which can be used to assess qualitatively the degree of central effects of drugs.

Gangloff and Monnier (1957) consider the EAD in intact cortex as a useful response for pharmacological studies on the brain, since it is sensitive to drug action. The fact that there is a paucity of studies on the role of neurohumoural factors involved in the generation and spread of epileptiform activity in the cerebral cortex gives some impetus for drug studies on isolated cortex which bear on this aspect.

A. Studies with Cholinergic Agents

Kristiansen and Courtois (1949) used local application of physostigmine and ACh to study their effects on the electrical activity

of acutely-isolated cortex. The application of cholinergic agents caused the appearance of regular rhythmic bursts with intervening periods in which electrical activity is almost completely abolished. This pattern differs from the usual form of EADs elicited by direct cortical stimulation which show a more continuous spiking. Echlin et al. (1952) studied epileptiform activity in chronically, partially-isolated cortex and its relation to ACh sensitivity. These authors observed that such areas of cortex require much lower concentrations of topically-applied ACh to elicit abnormal electrical activity. These authors (Echlin, 1959; Echlin and Battista, 1963) also mentioned observing similar responses on apparently partially-isolated cortex overlying brain tumours. On this basis they postulated that partial denervation, with apparent elimination of inhibitory mechanisms, renders this cortex more sensitive to the excitant actions of ACh. The mass of cortex studied by Echlin et al. (1952) was quite large, including many extracortical structures and also had increased sensitivity to topically applied strychnine.

Infantellina (1955) in acute experiments on isolated cortex showed that topical application of physostigmine (1%) by means of pledgets of drug-soaked filter paper resulted in suppression of Burns' burst response. Whenever this treatment was followed by the application of ACh (1%) intense 10 Hz spikes were observed. Similar results were noticed following intracarotid administration of ACh (0.01 - 0.2 μg/kg). Local application of atropine (0.2-1%) specifically blocked the responses elicited by the cholinergic drugs. Responses evoked by combined application of cholinergic drugs and strychnine were not blocked by atropine.

Maiti and Domino (1961) have investigated the effects of physostigmine and atropine on EAD duration in acute experiments on isolated cortex in dogs. Low doses of physostigmine (0.05-0.2 mg/kg) cause significant increases in duration within a short time. Larger doses of physostigmine (0.5 mg/kg) shorten EAD duration. On its own, atropine sulfate (1 mg/kg) does not appear to alter the duration of EADs, but it is effective in blocking the effect of subsequently administered physostigmine. Atropine was effective in antagonizing physostigmine when injected after the cholinergic drug.

In a similar study (Rech and Domino, 1960) several nicotinic drugs were tested, and only topically applied d-tubocurarine elicited spiking activity, similar to that evoked by strychnine application. Atropine did not antagonize this activity. Decamethonium, gallamine, hexamethonium, and mecamylamine when topically-applied were ineffective. Echlin _et al_. (1952) reported potentiation of the EAD by curare, whereas Preston (1955) observed no changes in activity of the slab.

Rech and Domino (1960) reported that systemic administration or topical application of physostigmine increases frequency and reduces amplitude of the ECoG, effects which are blocked by atropine. Physostigmine did not alter the strychnine-spiking patterns of isolated cortical slabs. These authors also injected low doses of ACh (2-6 μg/kg); arecoline (10-20 μg/kg) and nicotine (10-20 μg/kg). In addition to marked cardiovascular changes, these agents transiently decreased the frequency of strychnine spikes. This latter effect was prevented equally by atropine sulfate or methyl atropine nitrate (0.5-1.0 mg/kg). Since the charged molecule of atropine did not produce

any characteristic slowing of the ECoG it was concluded by the authors that the cholinergic drugs have no direct central actions. Halpern (1961), in his dissertation, reported that atropine (5 mg/kg) caused the appearance of high-voltage spikes resembling some types of spontaneous paroxysmal activity in chronically isolated cortex.

Gol and Kellaway (1963) have done acute experiments on isolated cerebral hemispheres in cats to study the effects of cholinergic drugs on the suppression-burst activity (Ingvar, 1955a; i.e. continuous high voltage slow activity intermixed with bursts of fast spiking). In a brief abstract these authors reported that ACh and physostigmine at certain critical concentrations convert irregular paroxysmal electrical activity to a regular (8-12 Hz) alpha rhythm, without eliciting a seizure discharge. Abnormal, irregular discharges were elicited with high concentrations of the cholinergic agents.

On the basis of earlier reports (Echlin, 1959; Stavraky, 1961) that suggested denervation supersensitivity to ACh as a mechanism in epileptogenesis (Echlin and Battista, 1963), Reiffenstein (1964) in his dissertation has investigated, on acutely and chronically isolated cortex, the sensitivity of isolated cortex to ACh. In half of his experiments, physostigmine (0.1-1.0%) applied topically onto acute slabs raised the threshold required to elicit EADs. When ACh (1%) was applied after a cholinesterase inhibitor, it caused, after a latency of 5 min, "spontaneous" EADs in all four animals tested. In one animal atropine (0.1%) blocked EAD responses due to electrical stimulation.

Application of ACh (1%) onto one chronic slab resulted in a prolonged discharge (Reiffenstein, 1964). Atropine (1 mg) adminis-

tered intra-arterially also produced a convulsion immediately, which was blocked by gallamine. Higher doses of atropine (up to 5 mg) did not affect EADs in 1 acute and 1 chronic slab. Topical application of hemicholinium, an inhibitor of ACh synthesis, caused spontaneous discharges similar to the ACh-induced activity. On the basis of the patterns of the responses that resulted, Reiffenstein (1964) concluded that the ACh-seizures were more similar to positive bursts of afterbursting, than they were to the EAD responses. This led him to conclude that cholinergic pathways are not necessary for the production or maintenance of EADs.

Infantellina (1955) has reported that physostigmine does not increase the ability of acutely-isolated cortex to produce EADs. Considering that in half of the experiments performed by Reiffenstein (1964) there is antagonism by ACh against epileptiform activity it would seem that there is some evidence for a modulator role of ACh controlling the duration of experimental seizure discharges (EADs).

Experiments with cholinergic and anticholinergic drugs similar to those reported earlier by Maiti and Domino (1961), have been published by Nickander and Yim (1964). There is some agreement of these latter findings with the former study. Physostigmine (0.1-0.2 mg/kg) caused significant increases in mean EAD duration, which were reversed by subsequently administered atropine. In half the animals (5 out of 9, 7 experiments) tremorine (1-6 mg/kg) caused mean decreases in EAD duration of 7.8 sec (0.2-17.3 sec), whereas in the remaining animals (4 out of 9, 5 experiments) mean increases of 4.8 sec (1.2-12.2 sec) were obtained. Nickander and Yim (1964) also administered arecoline (0.05-1 mg/kg) and measured significant increases (< 2 sec) and slight

decreases in duration, but the results are quite inconsistent.

Their experiments with nicotine (1 - 50 μg/kg) showed more significant increases in EAD duration (1.5-25 sec). Unfortunately, half of these experiments (3 out of 6 animals) were performed on animals that had received other drugs previously. In the majority of these experiments (7 out of 12) the increases were less than 3 seconds. Although some of these small differences were statistically significant one must be cautious interpreting the significance of such small differences in EAD duration.

These authors usually administered atropine (1 mg/kg) following the various cholinergic drugs, and in the majority of experiments (8 out of 14) there were increases in EAD duration when compared to control. Nickander and Yim (1964) considered that as atropine produced little or no change in EAD duration in 6 out of 14 experiments, it was blocking the action of the cholinergic drugs. They appear never to have pretreated with atropine to determine its action by itself and its influence on subsequent treatment of cholinergic drugs.

More recently, Spehlmann et al. (1970) have reported, based on both macro and microelectrode studies, that partial deafferentation of cat visual cortex contributes to cortical epileptogenesis, but that there is no resultant ACh supersensitivity. Reiffenstein (1970) in a preliminary report mentions that topically applied cholinergic drugs provoke EADs in acute and chronic slabs. Atropine arrests discharges only on acute slabs. Chronic slabs were more sensitive to pilocarpine, but responded with a different pattern and the response was apparently prevented by higher concentrations of atropine. Krnjevic _et al._ (1970)

have reported that only 20 percent of neurons in long isolated slabs respond to microiontophoretically applied ACh. Since these authors also observed almost a complete loss of AChE, as determined by histochemical methods in the chronic slabs, they argued that the hyperexcitability of chronically-isolated cortex cannot be ascribed to cholinergic structures. On this basis they explain the depression of unit activity produced by the iontophoretic application of eserine as a direct effect, unrelated to the anticholinesterase activity of the drug.

Other authors (Rosenberg and Echlin, 1965, 1968; Duncan *et al.*, 1968) have reported that marked changes in electrical activity and increased sensitivity to ACh of isolated cortex appear at the same time as the greatest changes in AChE levels. These authors and others (Green *et al.*, 1970), based on radiochemical analysis, report that AChE levels are about 50% of the level found in control tissues. Green *et al.* (1970) report further that there is a marked concomittant decrease (35% of control) in ChAc activity, which suggested to them that there was even less likelihood of ACh involvement in the hyperexcitability of chronically-isolated cortex. However, if there are only one-fifth as many cholinergic neurons, as suggested by Krnjevic *et al.* (1970), the 50 percent reduction in cholinergic catabolic enzymes may not be relevant in terms of the final concentrations of ACh presented to the remaining responding neurons.

B. Studies with Monoamines

a. Serotonin

Drug studies on isolated cortex in which biogenic amines have been administered are very few. Crepax and Infantellina (1957)

have applied serotonin onto the acutely-isolated cortex of cats and have reported two types of responses, dependent on the concentration applied. Low (0.01-0.1%) concentrations of serotonin increased spontaneously occurring electrical activity, whereas higher (0.2-2%) concentrations exerted a depressant effect. Close arterial injection of serotonin (0.020-0.060 mg/kg) produced a similar biphasic response, dependent on drug concentration. Rech and Domino (1960) administered serotonin systemically and reported that the drug decreases the frequency of strychnine or d-tubocurarine spiking in the acutely-isolated cortex of dogs.

b. Catecholamines

Rech and Domino (1960) have injected adrenaline or noradrenaline (2-6 μg/kg) to study the effects of catecholamines upon the electrical activity of acutely-isolated cortex in dogs. These authors were able to dissociate the cardiovascular effects of these agents from the observed electroencephalographic changes. Both of these agents, but especially adrenaline, were effective in decreasing the frequency of the large strychnine-evoked spike and burst responses. Adrenaline was as effective in this regard as serotonin. Potentiation of the inhibitory effect of adrenaline was observed following pretreatment with phenoxybenzamine (2 mg/kg).

A curious effect was observed by Rech and Domino (1960), in that after a series of injections of adrenaline, the susceptibility of isolated cortex to produce chemically-evoked seizures was apparently enhanced. Such an effect was also observed with respect to EADs elicited by repetitive electrical stimulation. Close arterial injection of 1-2 μg/kg of adrenaline depressed the afterdischarge for a period of

less than 10 minutes. After several (4-6) such serial injections, EAD duration was now increased with respect to control, even though depression of the response was seen immediately following the injection. As there was considerable variability between the responses obtained from different animals, no data were presented in tabular or graphical form. These results are in accord with earlier studies on EADs elicited in intact cortex (Minz and Domino, 1953).

In another study (Maiti and Domino, 1961) it has been reported that d-amphetamine (0.5 mg/kg) injected intravenously causes marked increases in blood pressure, but there is no consistent effect on EAD duration. In a similar manner, no clear effects for adrenaline (10 μg/kg) could be detected.

C. Studies With Other Pharmacological Agents

a. Central stimulants and convulsants

Preston (1955) has compared the effects of metrazol and thiosemicarbizide on the ECoG of intact and isolated cortex in cats. It was noted that metrazol produced immediate and simultaneous changes in both intact and isolated cortex, whereas the changes attributed to thiosemicarbizide had a much longer latency, regardless of whether thiosemicarbizide was administered systemically or applied topically. In a subsequent study Killam and Bain (1957) demonstrated that following thiosemicarbizide, the development of electrical seizure activity in isolated or intact cortex and subcortical tissues correlated well with the progressive enzymatic inhibition of l-glutamic acid decarboxylase.

Infantellina (1956) applied strychnine (0.2-2%) onto isolated

cortex and reported that no response was elicited by this treatment. However, it was observed that thresholds for responses to single and repetitive direct electrical stimuli were lowered and the evoked activity was considerably prolonged. When the treatment was strychnine with physostigmine or strychnine with ACh, spiking activity resulted and was now observed. These spike discharges were not antagonized by atropine. Studying chronically-isolated cortex in immature kittens, Purpura and Housepain (1961) have reported that strychnine (0.1%) application depressed evoked surface positive bursts.

Rech and Domino (1960) have applied strychnine (0.1-1%) onto the isolated cortex of dogs and recorded various responses, depending on the concentration applied. Low concentrations produced small negative spikes at irregular intervals. Intermediate concentrations caused larger diphasic (negative-positive) spikes, while at high concentrations a triphasic (positive-negative-positive) sequence was elicited. Several applications of high (1%) concentrations resulted in a repetitive discharge, which was usually preceded by burst activity. This response was not affected by intravenous atropine (0.5-1 mg/kg). Similar effects could be produced on intact cortex but the amplitude was reduced and frequency increased. The authors speculated that there might be a relation between the bursts observed in their experiments and the burst responses observed by Burns (1954), which were suggested to spread non-decrementally in layers IV and V of the cortex.

Rech and Domino (1960) also studied metrazol by various routes of administration and showed that it causes hyperactivity similar to that following strychnine, but small spikes and waves were observed

to alternate with the larger spikes. Isolated cortex was more sensitive at low doses (10-20 mg/kg) for this type of response. Picrotoxin was applied (0.1-0.25%) in some experiments and produced responses slightly different from those after strychnine or metrazol, although there was some similarity in the patterns. The seizure activity was much prolonged in duration compared to untreated cortex following picrotoxin. Halpern (1961) observed that metrazol directly induced activation of isolated cortex only at high doses. At significantly lower doses abnormal activity was always induced in subcortical centres. Sanders and Pinsky (1964) have studied the effects of the convulsant drugs metrazol and hexafluorodiethyl ether upon the EAD response in acutely isolated slabs of cerebral cortex in cats. These authors found that metrazol lowered the threshold for the production of EADs. The convulsant ether compound produced variable effects on this parameter, but appeared to initiate inhibitory effects.

The methylated xanthines, caffeine, theophylline, and theobromine, have been shown to increase EAD duration by 20 sec in the isolated cortex of the dog (Maiti and Domino, 1961). Caffeine was found to be more potent and consistent in this respect, and was compared, using the EAD response, with cholinergic and anticholinergic drugs. In experiments where large doses of physostigmine were administered first, this resulted in a significant decrease in EAD duration. Small doses of physostigmine (50-200 μg/kg) had the opposite effect and prolonged afterdischarge duration markedly. Whenever either of these treatments was followed by an injection of caffeine (0.5 mg/kg), there was always an increase in afterdischarge duration, i.e. whether afterdischarge duration was shortened or prolonged, caffeine was still

effective in increasing the response. Atropine injected before or after low doses of physostigmine blocked the expected prolonged response to low doses of physostigmine. Atropine administered before injection of caffeine blocked the usual increase in duration resulting from caffeine. Atropine following an injection of caffeine, that increased afterdischarge duration, had no further effect. On its own atropine had no clear effects on EAD duration. Caffeine was more effective than the low doses of physostigmine in prolonging afterdischarge duration.

These authors (Maiti and Domino, 1961) have proposed an interesting possibility concerning the ability of caffeine to prolong EAD duration in isolated cortex. Since low doses of physostigmine also prolong the duration of EADs, although not to the same extent, this finding suggests a common cholinergic mechanism. Also pretreatment with atropine blocks increases in duration due to caffeine or low doses of physostigmine, this strengthens the involvement of a common mechanism. However, atropine given after either of these agents, only antagonizes the effects of low doses of physostigmine, but not those of caffeine. This finding suggests that some other cortical structures are involved. Perhaps if higher doses of atropine had been injected afterdischarges might have been affected.

Ochs *et al*. (1962) found that mescaline (1%) topically applied to intact, acute or chronic isolated cortex of cats and rabbits elicits paroxysmal spiking patterns of long duration. This "convulsant" response was different from the DCRs, which were themselves depressed by the mescaline. It was further observed that high intravenous doses of

pentobarbital (30 mg) abolished these mescaline-spikes, while the DCR remains.

Frank and Jhamandas (1970) have studied several central stimulant agents on evoked responses in isolated cortex. It was reported that all such agents cause facilitation of the surface positive response and lower stimulus threshold. Glutamate (2%) applied locally causes transient, spontaneous bursting that is soon followed by spreading depression. The stimulants bemegride, picrotoxin, caffeine, and strychnine also produced convulsive discharges, while nikethamide did not. However, since each agent produced characteristically different types of electrical responses, these authors suggested that each one of these agents acts through a different mechanism.

b. Gamma-aminobutyric acid (GABA)

GABA has not escaped being studied on the isolated cortex. Goldring et al. (1961) have applied GABA (0.5%) topically and observed depression of negative components of the DCR, which was similar to the effect observed following pentobarbital (0.5%) application. Purpura and Housepain (1961) have studied the effects of topical GABA (1%) on the chronically-isolated cortex of immature kittens. These investigators observed that GABA first augments, then eliminates repetitive surface positive responses which could only be elicited in the chronic preparations. Ochs et al. (1962) found that mescaline-evoked spikes and DCRs were blocked by GABA (1%) application, and that GABA caused inversion of the DCR.

The isolated cortex of dogs has been utilized by Rech and Domino (1960a) to evaluate the effects of topically applied GABA on

potentials evoked by the CNS stimulant agents strychnine, d-tubocurarine, metrazol, or picrotoxin, and responses elicited by single electrical shocks. GABA (0.1%) inverts the surface negative potential produced by CNS stimulants. The same concentration of GABA inverts only the DCR elicited in superficial cortical layers, whereas higher concentrations of GABA (0.5%) are required to cause inversion of negative waves in deeper layers of isolated cortex.

Gottesfeld et al. (1971) have investigated the effects of GABA and glutamate injected systemically, on unit activity in chronically-isolated cortex. Neither of these agents altered unit firing in isolated or control cortex in a predictable fashion. On the basis of these results these authors proposed that there is no basis for altered permeability in cortical slabs as a mechanism for epileptiform activity. No records of surface discharge activity were obtained and it is not clear whether such unit activity has any bearing on surface records of seizure activity, as has been questioned (Halpern and Ward, 1969). Previous work in the former laboratory (Krnjevic et al., 1970a) had demonstrated that intravenous GABA in high doses (1 gm) would arrest repetitive unit activity and presumably seizure discharges at the surface of the cortex. Moreover, it is not clear as to the role of altered metabolism in chronically-isolated cortex, as Krass et al. (1968) have reported that there is an increase in glycolysis in isolated cortical slabs. Altered metabolic disposition of GABA in such cortical tissue may further complicate an already confused situation.

c. CNS depressants

Crepax and Infantellina (1956) have studied the effects of morphine (1%) applied to isolated cortical slabs. The threshold for surface DCRs was lowered and excitatory effects were observed following administration of this drug. Afterdischarge responses were enhanced and prolonged. When physostigmine (1%) was combined with morphine for topical application, intense 10 Hz spikes were observed, similar to those reported when ACh (1%) and physostigmine were combined. Topically applied atropine antagonized the response to cholinergic drugs alone but did not antagonize the repetitive response induced by combined physostigmine and morphine.

Domino (1957) has compared the effects of pentobarbital and a well-known convulsant barbiturate DMBB on the activity in partially-isolated cortex in the cat. Low doses (2 mg/kg) of the convulsant barbiturate caused increases in the frequency of spontaneous activity in isolated cortex, whereas larger doses depressed all electrical activity in the slab. Pentobarbital (5 mg/kg) caused similar effects and was remarkably similar to the convulsant compound, but pentobarbital did cause more spindle bursts of activity, i.e. the suppression burst discharges consisting of increased voltage, decreased frequency and spiking behaviour (Domino, 1956).

Goldring *et al.* (1961) applied pentobarbital (0.5%) onto acutely-and chronically-isolated cortices and observed that only the primary and second negative waves were depressed, while after-positivity and slow negativity were increased or unchanged. Topical procaine (0.5%) differed in its effects in that it raised stimulus threshold

equally for all DCR components. When injected intravenously pentobarbital (up to 45 mg) had the same effect as it did topically, but procaine (up to 110 mg) now depressed the slow negativity. Ochs et al. (1962) have reported observations on both acute and chronic slabs that pentobarbital (20-30 mg) blocked mescaline-induced spiking, while DCRs remain unaffected.

Recently, general depressant drugs have been investigated with respect to their effects on the electrical responses of isolated cortex following topical application (Frank and Jhamandas, 1970a). All of the agents tested depressed or abolished the surface negative and surface positive response, and raised stimulus threshold for the positive burst response. These findings are in accord for previous experiments (Frank and Sanders, 1963) with general and local anaesthetics on the same preparation. In high concentrations chlorpromazine caused spontaneous activity (Frank and Jhamandas, 1970a) as did intravenous pethidine, although this latter response resembled convulsant activity. These authors concluded on the basis of their results that depressant compounds produce anaesthetic-like effects on central neurons.

d. Anticonvulsant drugs

Perhaps the only studies on chronically-isolated cortex conducted with unanaesthetized, unrestrained animals bearing permanently implanted electrodes, are those reported by Halpern (1961) and Sharpless and Halpern (1962). These authors neuronally isolated a portion of left suprasylvian gyrus in cats, and permanently implanted a plate assembly of stimulating and recording electrodes. A modification of this method has been used in the present thesis. Under these condi-

tions these authors have studied, repeatedly, the effects of anticonvulsant drugs on afterdischarge duration.

Phenobarbital (25 mg/kg intraperitoneally, IP) increased threshold and decreased afterdischarge duration in isolated and intact cortex. Trimethadione (300 mg/kg) and acetazolamide (10 mg/kg) had little or no effect on the duration of elicited EADs in either region. The experiments with diphenylhydantoin (10-20 mg/kg IP) demonstrated significant reductions in EAD duration on intact cortex, but although marked reductions in EAD duration on chronically-isolated cortex were observed, there was an accompanying element of fatigue which did not permit these authors to state definitively whether the reduced duration was real and due to specific effects of diphenylhydantoin on EAD duration.

Sanders and Gravlin (1968) have utilized the PBR and EAD responses in chronically and acutely-isolated cortex of cats prepared with lesions in the brain stem for acute studies of anti-epileptic drugs. The rationale in this investigation was that the PBR is a component of normal activity in the intact brain whereas the EAD exemplifies the pathogenesis of epilepsy. From their results showing that diphenylhydantoin, phenobarbital and procaine decreased epileptiform activity without altering the PBR, it was inferred that these drugs act preferentially on the epileptogenic rather than physiological mechanisms. Thiopental suppressed the PBR in isolated cortex, but not the EAD. Their results on chronically-isolated cortex reveal that these drugs were less effective than they were on acute cortical slabs. Since the anti-*petit mal* drug trimethadione was ineffective against the EAD

it was suggested by these authors that the isolated cerebral cortex of the cat is a useful experimental preparation for the screening of anti-epileptic drugs useful in the clinical management of *grand mal* epilepsy.

STATEMENT OF THE PROBLEM

The body of this dissertation is an attempt to study the role played on the modulation of electrocortical potentials by the chemical mediators known to be active in the periphery. Most of the literature on this problem is based either on acute experiments on intact cortex, which is under the influence of many extracortical structures, or on single cell studies, which do not provide correlative information on the behavior of a population of neurons.

The neuronally isolated slab of cerebral cortex permits the investigation *in situ* of a population of viable neurons devoid of extra-cortical influences. Using the strategy of administering pharmacological agonists and antagonists in unanaesthetized and unrestrained animals with chronically isolated cortical slabs provides an experimental model which makes it easier to decipher the possible role of neurohumoural substances in regulating the electrical excitability of the cerebral cortex.

Hitherto, most investigators have reported that epileptiform afterdischarge duration (EAD) in isolated cortex is a variable parameter. The stabilization of afterdischarges would be the first important development necessary in order to conduct extensive and meaningful drug studies.

Since the EAD is supposed to be an index of the epiletogenicity of cortical tissue it would seem reasonable that a study of the action of various classes of autonomic and anticonvulsant drugs upon EAD duration may help to outline some of the pharmacological mechanisms involved in the limitation of cortical epileptiform potentials.

Interactions between the different drugs administered would provide additional clues as to possible anti-epileptic mechanisms

operative at the level of the cortex. This knowledge may prove to be useful in evaluating the effects of clinically useful anticonvulsants and why some seizures are refractory to drug therapy. Hopefully, a better understanding of the basic pharmacology of anti-epileptic drugs would lead to better testing of their efficacy and to the development of more effective agents with less toxicity. In addition, the results may help to explain some of the synaptic mechanisms operative in normal cerebral cortex.

The objectives of this investigation were:

a) To study the role played in the modulation of cortical activity by chemical mediators known to be operative in the periphery.

b) To study the role, if any, played by these mediators and modulators in the spread and termination of paroxysmal activity at the cortical level.

VIII. GENERAL METHODS

A. Selection and Maintenance of Animals

Male cats weighing between 2.5 and 4 kg were used throughout this investigation. Animals were selected before surgery and isolated in a separate holding room for a week. During this week they were observed daily for any signs of disease or infection, in which case they were treated. This isolation period was also utilized to innoculate healthy cats against feline distemper. Only healthy animals were used for surgery. Selected cats were fasted for at least 12 hours prior to surgery, but fluids were not withdrawn.

Operated cats were housed individually in large, clean, well-ventilated cages in a separate, chronic animal room. They were fed once a day with various kinds of canned cat foot obtained locally. Water was available continually and milk was provided on a regular basis. These animals were allowed to exercise within the confines of the chronic animal room at least once daily.

The author visited the chronic animal quarters daily and was personally involved with the cleaning of cages and feeding of animals. This daily contact between the experimenter and the animals greatly decreased the degree of apprehension, and lowered the level of general excitability of the animals during experimental sessions. The daily contact during subsequent drug studies was important in detecting and treating any delayed untoward effects resulting from the administration of some of the drugs used in this investigation.

B. Surgical Deafferentation of a Mass of Cerebral Cortex

a. Anaesthesia

Pentobarbital sodium (Nembutal[R] - Veterinary) was administered intraperitoneally in a dose of 30-35 mg/kg. This dose range provided the desired depth and duration of anaesthesia.

b. Pre-operative procedures

The operating room and table were clean, but not aseptic. All surgical instruments and accessories required for the operation were cleansed but not sterilized before use. Suture material (Ethicon[R]) was obtained in sealed sterilized packages. Absorbable gelatin sponge and film (Gelfoam[R], Gelfilm[R]) were likewise available in sterile packages. The electrode assembly and stainless steel stabilizing screws were first disinfected in a hexachlorophene solution (Hexaphenyl[R]), after which they were rinsed, and soaked in sterile physiological saline solution just before use.

Following the induction of surgical anaesthesia, the head of the animal was firmly supported in a Czermak Small Animal Head-holder (C.F. Palmer). The body of the anaesthetized animal was positioned comfortably over a heating device in the operating table. The head and neck region were shaved,and scrubbed thoroughly with a hexachlorophene cleansing solution. The surgical technique that followed was clean but not aseptic.

c. General surgery and craniotomy

A midline scalp incision extending from the junction of the coronal and saggital sutures to the lambdoidal crest was performed. The temporal muscle and fascia were bilaterally reflected, by blunt dissection, to the level of the zygomatic arch.

The operative field was now draped with a clean polyethylene sheet, cut so as to expose only a minimal portion of the skull. The drape was fastened at the cut edges of skin and muscle securely by means of towel clips. Care was taken not to keep the neck muscles taut and to avoid any possible compression of venous circulation in this region.

Employing a high-speed No. 8 dental bur, a small rectangular area of bone (3 mm x 10 mm), 10 mm from the midline in the left parietal bone, was excised. This craniotomy was enlarged anterio-posterially, and laterally with rongeurs, until the brain was exposed from the ansatus sulcus to the posterior lateral sulcus and from the lateral half of the marginal gyrus to the middle of the ectosylvian gyrus. Throughout the bone removal procedures, extensive and continuous irrigation with buffered Krebs-Henseleit physiological solution was employed to clear debris and haemorrhage from the operative field, and to minimize the danger of air emboli entering the cut ends of the diploe. Bone wax (hard paraffin 3-parts, yellow soft paraffin 1-part, and phenol-0.02 part) was applied extensively at all free edges of bone.

Using fine blunt-tipped dura scissors, a small flap of dura mater was cut exposing most of the suprasylvian gyrus and approximately 5 mm of adjacent cerebral cortex. The flap of dura mater was reflected carefully and the exposed pial surface was kept moist by a constant drip-flow of warm (37°C) buffered Krebs solution. Any bleeding of the dura mater was controlled by application of pledgets of absorbable gelatin sponge (Gelfoam[R]). The reflected dura was fastened now with stay sutures to the temporarily reflected and fixed temporalis muscle.

d. Complete neuronal isolation of a portion of the suprasylvian gyrus

Complete neuronal isolation of the left suprasylvian gyrus was achieved using a modification of the techniques previously described by Sharpless and Halpern (1962). The surgical isolation technique employed is, in most respects, similar to that employed in the preparation of acutely-isolated cortical slabs (Pinsky and Burns, 1962). A small area (approximately 3 mm x 3 mm) of the pia (and its superficial blood vessels) at the posterior end of the suprasylvian gyrus was electrocauterized (Birtcher Hyfrecator No. 703). A small drainage and sink-hole 2.5 mm in diameter was aspirated through this bloodless area into the lateral ventricle to provide a route for drainage, and entry and exit of the cortical slab isolating knives. The stages for the complete neuronal isolation of a slab of cerebral cortex in the cat left suprasylvian gyrus are outlined in diagrammatic fashion (Fig. 1).

For isolating the cortical slab a special set of wire knives and a spatula were utilized as previously described (Pinsky and Burns, 1962). The two longitudinal cuts (Lci and Lcii, Fig. 1) that sever the lateral connections of the slab were achieved with the End Cutting knife (Fig. 2). This wire knife, bent at right angles 3.5 mm from the end and contoured to form a smooth tip, as indicated (Fig. 2) was inserted into the sink-hole and the tip was manipulated to a subpial position such that the bent terminal 3.5 mm tip of the knife was perpendicular to the cortical surface, and just visible through the translucent pia.

Keeping the tip always visible in the subpial region (but with care so as not to damage the pia or any arachnoidal vessels) the

Figure 1. Stages for the Complete Neuronal Isolation of a Slab of Cerebral Cortex

I. LCi and LCii are separate cuts made with the End-Cutting Knife inserted via drain hole and guided subpially for 25 mm anteriad, and withdrawn via drain hole (cuts 1 & 2). Procedure repeated along LCii (cuts 3 & 4).

II. Area between LCi and LCii is undercut at a depth of 3.5 mm with Undercutting Knife inserted via drain hole. Undercut (Uc) is extended 25 mm anteriad using LCii as a guide subpially. Knife is withdrawn along same line (cuts 5 & 6). Cortical slab is now partially isolated.

III. Neuronal isolation is completed with Side-Cutting Knife inserted and guided 25 mm to forward-most point of LCi (Cut 7). Knife is then directed at right angle towards LCii along Sc for 4 mm to join with LCii at its forward-most point (cut 8). Knife is then drawn posteriad along LCii (cut 9) and withdrawn via the drain hole. Cortical slab is deprived of its afferent and efferent connections with the brain and is now completely neuronally isolated except for pial vasculature which perfuses the slab *in situ* with its usual blood supply.

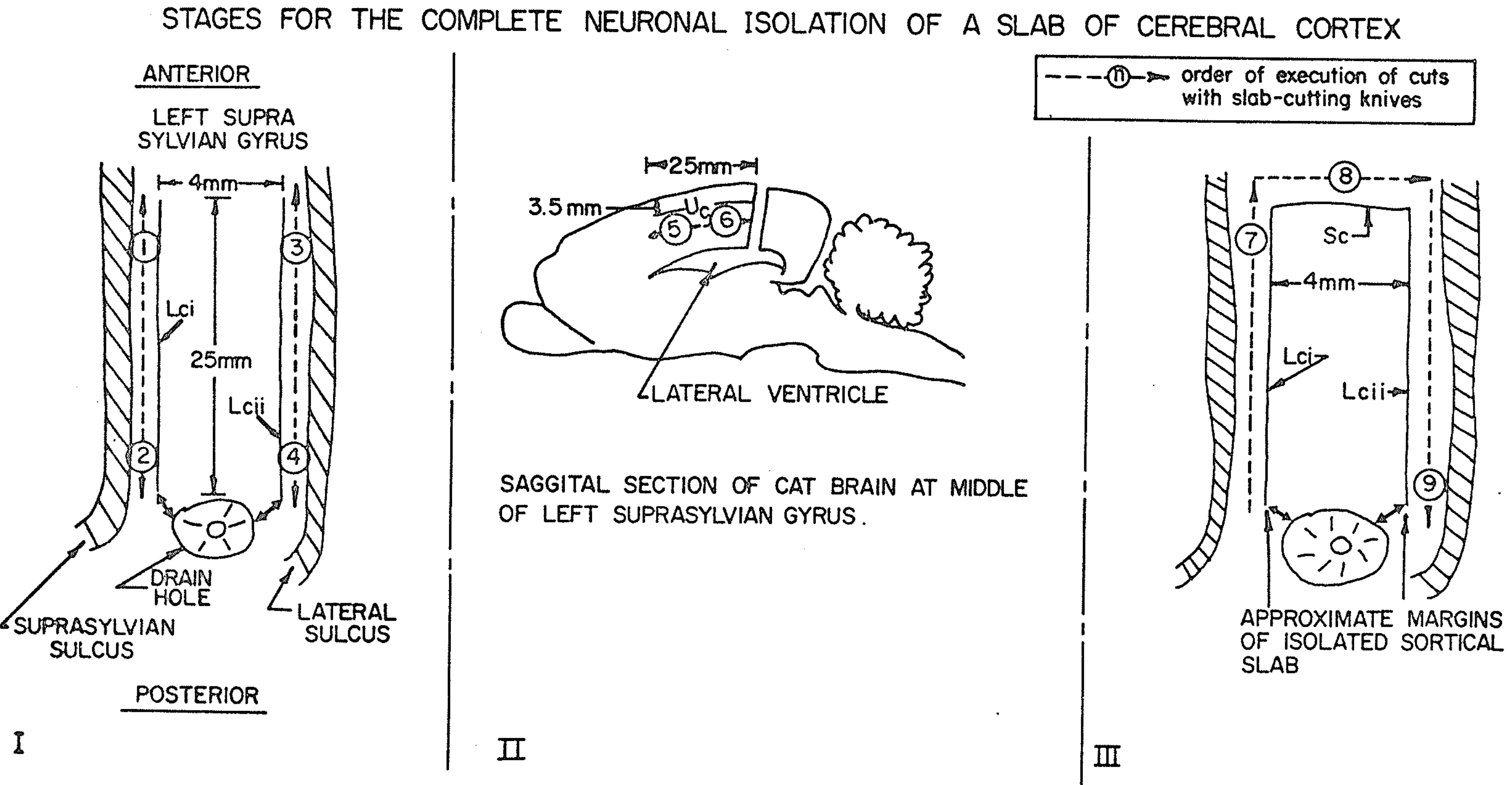


Figure 1.

Figure 2. Cortical Slab Isolation Knives

Diagrams showing shape and dimensions of the specially constructed stainless steel wire and spatula undercutting knives for isolating a slab of cerebral cortex. All tips are bent at a right angle 3.5 mm from the polished end to produce an isolated cortical slab approximately 3.5 mm in thickness. Note difference in leading edges betweeen End-Cutting Knife and Side-Cutting Knife.

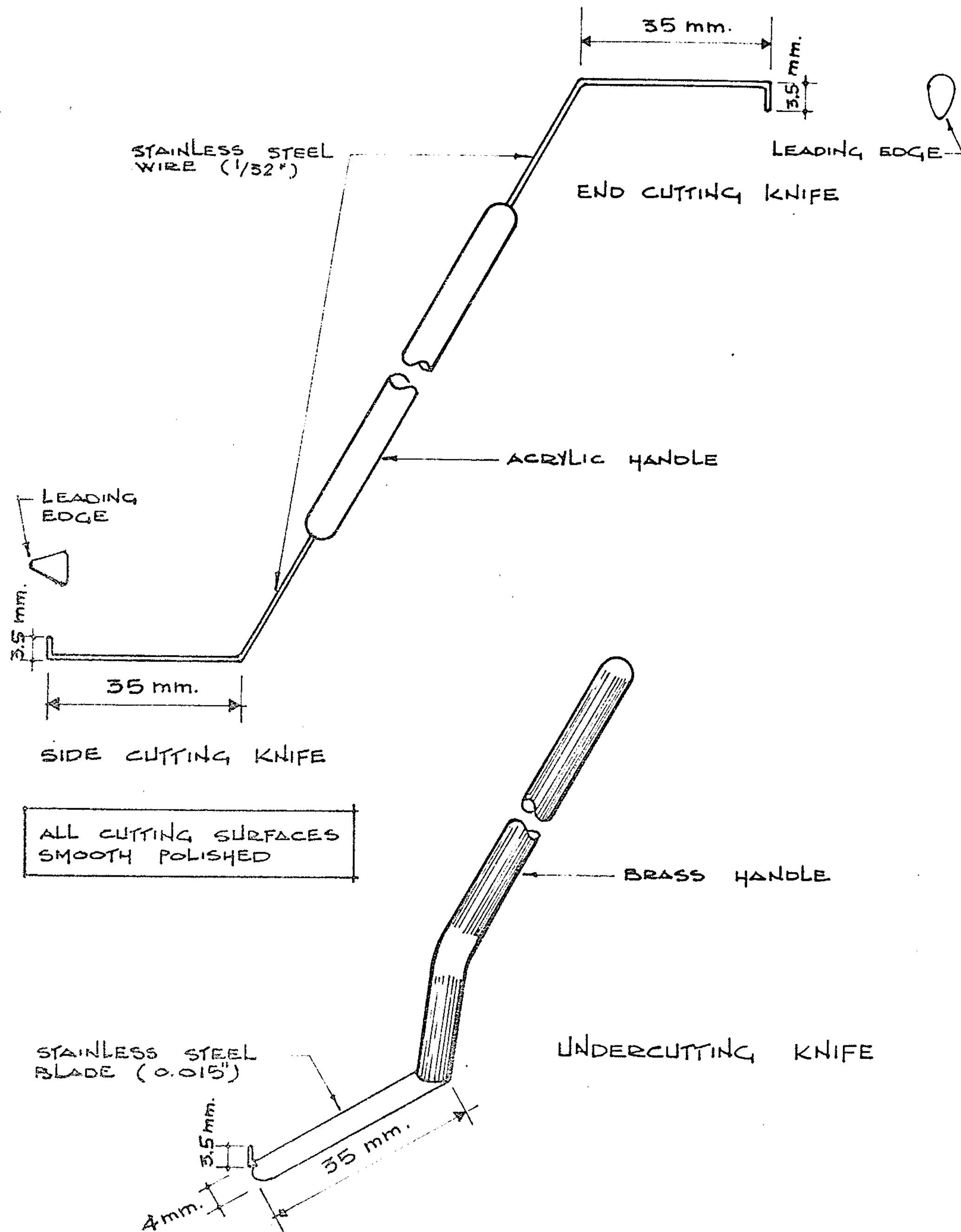


Figure 2.

End Cutting knife was gently advanced anteriorally for 25 mm, producing a longitudinal cut in the cortex (Lci, see Fig. 1) which was approximately parallel to the suprasylvian sulcus. The knife was then carefully withdrawn following the path of this cut. This procedure was duplicated to produce an identical approximately parallel cut 4 mm laterally near the lateral sulcus (Lcii, Fig. 1). At this stage the cortical slab was partially isolated from its lateral connections by the longitudinal cuts (Lci and Lcii, Fig. 1) and posteriorially by the drain hole. Care was taken to sever any remaining small intact neuronal bridges with the non-isolated cortex.

The Spatula Undercutting knife (Fig. 2) was then inserted, taking the same precautions as above. This knife was directed gently in the anteriad direction, using the vertical tip of the spatula as a guide, by keeping it just visible within one of the longitudinal previously made cuts (Lcii, Fig. 1). The undercut was made up to the forward-moist point of the parallel cuts and completed by withdrawing the spatula carefully, maintaining a depth of 3.5 mm and parallel to the cortical surface (Uc, Fig.1). At this stage the only remaining connections of the cortical slab with the rest of the brain were through its anterior end.

The final cut across the gyrus, between the most anterior portion of the parallel longitudinal cuts, was executed with the specially-constructed Side Cutting knife (Fig. 2). The cutting portion of this knife was also bent at a right angle 3.5 mm from the end and had a polished tip. This tip was positioned and manipulated carefully up one of the longitudinal cuts (Lci, Fig. 1), until it reached the for-

ward most portion of the cortical slab. By means of slow firm movements the Side Cutting knife was directed at a right angle away from the longitudinal (Lci, Fig. 1) cut towards (along Sc, Fig. 1) the leading edge of the other longitudinal cut (Lcii, Fig. 1) on the other side of the gyrus. If necessary, several passes were executed in this region to ensure complete neuronal isolation. This instrument was then carefully withdrawn following the other longitudinal parallel cut (Lcii, Fig.1). Thus, the slab was again undercut at the desired depth of 3.5 mm.

The uniformity of the slight subpial line of haemorrhage following the above procedures was usually indicative of neuronal isolation at the gross observation level. The completeness of neuronal isolation was confirmed later by histological methods (Weisman, 1969). The resultant completely isolated mass or slab of cerebral cortex was almost the entire width of the suprasylvian gyrus, but not so near to the sulci as to upset the extensive vasculature in this region. The overall approximate size of the completely neuronally isolated cortical slab was 25 mm long by 4 mm wide by 3.5 mm deep. The surface area of the slab and exposed intact cortex was more than adequate to accomodate the specially constructed electrode assembly of 4 pairs of stimulating-recording electrodes. This isolated cortical slab was separate from the rest of the brain, but retained an intact superficial cerebral circulatory network via pia-arachnoidal vessels which perfuse the slab.

C. Implantation of Indwelling Electrode Assembly

The area of the craniotomy was now prepared to receive the assembly of epicortical stimulating and recording electrodes (Fig. 3).

Figure 3. Silicone Rubber Electrode Assembly and Subminiature Connector

Photograph showing finished electrode assembly, flexible lead wires, and 9-contact subminiature connector. Details of construction given in Fig. 6. Note reference lead at opening where connector is fixed to right parietal bone of skull with small stainless steel screw. Note also smooth rounded electrode tips. Silicone rubber plate assembly (left-hand portion of photo) rests on cortical surface with small allowance for vertical movement. Thin silicone rubber sheet overlay is fixed with interrupted sutures (#6-0) to intact dura mater.

Figure 3A. Close-up of Assembly Removed Six Months Postoperatively

Photograph of silicone rubber electrode assembly removed from chronic animal six months after surgery and implantation procedures. Note smooth rounded electrode tips.

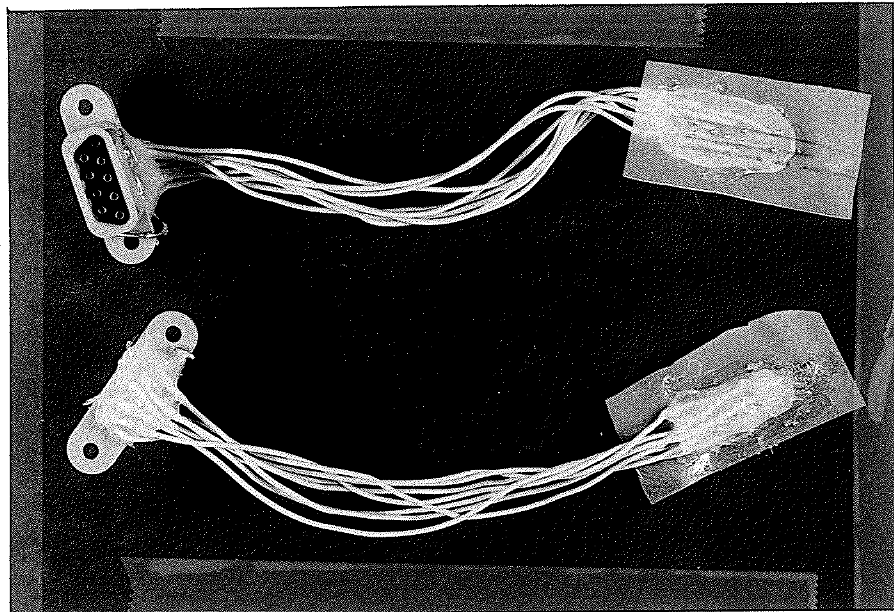


Figure 3.

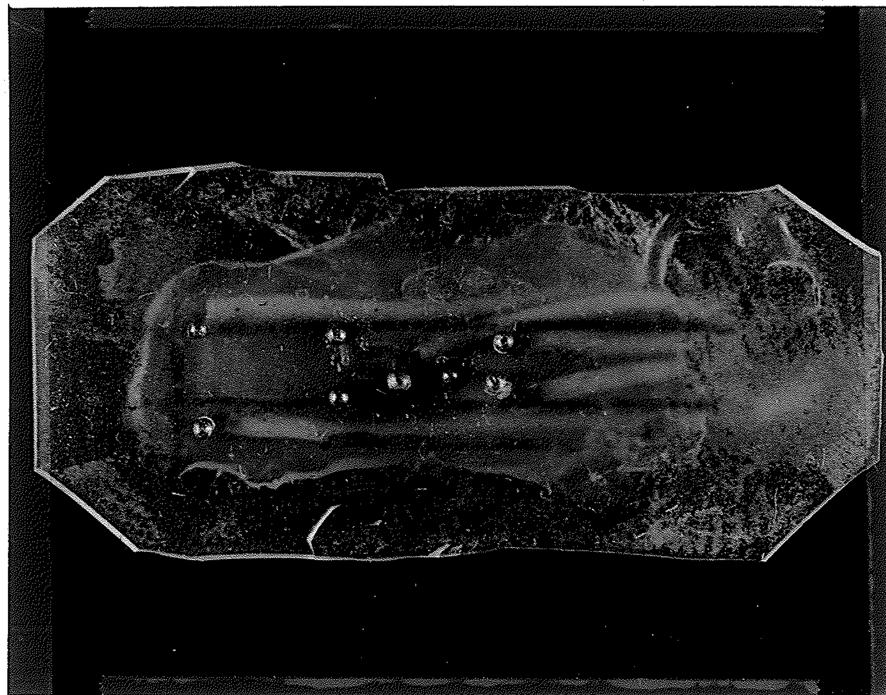


Figure 3A.

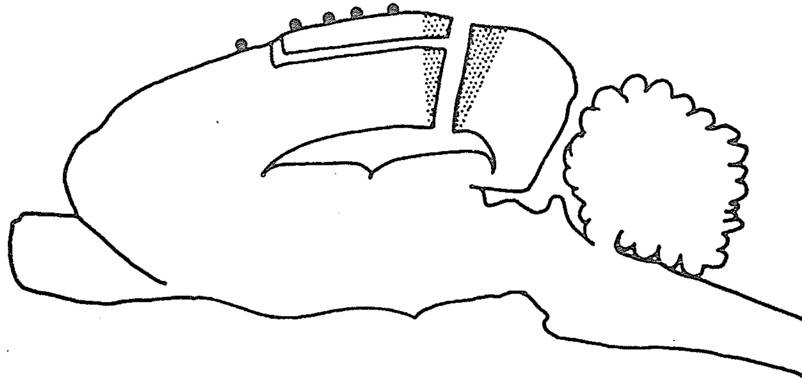
The anterior pair of recording electrodes were rested on intact cortex at least 5 mm beyond the anterior margin of the isolated margin of the isolated cortex (Fig. 4). In this way four recording and two stimulating electrodes rested entirely within the margins of the isolated slab. The positioning and stabilization of the electrodes required considerable patience and careful manipulation as the small marginal overlay of thin rubber sheeting (Silastic[R], 0.005" - 0.010") attached to the electrodes (Fig. 3) had to be carefully tucked in between the dura mater and the surface of the cortex. It was possible to see the margins of the isolated cortical slab through the thin translucent silicone electrode assembly and make final adjustments carefully, as required. The approximate configuration and final location of the recording-stimulating assembly of electrodes at the cortical surface is indicated diagrammatically (Fig. 4).

The stay sutures on the flap of dura were cut and the flap was returned approximately to its original position, covering now the electrode assembly. The dura was then sutured. The sutures were tied in a manner that brought the cut edges of the dura almost together and exerting a slight pressure on the electrode assembly. The orientation and position of the electrode assembly were checked once more. The electrode was now fixed with interrupted holding sutures (Ethicon No. 6-0) passed simultaneously through the dura and the thin silicone rubber overlay at several points.

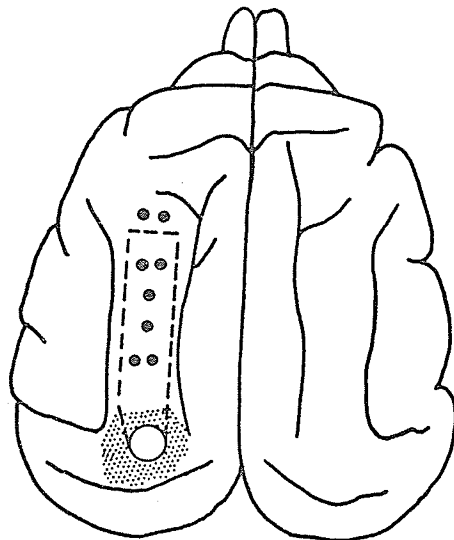
Although these sutures served to fix and stabilize the flexible electrode assembly by means of close attachment to the dura mater, an allowance was made to permit small movements in a vertical plane.

Figure 4. Approximate Location of Cortical Electrode Assembly

Diagram, saggital and dorsal views, showing approximate orientation and location of the 8 epicortical electrodes. Slab margin as indicated by broken line (dorsal view). Electrode assembly rests on cortical surface with small allowance for vertical movement. The isolated cortical slab contains 2 pairs of recording and 1 pair of stimulatory electrodes (bipolar mode) at its surface.



SAGGITAL VIEW OF CAT BRAIN NEAR LEFT SUPRASYLVIAN SULCUS



DORSAL VIEW OF CAT BRAIN

Figure 4.

This procedure prevented occlusion of the pial vessels by pressure of the electrode assembly.

The remainder of the procedures were designed to fix to the skull a miniature socket (Amphenol[R] No. 223 series - 9 contact) connected to the electrode tips by means of small, flexible multistranded cable (Siliflex[R] No. 29 - 51/46), and to protect the craniotomy and repair the scalp incision.

a. Repair of wounds

A suitably shaped piece of thin sterile absorbable gelatin film (Gelfilm[R]) was now carefully inserted over the sutured dura mater. The edges of this protective film were placed between the dura mater and the calvarium. Thus, there were now two barriers in place to minimize any effects of fibrous growth into the craniotomy. The thin gelatin film was temporary, since it is absorbed with time, but the thin silicone rubber sheet provided a more or less permanent seal over the exposed cerebrum. The area of the craniotomy was packed and covered with thick pieces of sterile absorbable gelatin sponges before applying a topical antibiotic ointment (Polymyxin B Sulfate - Neomycin, Neosporin[R]). This procedure coupled with the intramuscular injection of 300,000 IU of a depot form of penicillin G (Duracillin[R]) appeared to be adequate to prevent infections during the immediate post-operative period, and all wounds healed relatively quickly.

b. Stabilization and fixation of lead wires and connector

The flexible cables that connected the electrodes to miniature socket were arranged in a caudal direction and fixed with dental acrylic cement to a dry portion of exposed skull just posterior to their exit

from the craniotomy. The miniature plug-in socket connector was then fixed with two stainless steel screws to the right calvarium. The anterior screw was positioned in the frontal bone while the posterior screw was over parietal bone. One of the socket contacts was connected with this posterior screw and served as a reference lead for grounding purposes. Dental cement was applied around the base of the socket to envelope the anchoring screws and fill the gaps between the socket and the skull.

Once the acrylic cement had cured, the cut margins of the temporal muscles and fasciae were brought into apposition in the mid-line and repaired with interrupted sutures. The skin was also repaired with interrupted sutures. The miniature connector socket was exteriorized through a small incision. Twenty-five ml of freshly-prepared physiological poly-electrolyte solution were administered intraperitoneally a short time later. The animal was then placed in a warm cage in the chronic animal room. Prophylactic antibiotic in the form a depot intramuscular penicillin G (300,000 IU was administered every other day for a week.

The relative position and connection of the exteriorized miniature socket is indicated in the photograph included in Fig. 5.

D. Post-Operative Procedures

Recovery from the operation and the anaesthetic was usually rapid and uneventful, with the animal having regained locomotor ability, and not showing any neurological deficits (with the exception of slight lethargy) on the next day. Within 48 hours most operated cats were eating and behaving completely normally. Daily cleansing was sufficient

Figure 5. Example of Chronic Animal Connected to Stimulating-Recording Apparatus

Photograph showing location of exteriorized subminiature connector and mode of connecting electrode plate assembly to the electrical arrangements for the purpose of surface stimulation and recording. Note minimal degree of restraint imposed on animal and complete healing of skin around the subminiature plug-in connector located on the right side of the head.

Figure 5.

to cope with some few cases of slight infection of the wound margins. Without exception, all animals surviving the surgery and electrode implantation were used for the experimental studies. Animals were allowed to recover for at least 14 days before subjecting them to any experimental procedures. A few animals (6 cats) were left for longer periods before commencing studies.

Re-exposure of the chronically-isolated cortex was never required in the normal course of events. In most instances the wounds healed and sutures were removed approximately one week after the surgery. Each animal was used as long as electrical activity could be recorded and responses could be elicited. When an animal was no longer responding to stimulation it was sacrificed with an overdose of pentobarbital. The electrode assembly was exposed and removed carefully in order to examine the condition of the isolated cortical slab and adjacent cortex. Several specimens were removed for histological examination.

E. Removal of Cortex from Chronically Prepared Animals

In order to remove isolated cortical slabs for histological studies it was not possible to entirely reflect the temporal muscle and fascia from their origin without also tearing away the electrode assembly and damaging the exposed cortex in the region of the craniotomy. There was always much fibrous scar tissue and adhesions which were in close association with the flexible cable and superficial parts of the electrode assembly.

Therefore, it became necessary to tease out carefully the

small groups of tissue fibres. Underlying adhesions were cut with fine iris scissors. Providing that the dura remained well sealed and the marginal overlay was in place, very few problems were encountered with fibrous tissues infiltrating under the electrode assembly itself.

The craniotomy was expanded and enlarged with rongeurs and a similar exposure of the right hemisphere was also produced. A large block of cortex and underlying white matter was removed by means of cuts with a scalpel so as to include all of the isolated cortical slab and parts of adjacent gyri. The same procedure was employed for removing a similar portion of the homotopic right suprasylvian gyrus.

The specimens of intact and chronically isolated cortex were prepared for histological examination (Weisman, 1969) at various times post-operatively (3-6 months). Chronically-isolated cortex and contralateral homotopic cortex in 8 cats were removed by section through adjacent gyri and wide undercutting. The excised samples were rinsed with saline, fixed with freshly prepared potassium dichromate (0.5%) and osmic acid (0.2%) for 72 hours and stained using a modified Golgi Rapid Method (Weisman, 1969).

F. Electrode Design and Construction

The electrodes were eight platinum-iridium (90% - 10%, 0.010" diameter) wires beaded at the end that eventually rested on the pial surface. The beaded end was bent at right angles and inserted through small holes in a silicone stabilizing plate (0.005 - 0.010" thick), the beaded electrode tip extended 0.5 mm below the silicone plate (Fig. 3; Fig. 6). The electrodes were arranged so that the anterior pair was aligned at least 5 mm ahead of the three posterior or isolated cortex

Figure 6. Electrode Assembly Schematic

Diagram showing details and dimensions of silicone rubber plate electrode assembly, flexible lead wires and subminiature plug-in (female) connector. Note approximate location and separation of electrode tips on intact cortical and isolated cortical slab surface. The two electrodes in the centre of the slab were usually used to deliver the stimulus train to the isolated slab. Electrode tips are platinum (90%) - iridium (10%) with fire polished tips. The silicone rubber overlay is used to fix the assembly in the desired position on the cortex by means of interrupted sutures passing through the overlay and opposing intact dura mater.

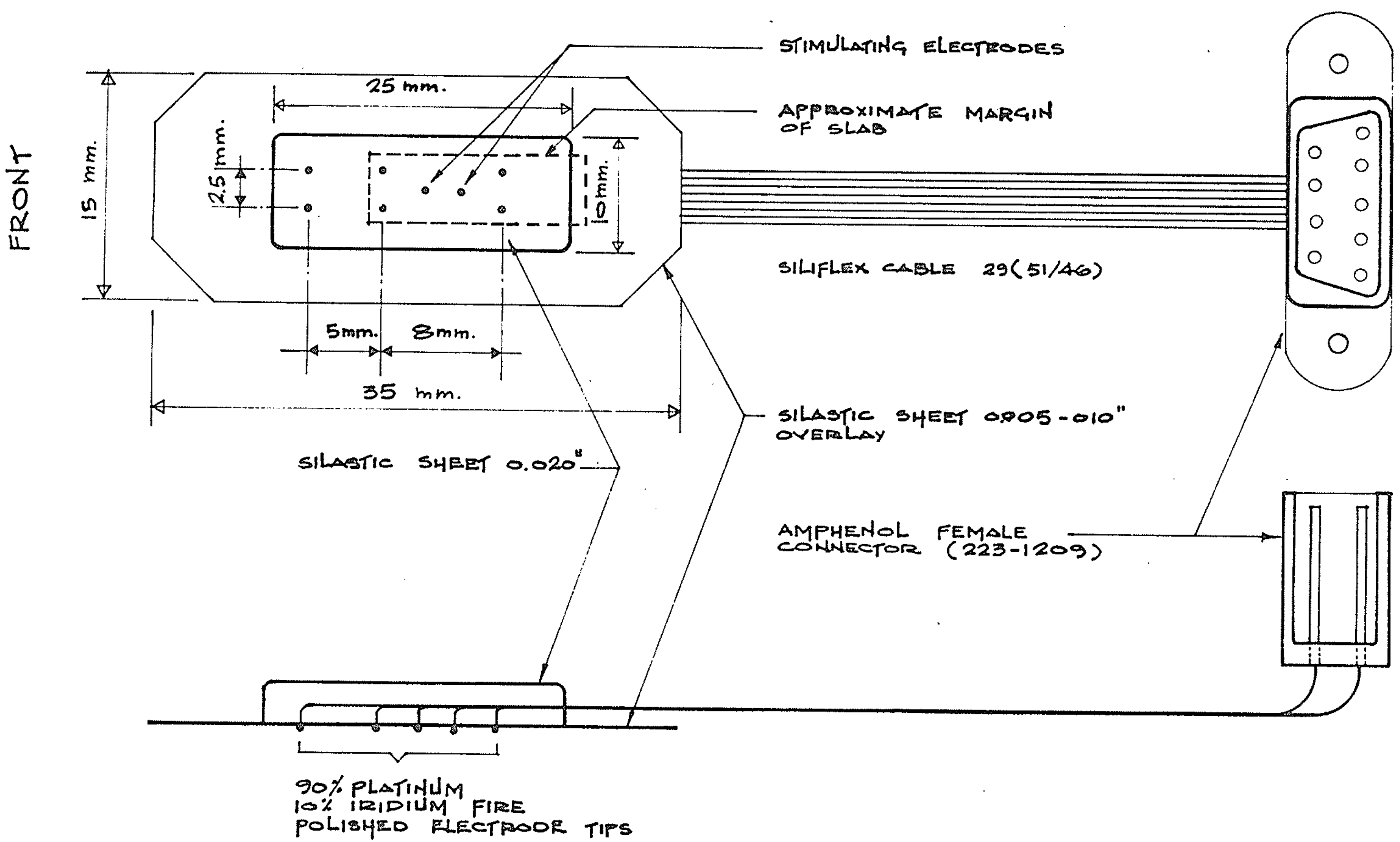


Figure 6.

electrodes. In this way the anterior pair rested or intact cortex approximately 4 mm rostral to the leading edge of the isolated cortex (Fig. 4; Fig. 7).

The 6 distal electrodes used for bipolar stimulation and recording from the cortical slab were arranged in pairs (Fig. 6). The frontal pair in a line across the isolated slab, the central pair in a line in the centre of the slab at right angles to the frontal and posterior pair of recording electrodes. The central pair of electrodes eventually resting in the centre of the isolated slab was usually used to deliver pulses from the stimulator (Fig. 7). The separation of electrode tips, except where indicated, was 2.5 mm.

The unfinished ends (not beaded) of the electrode tips were soldered to fine flexible lead wires (100 mm long) which rested flat against the stabilizing plate and extended behind the assembly. These wires were insulated with flexible silicone tubing. A piece of silicone rubber sheet (7 mm x 20 mm, 0.020" thick) was glued with silicone adhesive (Silastic[R] - type A) firmly over the area where the lead wires were soldered to the electrodes. Additional adhesive was applied such that this area was completely encapsulated and protected. The whole assembly was left undisturbed for 24 hours while the adhesive cured. The finished assembly consisted of a flexible plate approximately 3 mm thick (Fig. 3).

The free ends of the lead wires were crimped to connectors that were forced into a 9-contact Amphenol miniature socket (Fig. 3; Fig. 6). The ninth contact of the Amphenol socket was connected with the posterior screw used to fix the miniature socket to the skull (Fig. 7). The base of the socket where the lead wires entered was encapsulated

Figure 7. Block Diagram of Electrical Arrangements for Stimulation and Recording at Cortical and Isolated Cortical Surfaces

Diagram shows arrangement of electrical components to secure 3 channels of bipolar electrical records of the ECoG (1 channel from intact cortex, 2 channels from isolated cortex). Stimulating electrodes were used only to deliver the stimulus train. Duration of stimulus train determined by timer-switch. Stimulator output is floating and isolated from the isolated cortex during recording. Relays (Re) used to disconnect recording electrodes from cathode follower inputs during period of stimulation, at which point stimulator was switched into isolation unit and predetermined stimulus train was delivered from stimulus isolation unit outputs to electrodes 5 and 7. On completion of stimulus train, relays closed, switching recording electrodes back into the cathode follower inputs and stimulator output back to floating mode. Switches (S) permitted selection of capacitance or direct coupling for the recording electrodes.

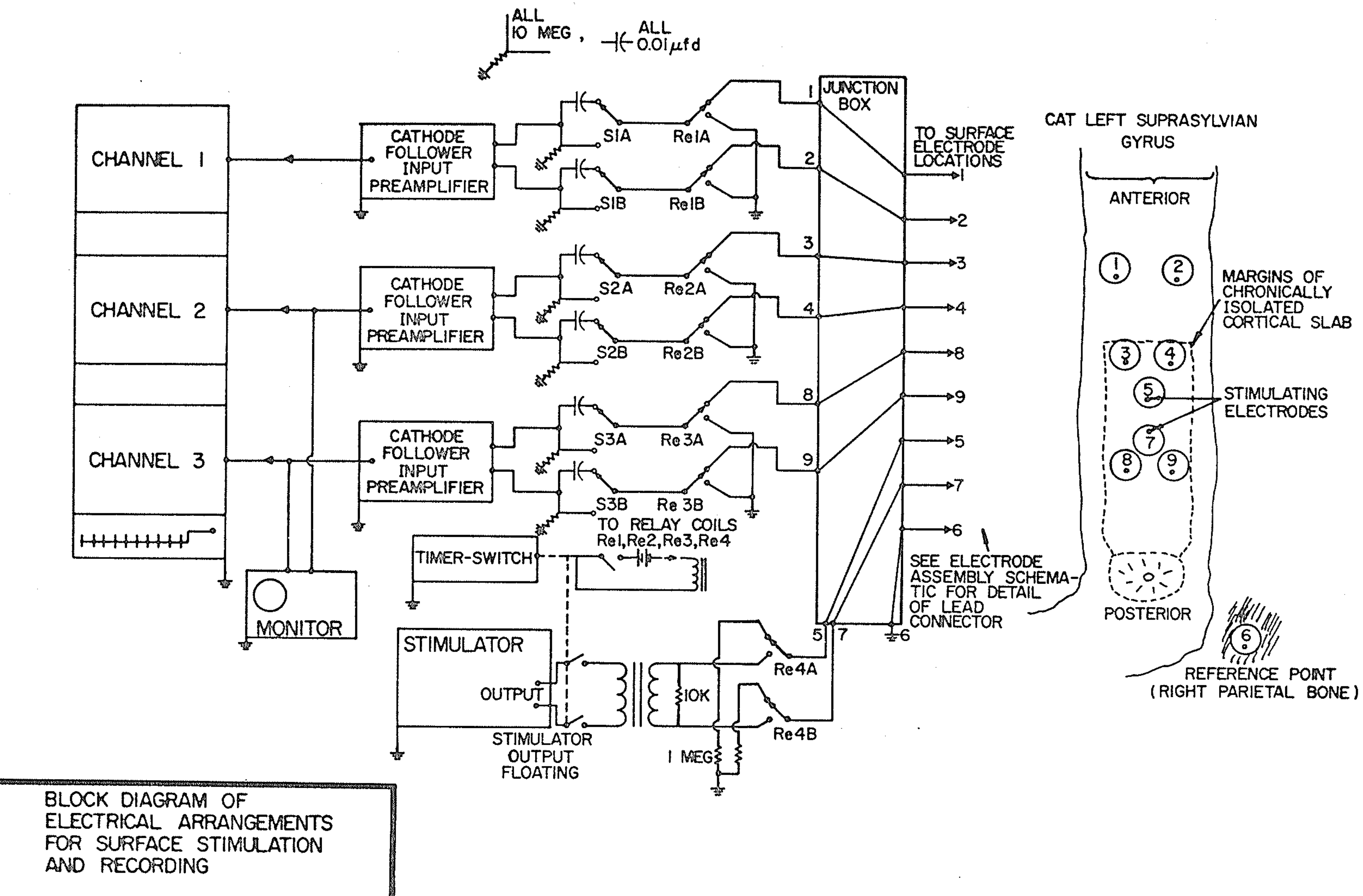


Figure 7.

with silicone adhesive.

The initial electrode assemblies were embedded in dental acrylic cement, but it was soon found that this design was unsatisfactory, due to its lack of flexibility and resultant excessive local tissue reaction to the indwelling implant. In order to overcome these problems and to increase the useful life span of the experimental preparation, the silicone electrode assembly described above was developed and found to be superior.

G. Stimulating-Recording Apparatus

For recording and stimulation, animals were placed individually inside a cubic box (1 m x 1 m x 1 m). Aside from minimal restraint imparted by the flexible coupling cable, the animals were free to move about within the chamber. Glass doors at the front of this cubic box permitted continuous observation of the behaviour and activity of the cat under study. Under these conditions it was possible to study simultaneously, the animal's ECoG, as well as its behaviour. The behavioural responses were useful as an index of the relative degree of central effects of drugs.

It was found that it was desirable to start the experimental sessions late in the afternoon because the animals were usually sedated and tolerated better the handling, stimulation, and drug injections.

The socket fixed to the head of the cat was connected to its male counterpart that led by means of a 9 conductor shielded cable to a grounded 9-jack junction box outside the recording chamber (Fig. 7). In the initial phases of this study a rotating cable-coupler filled with mercury (Sutton and Miller, 1963) was used at the top of the recording

chamber. This device also connected the cat to the junction box, but had the advantage that it would rotate when any tension was imparted to the flexible recording cable due to movements by the cat inside the chamber. However, this advantage was far out weighed by other serious problems attendant with the use of mercury pools in electronic circuitry.

A long, braided, shielded, and flexible cable (Siliflex^{R}) was found to be more practical. The 9 leads were of a low-noise, low-capacitance, miniature type and were also enclosed within a similar length of flexible plastic laboratory tubing which served to protect the leads from playful animals. The length of cable was arranged in a loop at the ceiling of the chamber by means of a rubber band to allow the experimental animal sufficient freedom to walk or lay down without discomfort or hindrance.

The junction box was simply a plug-in unit that permitted selection of any pair of electrodes for stimulation or recording. At the junction box the reference lead was connected to ground (Fig. 7). Usually the pair of electrodes over the centre of the isolated slab were connected with the isolated output of the stimulator (Grass SD5). The output of the stimulator was controlled by a relay activated electronic timer (Fig. 7). This relay maintained the output of the stimulator disconnected and grounded, while the preamplifiers were connected with the recording electrodes. At the time of stimulation the relay was closed and, simultaneously, the inputs of the preamplifiers were grounded and the output of the stimulator isolation unit was connected to the pair of stimulating electrodes (Fig. 7). Once the desired duration of stimulation was delivered the process was reversed, and the

stimulator was shunted to ground and the amplifiers were simultaneously switched back into the circuit. Thus, throughout the recording period (interstimulus interval) the stimulating electrodes were left floating. This arrangement had the advantage that it was possible to accurately reproduce stimulus train durations and it minimized the degree of amplifier blocking due to the stimulus train.

For recording purposes six recording leads (4 from within and 2 from without the slab) were connected in pairs to cathode follower stages in each of 3 preamplifiers (Grass P5 or Grass Dual P9). The output stage of each amplifier was distributed in parallel to an ink-writing oscillograph (Grass 5C-Polygraph), and for monitoring purposes to an oscilloscope (Tektronix 502). In this manner, bipolar electrical records of spontaneous activity from intact cortex (1 channel), and paroxysmal activity and EAD responses from isolated cortex (2 channels) were recorded simultaneously.

H. Parameters for Stimulation and Recording

Approximately two weeks after the surgery and implantation procedures control studies were initiated. Usually cats were stimulated using 3 sec trains of square pulses of 3 msec duration at a frequency of 30-40 Hz. In a few animals it was found that train durations less than, or greater than 3 sec (2-5 sec) were necessary to produce stable EADs. These parameters were checked in each cat during extensive control experiments prior to the drug studies.

In order to determine the threshold for each cat, stimulation was commenced using a value of 5V on the digital dial of the stimulator (Grass SD5). This value is very close to that reported by Grafstein

and Sastry (1957) for chronically-isolated cortex exposed at the time of investigation. Stimulus current strength, assumed to be proportional to pulse amplitude was increased step-wise by 10% until an EAD was elicited. In all experiments the interval between successive deliveries of stimulus trains was 20 minutes.

Once an EAD was elicited, the value was noted and increased a further 10%. This new value was designated threshold and maintained for the remainder of the experiment. Determination of threshold for each cat in this manner invariably ensured that succeeding stimulus trains, at 20 min intervals, elicited stable EAD responses on the isolated cortical slab throughout the experimental period. In most cats EAD responses could be evoked at stimulus strength reading between 10-30 volts on the stimulator digital voltage control.

With time, thresholds gradually increased. This was probably due to tissular changes (some fibrous growth) in the region of the electrode tips. When this occurred, control experiments were again performed to determine the threshold and stability of the response. Most operated cats could be stimulated for up to 6 months and respond with stable EADs.

I. Procedure for Recording and Measurement of Responses

Since the electrical activity recorded from the cat brain is subject to movement artifacts it was important that the animals remain relatively calm and undisturbed in the recording chamber. Minimal interference was achieved by commencing studies late in the afternoon. Attention to differences in behaviour between the different animals also made for 'cleaner' records.

Three channels of bipolar electrical activity were recorded simultaneously on an ink-writing oscillograph operated at a paper speed of 10 mm/sec. One channel recorded spontaneous activity from intact cortex rostral to the isolated slab, while the second and third channels recorded the activity from the other two pairs of electrodes which were resting on the isolated cortex (Fig. 7).

EADs were measured from the point of withdrawal of the stimulus train, i.e. after the last pulse, to the point of abrupt and usually well-defined termination of spiking activity. This point was relatively easy to detect and was noted on the recording paper.

A great deal of effort during the first few sessions with naive animals was devoted to experiments designed to determine the effect of repeated stimulation on the stability and reproducibility of the response. These experiments attempted to assess the role of fatigue and changes in duration of EADs, a problem frequently encountered in earlier studies (Rech and Domino, 1960; Sharpless and Halpern, 1962). Control injections of saline or distilled water (0.5 - 2.5 cc/kg) were also included to familiarize and condition the animals to the injection procedure.

Prior to commencing a daily stimulating recording session, calibration of the amplifiers and stimulator was performed. It was soon discovered that the output of the stimulator (Grass SD5) could vary from day to day. Consequently, appropriate corrections were made as required. It was assumed that the surface electrodes remained in contact at all times. If negative responses were encountered during an experiment the animal was rested overnight and a short control experi-

ment at the same threshold was conducted the next day. If the response had 'returned', it was assumed that the electrodes were in contact on the previous day and that a real effect had been produced, i.e. complete arrest of EAD duration had occurred. Otherwise, the experiment was discarded, as were those in which EAD duration was unstable.

J. Experimental Design and Protocol

Once the optimal level of stimulus parameters (just above threshold by approximately 10%) had been determined for each cat, each animal was stimulated using this pre-determined value 3 times, at inter-stimulus intervals of 20 min. This group of EADs composes the "Control Measurements" (Fig. 8). If these measurements agreed well with previous responses in that cat, the experiment was continued as follows: Fifteen minutes after the last Control Measurement, saline or drug-free vehicle was injected intraperitoneally (IP). Five minutes after this injection, 3 successive EADs were elicited using the same stimulus parameters and schedule in each cat. This group of 3 EADs constitutes the "Saline Control Measurements" (Fig. 8).

Fifteen minutes after the last Saline Control Measurement, the drug under study was injected IP. Five minutes after the drug injection, 3 successive, EADs were elicited still using the same stimulus parameters delivered at 20 min intervals. This group of EAD measurements constitutes "Drug Treatment-1" afterdischarge measurements.

This protocol was adhered to after each successive injection of drug, i.e. Treatment-2, Treatment-3, etc., in each experiment. Usually there were not more than 4 treatments in one experiment on each cat.

Figure 8. Protocol for Experimental Design

Block diagram showing procedure used to determine threshold stimulus intensity for an EAD in each animal. All stimulus trains administered at 20 min intervals. Once threshold was determined, stimulus train amplitude was increased by 10 percent and three stable EADs were determined (Control Measurements). Note control and saline control measurements to assess stability of EAD duration before proceeding with a drug study. Injections precede a stimulus train by approximately 5 min. Differences in EAD duration following treatments are obtained by subtracting Treatment EAD duration from appropriate control EAD duration.

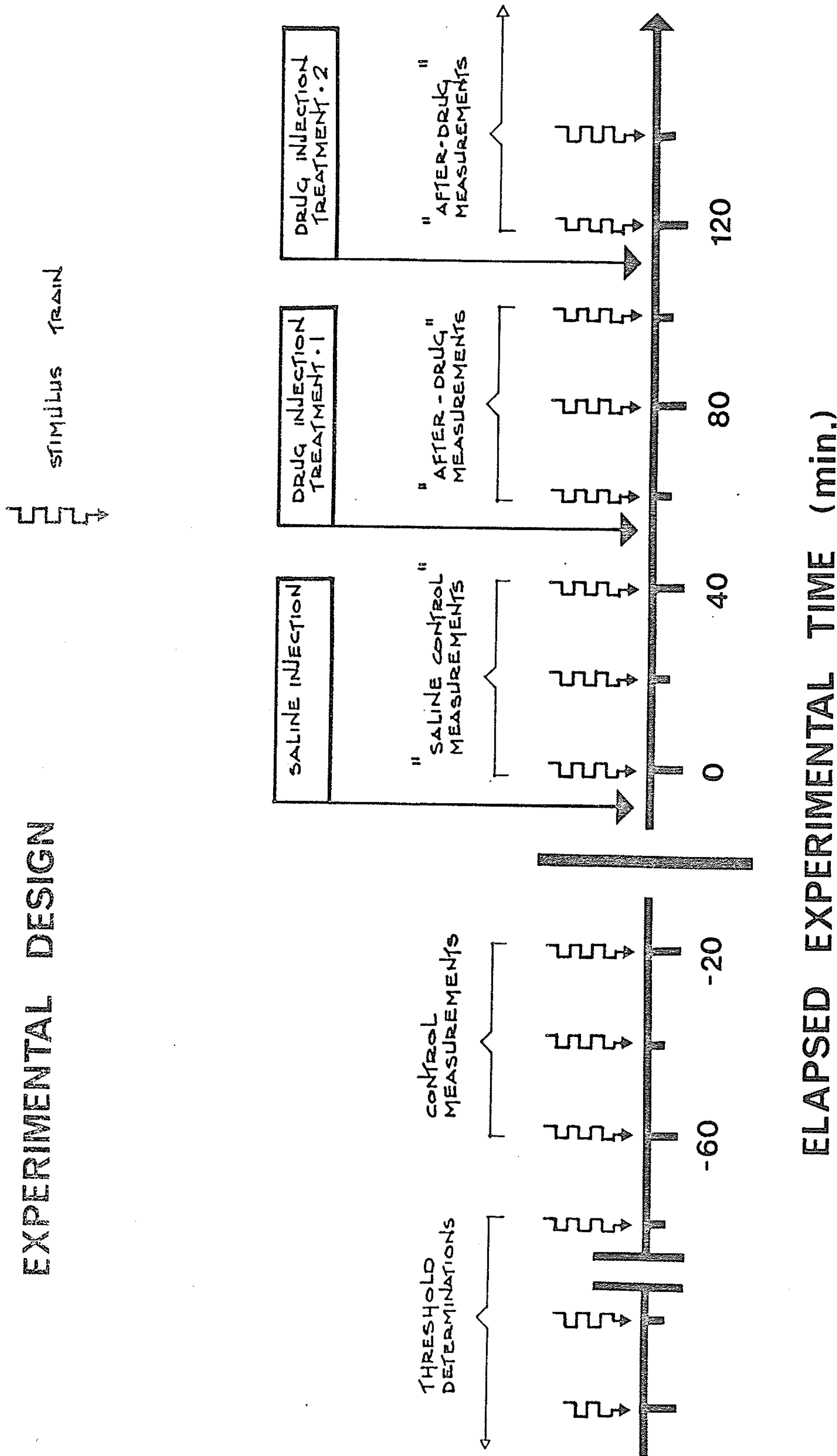


Figure 8.

If it became necessary to observe the effects of drugs for longer periods, it was customary to include more measurements in the particular Treatment Group. The responses were elicited as always, maintaining the predetermined fixed level of stimulus and delivery schedule in each cat, and 6 or more EADs were included in the Treatment Group.

A more thorough analysis of the parameters for stimulation was not undertaken as this has been the subject of previous studies (Pinsky and Burns, 1962; Reiffenstein, 1964; Sanders and Pinsky, 1967). If the Control and Saline Control Measurements were found to be unstable and differ significantly from each other, the experiment on that animal was terminated and a different cat was selected, and put through the same protocol. The Control Measurements compared to previous controls in each cat provided a good indication of the stability and reproducibility of the response during succeeding experiments. Administering saline instead of drug for several treatments and eliciting responses at the fixed parameters of stimulation for several hours of elapsed experimental time was also used as a check on the effects of repeated stimulation in each cat on EAD duration over a time period corresponding to an experimental session.

For the drug studies, experiments were repeated on several different cats to collect sufficient data for statistical analysis of the effects of a particular treatment. Each cat is it's own control.

K. Tabulation and Calculation of Data

For the purpose of estimating the variability of successively elicited EADs during an experiment, the durations of the three EADs

elicited during the "Saline Control" period (Fig. 8) were subtracted in the following manner from the durations of the three EADs elicited during the "Control Measurements" (Fig. 8): The duration of the first EAD elicited during the "Saline Control" was subtracted from the duration of the first EAD elicited during the "Control Measurement"; the duration of the second "Saline Control" EAD was subtracted from the duration of the second "Control Measurement" EAD; and the duration of the third "Saline Control" EAD was subtracted from the duration of the third "Control Measurement" EAD.

This calculation produces for each testing interval three "Control" differences in EAD duration. From the results of several experiments in each study these "control" differences in EAD duration were tabulated in order. For each point, i.e. the 0 min, 20 min, and 40 min of elapsed experimental time, the three differences in after-discharge duration for each cat were summed respectively to calculate the mean "Control" differences in EAD duration in sec, plus or minus the standard error (S.E.) of the mean at 0 min, 20 min, and 40 min of elapsed experimental time, i.e. a measure of the variability of our controls. Thus each animal was its own control.

For estimating the effects of drug treatment, the duration of the first EAD elicited during the "Treatment-1" period, was subtracted from the duration of the first EAD elicited during the "Control Measurement", the duration of the second "Treatment-1" EAD was subtracted from the duration of the second "Control Measurement" EAD, and the duration of the third "Treatment-1" EAD from the duration of the third "Control Measurement" EAD. The same procedure was applied if there was more

than one drug treatment. The EAD durations obtained under "Treatment-2", or "Treatment-3", etc. were always subtracted from the corresponding (in order of elicitation) EAD durations of the "Control Measurements" in the same way.

In this way were obtained the differences in EAD duration in sec for each testing interval after a drug was administered. These differences from "Control" in EAD duration were then tabulated in order, for each testing interval, and the results from several experiments were used to calculate the mean difference from "Control" in EAD duration, during a treatment, plus or minus the standard error of the mean (Steel and Torrie, 1960).

Calculating the data in this manner was found to be a better method for subsequent analysis of data since the changes in EAD duration provided a more consistent index of drug effects on afterdischarges, than did the use of proportional factors. Since the calculated mean differences in EAD duration in sec corresponded to the points in time at which EADs were elicited, they were also used to plot the results of my studies.

In some few cases the absolute values for EAD duration in seconds were tabulated in order for each experiment. For each point the mean EAD duration in seconds plus or minus its standard error was then calculated and plotted.

L. Statistical Analysis of Results

These experiments were designed such that 3 replications of an EAD measurement were obtained under a specified condition or treatment, i.e. a treatment group. The replications were tabulated in order

of their elicitation for each experiment. For each drug study the EAD durations of all experiments (at least 4 cats) were arranged in tables respectively. The data were then grouped and designated in the table according to the particular treatments.

Classifying the results of several experiments in this manner was adopted to facilitate statistical analysis of the data with a randomized complete block analysis of variance with subsampling (multiple entries per cell; Steel and Torrie, 1960). The level of significance for changes in EAD durations between a treatment group and its own control was determined from the studentized ranges calculated for each treatment group. The level of significance was obtained from significant studentized ranges for the 5 percent and 1 percent level (Steel and Torrie, 1960), based on the number of means in the comparison. Multiple comparisons between the various treatments on the experiments in a drug study were performed at the same time with Duncan's new multiple range test (Steel and Torrie, 1960).

M. Graphical Presentation of Results

Elapsed experimental time in min is plotted along the abscissa. Either the calculated mean EAD duration of mean difference in EAD duration from control is plotted along the ordinate in seconds for every 20 min of elapsed experimental time. The variability for each point (20 min inter-stimulus interval) is estimated by a pooled standard error (S.E.) for all the experiments in that study by an "X" (Steel and Torrie, 1960). This estimate of error is smaller than the individual S.E. of the mean or mean difference in sec for each point on the graph, but has the advantage that it is calculated on the basis of taking into account

all the measurements of the experiments in the given study. Thus, it incorporates a better assessment of variability and error in EAD measurements as they occur throughout the whole study and relates it to all points. Since this S.E. is obtained from the analysis of variance calculation it is relatively easy to obtain and facilitates production of the graphs. The graphs depicting the results of drug and other studies represent the course of mean changes in EAD duration measured on the oscillograph records.

In some cases only a few experiments (1-3 cats) were completed. In these cases the results are presented in tabular form throughout the results section. These results were subjected to the same calculations and the appropriate significance, based on the fewer observations, are presented. In all cases $P < 0.01 - 0.05$ was selected as the significant probability.

N. Drug Studies

The following drugs in the form designated were used in the experiments reported in this thesis:

a. Cholinergic drugs

1. Acetyl-β-methylcholine chloride; (Methacholine, Sigma Co.)
2. Arecoline hydrochloride; (Sigma Co.)
3. Carbamylcholine chloride; (Carbachol, Koch-Light Labs)
4. dl-Muscarine iodide; (Geigy Ltd.)
5. Physostigmine salicylate; (Eserine, Sigma Co.)
6. Pilocarpine hydrochloride; (Sigma Co.)
7. Oxotremorine sesquifumarate; (Aldrich Chemical)
8. 4-(m-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride; (McN-A-343, McNeil Labs)

9. N-benzyl-3-pyrrolidyl acetate methobromide; (AHR-602; A.H. Robins Co.)

10. Lobeline sulfate; (Sigma Co.)

11. Nicotine sulfate; (Sigma Co. obtained in solution)

b. Anticholinergic drugs

1. Atropine methylnitrate; (Sigma Co.)

2. Atropine sulfate; (Sigma Co.)

3. Procyclidine hydrochloride; (Kemadrin, Burroughs & Wellcome Ltd.)

4. Scopolamine hydrochloride; (hyoscine, Sigma Co.)

5. Trihexyphenidyl hydrochloride; (Benzhexol, Lederle Labs.)

6. Dihydro-β-erythroidine hydrobromide; (Merck, Sharp & Dohme, Ltd.)

7. Mecamylamine hydrochloride; (Inversine, Merck, Sharp & Dohme, Ltd.)

c. Sympathomimetic drugs

1. d-amphetamine sulfate; (Sigma Co.)

2. 2-(2,6-Dichlorophenylamino)-2-imidazoline hydrochloride; (Clonidine, Boehringer Ingelheim Labs.)

3. 1-β-3,4-dihydroxyphenylalanine; (1-dopa, Sigma Co.)

4. 1-ephedrine sulfate; (Sigma Co.)

5. Hydroxyamphetamine hydrochloride; (Smith, Kline & French Labs.)

6. Methamphetamine hydrochloride; (Desoxyn, Abbott Labs.)

7. Methoxamine hydrochloride; (Vasoxyl, Burroughs & Wellcome Ltd.)

8. Pargyline hydrochloride; (Eutonyl, Abbott Labs.)

9. 1-phenylephrine hydrochloride; (Neo-Synephrine, Winthrop Labs.)

10. Tyramine hydrochloride; (Koch-Light Labs.)

11. dl-isoproterenol sulfate; (Sigma Co.)

d. Adrenergic receptor blocking drugs

1. Chlorpromazine hydrochloride; (Largactil, Poulenc Ltd.)

2. 1,4-Bis(1,4-benzodioxan-2-ylmethyl) piperazine; (Dibozane, McNeil Labs.)

3. Haloperidol; (Haldol, McNeil Labs.)

4. Phenoxybenzamine hydrochloride; (Dibenzyline, Smith, Kline & French Labs.)

5. Phentolamine hydrochloride; (Rogitine, Ciba Ltd.)

6. 4-(2-dimethylaminoethyl)-5-isopropyl-2-methyl phenyl acetate; (Thymoxamine W.R. Warner Ltd.)

7. Tolazoline hydrochloride; (Priscoline, Ciba Ltd.)

8. Dichloroisoproterenal hydrochloride; (Sigma Co.)

9. D(-)-1-(4-nitrophenyl)-2-isopropylaminoethanol hydrochloride; (D-INPEA, Selvi Ltd.)

10. L(+)-1-(4-nitrophenyl)-2-isopropylaminoethanol hydrochloride; (L+INPEA, Selvi Ltd.)

11. Practolol; (Ayerst Labs.)

12. Pronethalol (Ayerst Labs.)

13. d-propranolol; (Ayerst Labs.)

14. dl-propranolol; (Inderal, Ayerst Labs.)

e. Catecholamine depleting agents

1. Disulfiram; (Antabuse, Ayerst Labs.)

2. Bis(4-methyl-1-homopiperazinyl-thiocarbonyl) disulfide; (FLA-63; AB-ASTRA Ltd.)

3. dl-1-methyltyrosine methylester hydrochloride; (H 44/68; AB-Biotec Ltd.)

f. Serotonin drugs

1. 5-hydroxy-dl-tryptophan; (5-HTP, Sigma Co.)

2. 5-hydroxytryptamine Creatinine sulfate complex; (5-HT, Sigma Co.)

3. Serotonin hydrogen maleate; (Sandoz Ltd.)

g. Serotonin antagonist drugs

1. benzo (4,5) cyclohepta (1,2-b) thiophene; (BC-105, Sandoz Ltd.)

2. 2-bromo-lysergic acid diethylamide; (BOL, Sandoz Ltd.)

3. Cyproheptadine hydrochloride; (Periactin, Merck, Sharp & Dohme Ltd.)

4. 1-methyl-8 β-carbobenzyloxy-aminomethyl-10 α ergoline; (Methergoline, Burroughs & Wellcome Labs.)

5. 1-methyl-d-lysergic acid butanolamide hydrogenmaleate; (Methysergide, Sandoz Ltd.)

h. Serotonin depleting agents

1. DL-p-chloroamphetamine hydrochloride; (Regis Chemicals)

2. p-Bromo-methamphetamine hydrobromide; (Chinoin Pharm.)

3. p-Chloro-dl-phenylalanine; (Sigma Co.)

4. α-propyldopacetamide; (H22/54, Aldrich Chemicals)

i. Extracerebral decarboxylase inhibitor

1. N(DL-seryl) N-2,3,4-trihydroxybenzyl) Hydrazine; (Ro4-4602, Hoffmann-LaRoche Co.)

j. Anticonvulsant drugs

1. Diphenylhydantoin sodium; (Dilantin, Parke Davis & Co.)

2. Ethosuximide; (Zarontin, Parke Davis & Co.)

3. Pentobarbital sodium; (Nembutal, Abbott Labs.)

4. Phenobarbital sodium; (British Drug Houses Ltd.)

5. Sodium bromide; (British Drug Houses Ltd.)

6. Trimethadione; (Trimedone, Abbott Labs.)

k. Benzodiazepine derivatives

1. Chlordiazepoxide hydrochloride; (Librium, Hoffmann-LaRoche Ltd.)

2. 7-nitro-5-(2-chlorophenyl)-3H-1,4 benzodiazepine-2(IH) one; (Ro5-4023, Clonazepam, Hoffmann-LaRoche Ltd.)

3. 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepine-2-one; (Diazepam, Valium, Hoffmann-LaRoche Ltd.)

1. Miscellaneous compounds

1. dl-4-gamma-amino butyric acid (GABA, Koch-Light Labs.)

2. Ethyl alcohol; (Ethanol, British Drug Houses Ltd.)

3. 5-ethyl-5(1,3-dimethylbutyl) barbituric acid sodium, (DMBB, Lilly Research Labs.)

4. 5-(1,3-dimethylbut-2-enyl)-5-ethyl barbituric acid; McN-481, McNeil Labs.)

O. Vehicles for Solubilizing Drugs

For the majority of the drugs injected, simply dissolving the substance in sterile saline or water for injection was the usual procedure. All solutions were made up fresh daily at the appropriate concentration required, and any excess was usually discarded at the conclusion of experiments. Drug concentration was adjusted so that maximal injection volumes never exceeded 1 cc/kg.

In certain cases substances proved to be very difficult to dissolve and it became necessary to resort to using a multicomponent vehicle which would solubilize the required concentration of drug. Whenever these vehicles were utilized they were tested out extensively in preliminary control studies to determine if there was any effect on EAD duration of the drug-free vehicle, in amounts that corresponded to an injection volume for the required dose of drug.

One vehicle that proved very useful was the one used to solubilize diphenylhydantoin sodium (DPH). This had the following composition:

Propylene glycol	-	40 ml
Ethyl alcohol	-	10 ml
Distilled water	-	50 ml

As DPH is an insoluble substance it is recommended that the hydrogen ion

concentration of this vehicle be adjusted to pH 12. In my experience this last step was omitted and DPH was dissolved to give a final concentration of 30 mg/ml. This solution was stable for up to 5 hours. Fresh solutions were always made up before each injection to avoid using solutions that may have contained precipitated crystals of drug.

The vehicle used to solubilize haloperidol had the following composition:

Lactic acid	-	0.1 ml
Hot distilled water	-	20.0 ml
Propylparaben		0.1 mg
Methylparaben		10.0 mg

This 20 ml of vehicle was used to dissolve 100 mg of haloperidol pure substance, producing a final concentration of haloperidol 5 mg/ml.

The vehicle used to solubilize diazepam had the following composition:

Propylene glycol	-	40	ml
Ethyl alcohol	-	10	ml
Sodium benzoate & Benzoic acid	-	5	gm
Benzyl alcohol	-	1.5	ml
Distilled water	-	100	ml

Diazepam dissolved in this vehicle to produce a final concentration of 2 mg/ml.

P. Agents Requiring Special Vehicles for Dissolution

Phenoxybenzamine hydrochloride was dissolved in propylene glycol to produce a final concentration of 50 mg/ml. This solution was acidified with 0.1 N HCl, 0.1 ml per 20 ml of drug solution and prepared daily as required.

Disulfiram was solubilized in 1% carboxymethylcellulose and 0.4% of Tween 80 in saline to produce a final concentration of 200 mg/ml.

At best, this resulted in a fine suspension which was used for IP administration.

The serotonin depleting agents p-chlorophenylalanine was dissolved in the following vehicle described by Wada and Terao (1970).

Propylene glycol	-	2 ml
Sodium hydroxide 3N to	-	pH 12
Hydrochloric acid 3N to	-	pH 7
Distilled water	-	3 ml

The solution was diluted with water to give a final concentration of para-chlorophenylalanine 50 mg/ml.

The following agents were dissolved using the DPH vehicle:

i -	Diphenylhydantoin sodium	-	30 mg/ml
ii -	Trimethadione	-	200 mg/ml
iii -	Clonazepam, Ro5-4023	-	1 mg/ml

Haloperidol and diazepam were used in the vehicles described earlier. These are the vehicles in which these compounds are supplied commercially for parenteral administration.

IX. EFFECTS OF CHRONIC ISOLATION ON CEREBRAL CORTEX

A. Histological Changes

Previous work in this laboratory (Reiffenstein, 1964; Weisman, 1969) has shown that chronically-isolated slabs of cerebral cortex undergo shrinkage and tissue deformation. In coronal sections the regular rectangular isolated slab, after chronic isolation appears to be transformed into a triangular-shaped mass with the apex directed towards the white matter (Weisman, 1969). A similar result was observed in the present study (Fig. 9A, 9B). The staining technique employed was a modification of Golgi's Rapid Method (Weisman, 1969). Superficially, there was a mild and localized depression over the isolated slab and part of the left suprasylvian gyrus, extending laterally to the marginal and ectosylvian gyri and anteriad to the ansatus sulcus. Thus, the surface of the cortex in the isolated region becomes slightly flattened, probably due in part to the presence of the electrode assembly.

The area of cortex, including the slab, covered by the electrode assembly appeared adequately vascularized (Fig. 10A) and no gross discrepancies between the intact and isolated cortex in this respect were observed. The slab outline was reasonably visible, and the isolated region was readily differentiated from adjacent cortex. Blood vessels were present throughout the slab and adjacent regions and apparently were filled with blood cells (Fig. 10A). Structures resembling small blood vessels could be seen coursing down into the cortex (Fig. 10A). The pial surface area directly under the electrodes showed some thickening (Fig. 10A; 10B) and was covered with tough leptomeninges. Changes of the pia were for the most part minimal, with some thickening and fibrosis in evidence.

Coronal Sections of Isolated Cortical Slab and Adjacent Tissue

Figure 9. ⌊__________⌋ (1 mm), magnification X3 (#GK-12). Cortical Slab removed 4.5 months after surgical deafferentation. Note completeness of isolation and separation of grey and white matter. Notice also uniform impregnation of neuronal elements in isolated slab and adjacent intact cortex. Pia mater over cortical slab is continuous with adjacent pia mater.

Figure 9A. ⌊______________⌋ (1.5 mm), magnification X10 (#K-16). Cortical slab removed 6 months after isolation. Note completeness of isolation and blood vessels coursing down from continuous pia into superficial layers of cortical slab.

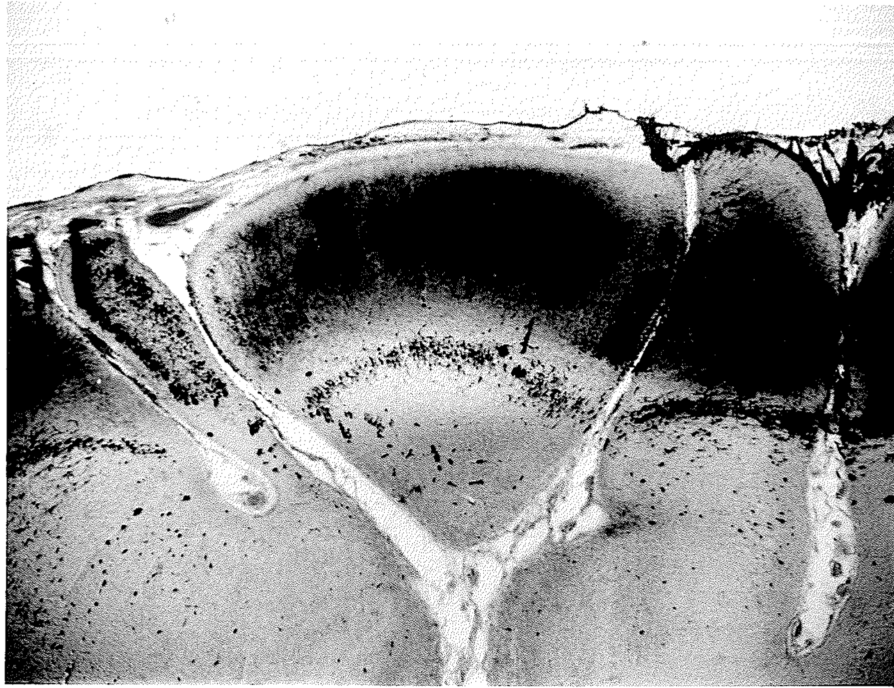


Figure 9.

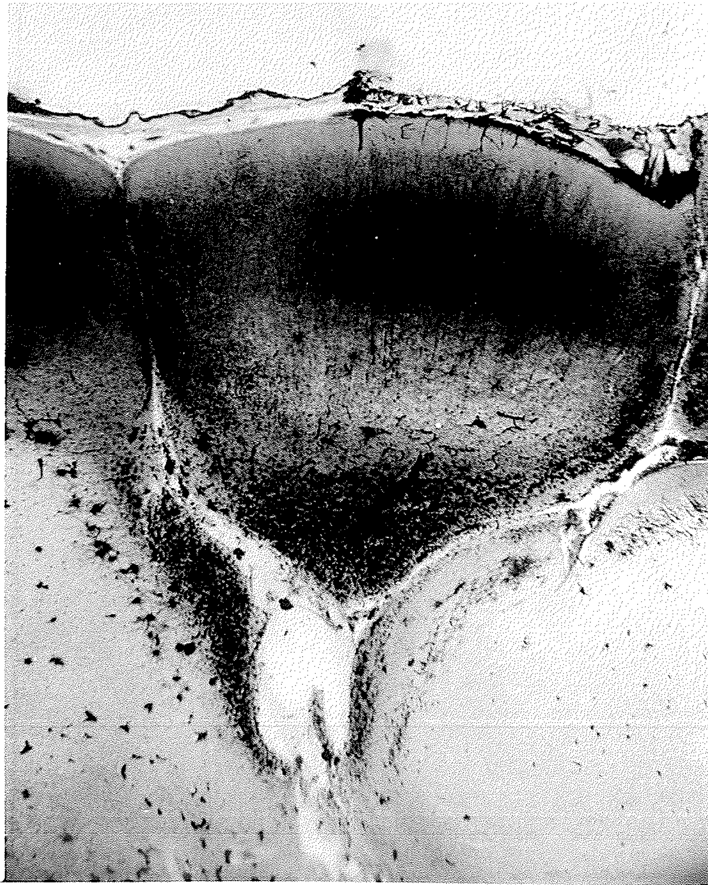


Figure 9A.

Figure 10A. |______________________| (200 microns), magnification X100 (#G-4). Note engorged lumen of pial vessel just above isolated cortex. Smaller pial vessels are seen to pass down into the superficial layers of the isolated slab of cerebral cortex. Note relative paucity of cortical elements in this region.

Figure 10B. |______________________| (0.5 mm), magnification X40 (#GK-12). Note small pyramidal cells in layer V and relative absence of cortical elements in layer I. A large impregnated protoplasmic astrocyte is visible just below layer V. Another zone apparently devoid of cortical elements extends below layer V for about 0.25 mm to a discrete layer of spindle cells in the white matter (refer also to Fig. 9). Note thickness of fibrotic tissue on the cortical surface.

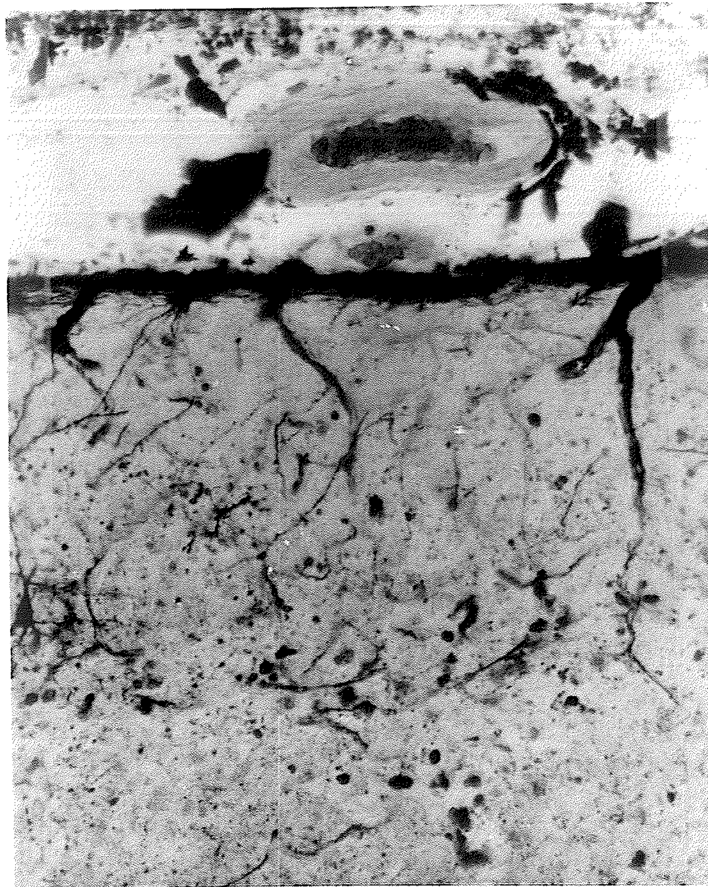


Figure 10A.

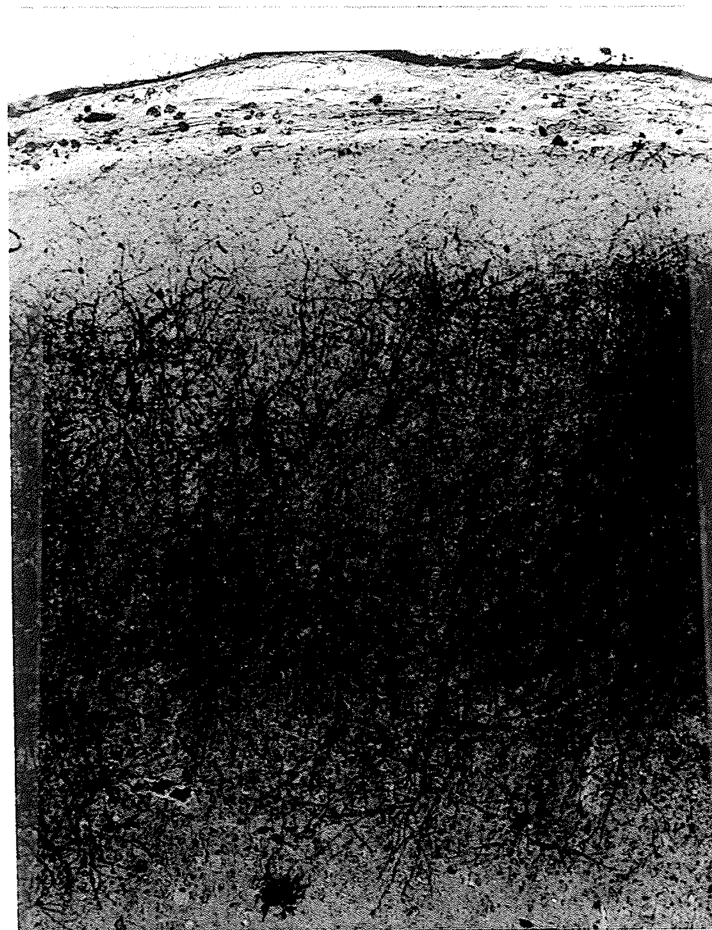


Figure 10B.

The lower two-thirds of the slab show large cysts or glial scars at the site of the original lesion (Fig. 9A; 9A) and are apparently devoid of normal cellular elements. There is some disruption of the laminar organization due to infiltration with neuroglia, but the lamination is still evident. There is disappearance of large pyramidal cells in layer V which agrees with previous findings (Reiffenstein, 1964; Weisman, 1969; Krnjevic _et al._, 1970) and an increase in observable astrocytes, especially in the lower area. The total number of neurons is decreased.

There were no signs of neural continuity with intact cortex by means of intact microscopic bridges in the region of the molecular layer. No neural connections between the slab and the cortex adjacent to the slab were ever seen in the coronal sections (Fig. 9A, 9B). A short distance beyond each cut there was generally no cellular distruction (Fig. 9B).

It is clear that many neurons remain in cortex isolated for periods up to 6 months (Fig. 10B). There is an increase in the number of glial cells, primarily astrocytes and microglia (Fig. 10B; 11A; 11B). The difference between neurons and astrocytes is quite clear, with many of the neurons showing a conical dendritic processes. The astrocyte cell body tended to be comparatively amorphous with more processes extending from it in a highly disorganized pattern, i.e. fibrous astrocytes.

A major visible effect of chronic isolation appears to be the almost complete loss of large pyramidal cells which are normally found in Layer V. There remain a number of small pyramidal cells in this layer (Fig. 10B, 11B). There is evidence of some lamination of cortical

Coronal Sections from Non-isolated Homotopic Cerebral Cortex

Figure 10C. Photograph shows layer of spindle cells at point of division of grey and white matter. Note relatively small region of stain-free elements between layer V and spindle cell layer.

Figure 10D. Photograph shows elements present in non-isolated homotopic cortex. Note profusion of long dendrites with pronounced arborization in superficial layers of the cortex. Notice also the density of elements in middle and deep portions of cerebral cortex.

Figure 10C.

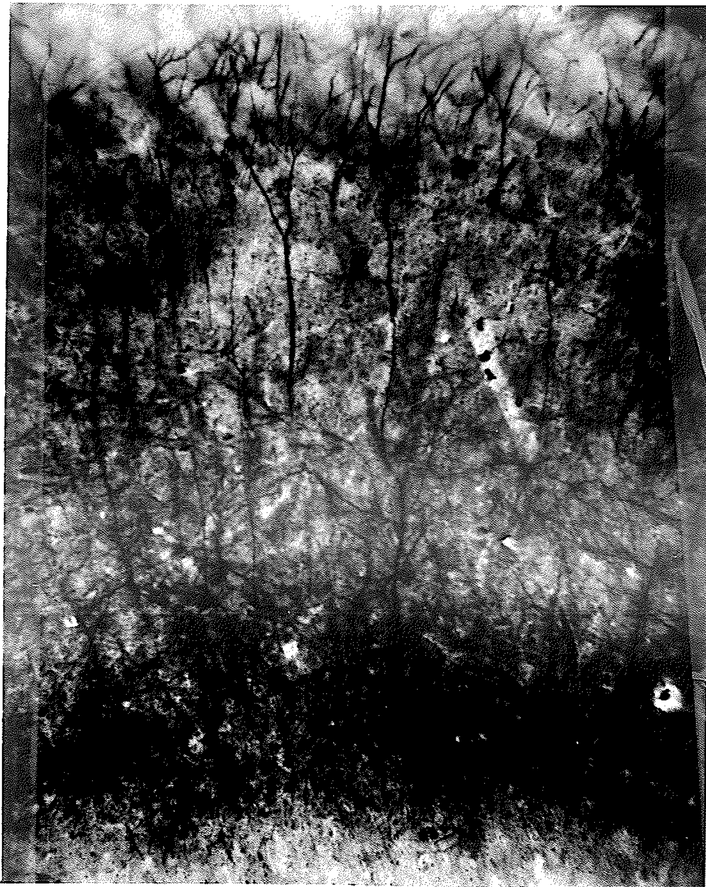


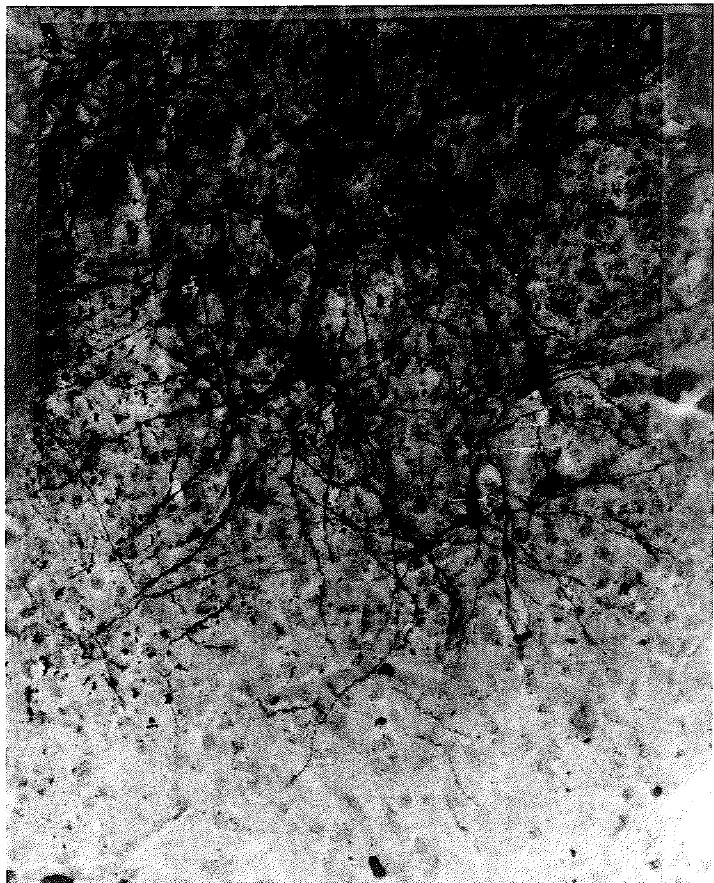
Figure 10D.

Coronal Sections from Isolated Cortex

Figure 11A. ⌊________________⌋ (200 microns), magnification X100 (#GK-12). Photograph showing small pyramidal cells in layer II. Dendrites are continuous with neuronal soma, but do not show extensive terminal arborizations into layer I. Protoplasmic astrocyte is present in subpial cortex. Some dense fibrotic tissue is present in the pia.

Figure 11B. ⌊__________⌋ (120 microns), magnification X100 (#GK-12). Photograph of coronal section showing numerous small pyramidal cells in layer V. Note basilar dendrites extending tangentially from pyramidal cell bodies. Notice also relatively large region below layer V not staining for cortical elements and which extends downward for about 300 microns up to spindle cell layer (not shown).

Figure 11A.



cell types, but on the whole there is disorganization. Chronically isolated cortical slabs undergo shrinkage in all aspects (Reiffenstein, 1964; Weisman, 1969), however, except for the loss of the major cell types it is not clear in which of the cortical layers the major portion of this shrinkage occurs.

Comparison of coronal sections from isolated regions with those of non-isolated cortex in the homotopic right suprasylvian gyrus gives some indication of other changes that take place in chronically isolated cortex. Beneath layer V in the isolated slab there is a relatively large gap apparently devoid of neurons which extends to the layer of spindle cells demarcating the white matter (Fig. 10C). In control cortex this gap is approximately 200 microns wide, whereas in isolated cortex (Fig. 9A, also measured in 10B) this gap is now 400-500 microns wide. This indicates that a great majority of the cells in the cortical depths have degenerated following chronic isolation.

There appears to be a major loss of dendritic and cellular elements from more superficial (layer I) regions of the isolated region. In a control section (Fig. 10D) it is clear that there are extensive dendritic arborizations in the area just below the pia. Contrasting this to the same region in isolated cortex (Fig. 10A; 10B; 11A), it is readily apparent that these elements are no longer demonstrated and this region is comparatively devoid of recognizable cells. Thus, it appears that the majority of cortical cells remaining are located in the central portions of the isolated region.

The technique of replacing the dura mater and packing the osteotomy with absorbable gelatin film and sponge (Halpern, 1961) prevented and delayed, to a great extent, the development of thick fibrous

membranes often observed over the isolated region (Halpern, 1961). This practice extended the useful life of the implanted electrode assembly since direct apposition between the electrodes and the cortical surface is essential for function and sustained performance. Examination, at autopsy, in a few animals that ceased to respond to stimulation within a short time post-operatively (< 5 weeks) revealed either a highly organized clot (1 cat), marked fibrotic encapsulation at the electrode assembly (5 cats), and unknown factors (1 cat).

B. Electrophysiological Behaviour

During the first few days post-operatively, only intermittent bursts of spontaneous activity could be recorded from the isolated slab. After a week of isolation the isolated cortex shows irregular and abnormal spontaneous bursts, which resemble the spontaneous "after-bursts" reported by Burns (1954) on acutely-isolated cortex previously conditioned with several weak stimuli. These bursts of spontaneous electrical activity were observed whether the operated cats were stimulated or not.

Two to four weeks post-operatively a readily apparent and much more regularly structured form of spontaneous electrical hyper-activity develops on the isolated cortex of animals not receiving any previous direct stimulation. This activity is characterized by slow waves of 100-150 µV at frequencies of 1-2Hz, rapid spike-like excursions, and short bursts of high frequency, that can be seen in the control records before stimulation (Fig. 12A).

a. Epileptiform afterdischarges

Following the withdrawal of a threshold stimulus, (3 msec monophasic pulse, 30-40 Hz, and 2-5 sec train duration) the EAD consists

Epileptiform Afterdischarges (EADs)

Figure 12A. Examples of afterdischarges elicited from 3 different chronically neuronally isolated slabs of cerebral cortex. The upper most tracing in each of A, B and C is the ECoG recorded from adjacent non-isolated cortex. Note the difference in patterns between non-isolated and isolated regions. Notice also abrupt termination of epileptiform afterdischarge. EAD duration of approximately 40 sec, is the average duration recorded from these preparations.

Figure 12B. Example of an EAD showing typical "clonic" bursts interrupted by relatively short (< 2 sec) periods of apparent electrical silence towards latter stages. Stimulation at S.

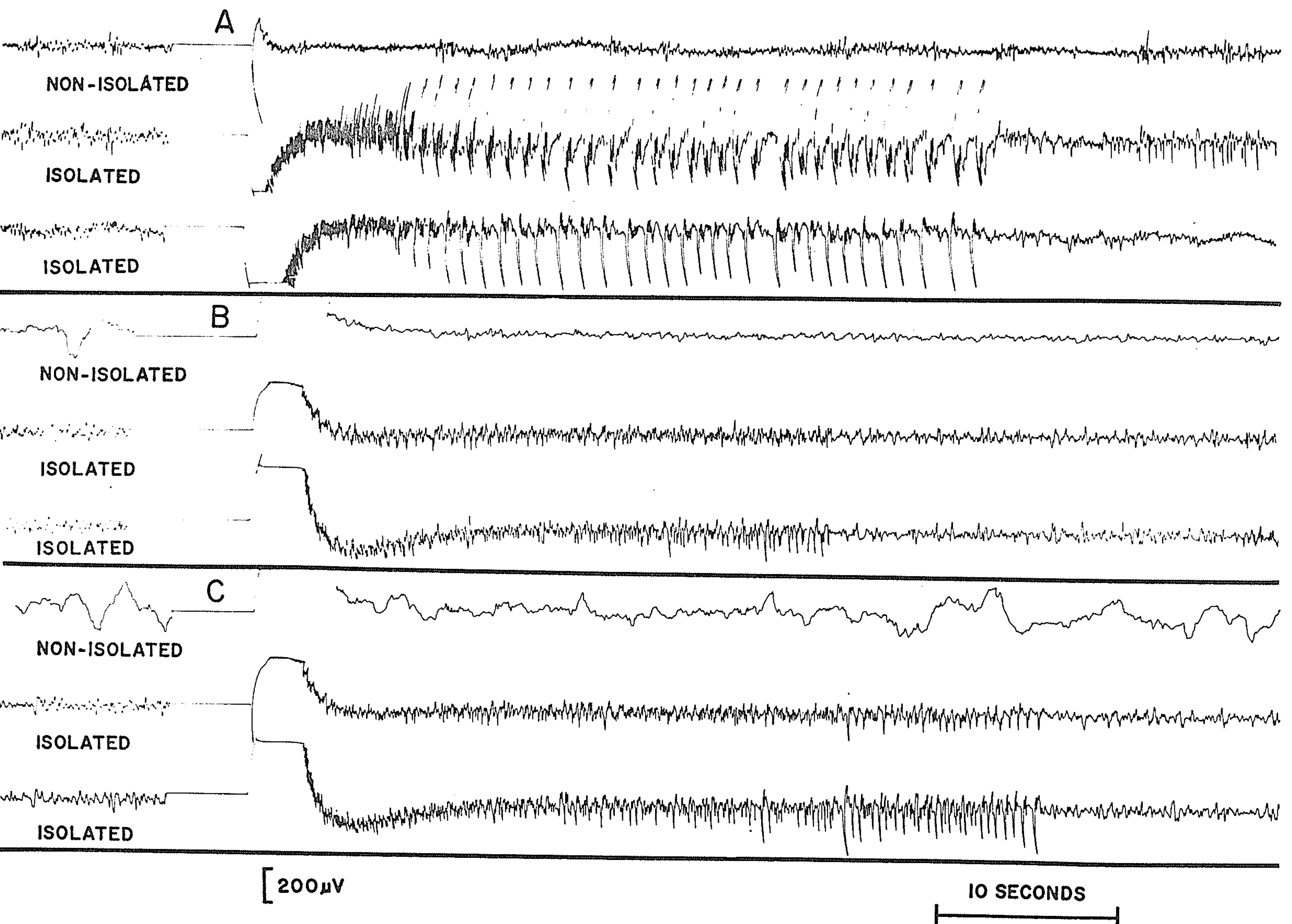


Figure 12A.

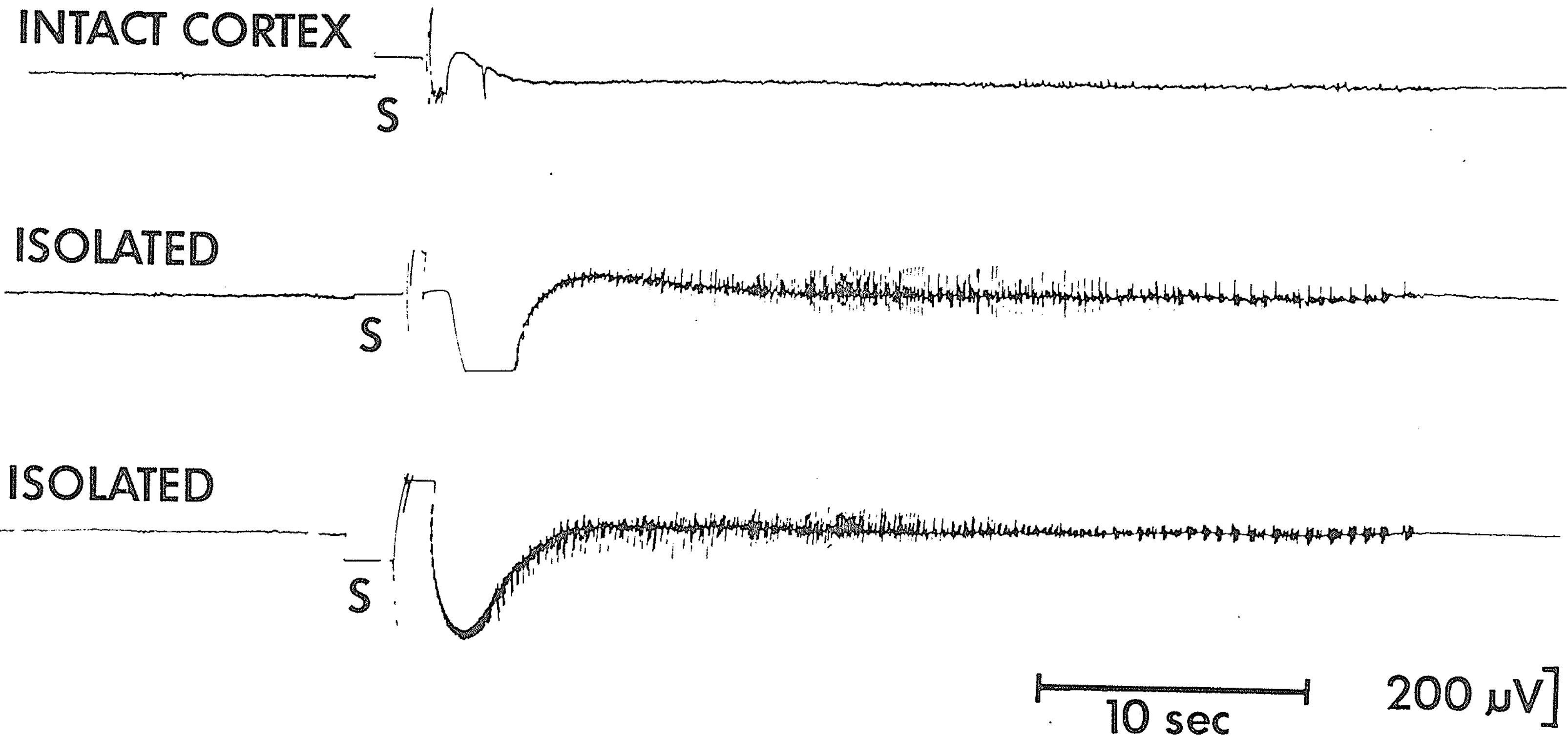


Figure 12B.

of a short period of high-frequency, ($>$ 20 Hz) spikes which gradually increase in voltage (Fig. 12A; 12B). The maximum amplitude of this early phase ("tonic phase") can reach 500 μV or greater. The tonic phase shifts abruptly to a period of large spikes, at least 500 μV in size, at a frequency of 2 Hz ("clonic phase"). On the ascending portion of the large spikes is superimposed a burst of low voltage ($<$ 200 μV), high frequency (25 Hz) repetitive spikes. This activity terminates abruptly and pre-stimulus or control background activity resumes. In some cases the response is followed by a short period of post-ictal depression, but this is not always the case. In other cases, paroxysmal activity re-commences. If the latency of this re-appearance or the relative period of post-ictal silence was greater than 10 sec, the EAD was considered terminated at the first end point. In the majority of cases there was no re-appearance of the response and the duration of the EAD was relatively easy to measure, the end-point being well defined.

b. EAD patterns

The generalized paroxysmal spiking and multiple spike and wave discharges that characterize an EAD, in these experiments, occurred with an uncertain latency soon after abrupt cessation of the predetermined threshold stimulus train. On the whole, the EAD is evident simultaneously and with similar characteristics throughout the isolated slab. Usually, there were no observable alterations in electrographic records other than a large DC swing due to amplifier block during the first few seconds following termination of the stimulus train. This initial interference was not evident in some records, suggesting that it was related to electrode configuration and location. As measurement of after-discharge duration always commenced at the point of withdrawal of the

stimulus train and switching back into the circuit of the amplifiers, the reference starting point was always clear. The isoelectric period seen following the EAD response in records of EADs on normal cortex (Maiti and Domino, 1961) is hardly ever observed in records from isolated cortex (Fig. 12A). Maiti and Domino (1961), and Sharpless and Halpern (1962) reported similar findings on isolated cortex. In the present experiments the post-ictal record often resembles the pre-stimulus (interictal) or control record of the ECoG.

The form and frequency of the spontaneous activity recorded from the isolated cortex after the second post-operative week could be differentiated from electrical rhythms recorded on intact cortex. Different patterns of electrical activity were sometimes recorded simultaneously from two different points on the isolated cortex. In most cases there was marked similarity of the simultaneously recorded patterns between the two pairs of recording electrodes. With increasing post-operative time (months), progressive reductions in amplitude of the ECoG took place. As these changes appeared uniformly in all animals, from 2-6 months post-operatively, the effect was presumed to be due to tissue-foreign body reaction at the electrode tips resulting in increasing amounts of fibrous growth around the recording electrodes and acting as an insulator. At the same time it was necessary to increase a little the intensity of stimulus voltage in order to continue to elicit the EAD. On histological examination there was only slight evidence of gross deleterious effects in the cortex at the area surrounding the electrode tips, fibrous tissue being the most prominent feature.

In the normal course of events, as the period of chronic

isolation was extended (beyond 2 weeks post-operatively), EADs were much prolonged in duration when compared to EADs elicited on acute cortical slabs (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962). The response elicited from chronic slabs lasts approximately 60 sec or more as compared to 10-15 sec in acutely isolated cortex. In addition, the initial tonic phase of the EAD becomes prolonged and there is a gradual shift to slower frequencies. The clonic period may be followed after a short time (< 10 sec) by a further, but shorter period of tonic activity. However, the response invariably terminates abruptly during clonic activity.

c. Prolonged afterdischarges

In several animals (6 cats) that were left unstimulated for long periods (5 weeks or more) afterdischarges lasting several hours (3-6 hours) occurred in response to a single threshold stimulus. An example of such a prolonged EAD is shown in Fig. 13. These unique responses usually begin with high frequency repetitive spikes, followed in a few minutes by lower frequency, high amplitude spikes, accompanied by short bursts of higher frequency small spikes. This latter pattern persists throughout most of the long EADs. As the point of after-discharge termination is reached, all forms of paroxysmal electrical activity gradually disappear, except for the large spikes.

An interesting result observed was that stimulation of the slab 1-2 hours after the initiation of a prolonged afterdischarge resulted in a short (< 60 sec) typical EAD response. This happened on repeated stimulation at 30 min intervals for at least 1 hour. During three of these responses various drugs (arecoline 2 mg/kg, diphenyl-hydantoin 10 mg/kg and eserine 0.5 mg/kg) were injected individually

Prolonged Afterdischarge

Figure 13. Example of a prolonged afterdischarge elicited in a chronically isolated cortical slab that had not been stimulated for at least 1 month. Both tracings at 0, 3 and 5 hours are records from two areas on the same isolated slab. Note the difference in electrocortical pattern of the EAD recorded from within the same slab, 7.5 mm apart. This response lasted approximately 5 hours.

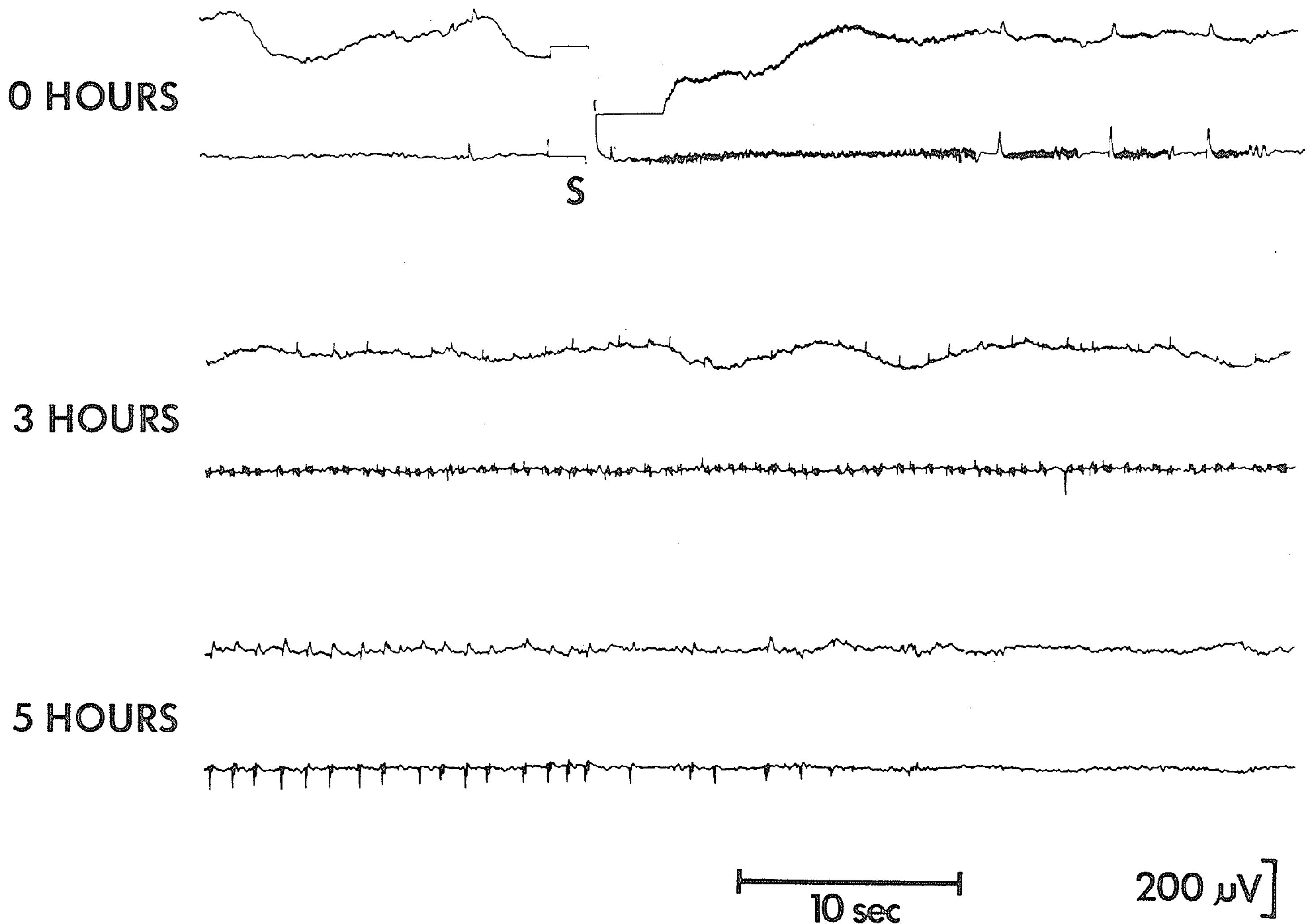


Figure 13.

at 60 min intervals but this treatment never arrested ongoing epileptiform activity as these responses continued for several hours, apparently terminating on their own.

d. Reproducibility of responses

In most cats with chronically isolated slabs that were stimulated at regular weekly intervals (usually not more than 20 presentations of threshold stimulus trains at just above threshold parameters per experiment) the duration of elicited EADs ranged from 30-80 sec (61 out of 93 cats, Fig. 14). In approximately 10 percent of cats (8 out of 93) cats, the control responses were greater than 80 sec, while in 25 percent of cats (24 out of 93 cats) the response was less than 30 sec. In each of these three arbitrary groups of cats (Fig. 14), the EAD response remained relatively constant for a testing period of several hours (EADs elicited at 20 min intervals; Fig. 15). Resumption of control tests the following day (+ 20 hours) revealed little change in EAD duration. The usual response has a mean value of 45 sec, which is significantly greater than that reported for acutely-isolated cortex in the cat (Grafstein and Sastry, 1957; Pinsky and Burns, 1962), and agrees with the results reported earlier by Sharpless and Halpern (1962) for EADs elicited on chronically isolated cortex. Whenever possible, drug studies were performed on cats having EAD durations in this intermediate range, i.e. 45 sec.

Durations of EADs elicited at 15-20 min intervals remained stable throughout experimental periods of 3-5 hours (Fig. 14; 15).

When the inter-stimulus intervals was 10 min or less, there appeared to be a considerable reduction in the capability of the isolated region to sustain an EAD response longer than about 10 sec, even

Figure 14. Three arbitrary groups of EAD durations measured during the course of control experiments. Mean afterdischarge duration shown in sec along ordinate and elapsed experimental time in min along abcissa. EADs were elicited (as indicated by data points) at 20 min intervals. X designates a pooled standard error (S.E.) for all the mean EAD durations in each group. The points were calculated from the results of experiments on 8 cats for each group. Note the relative stability of the response for over 3 hours and longer.

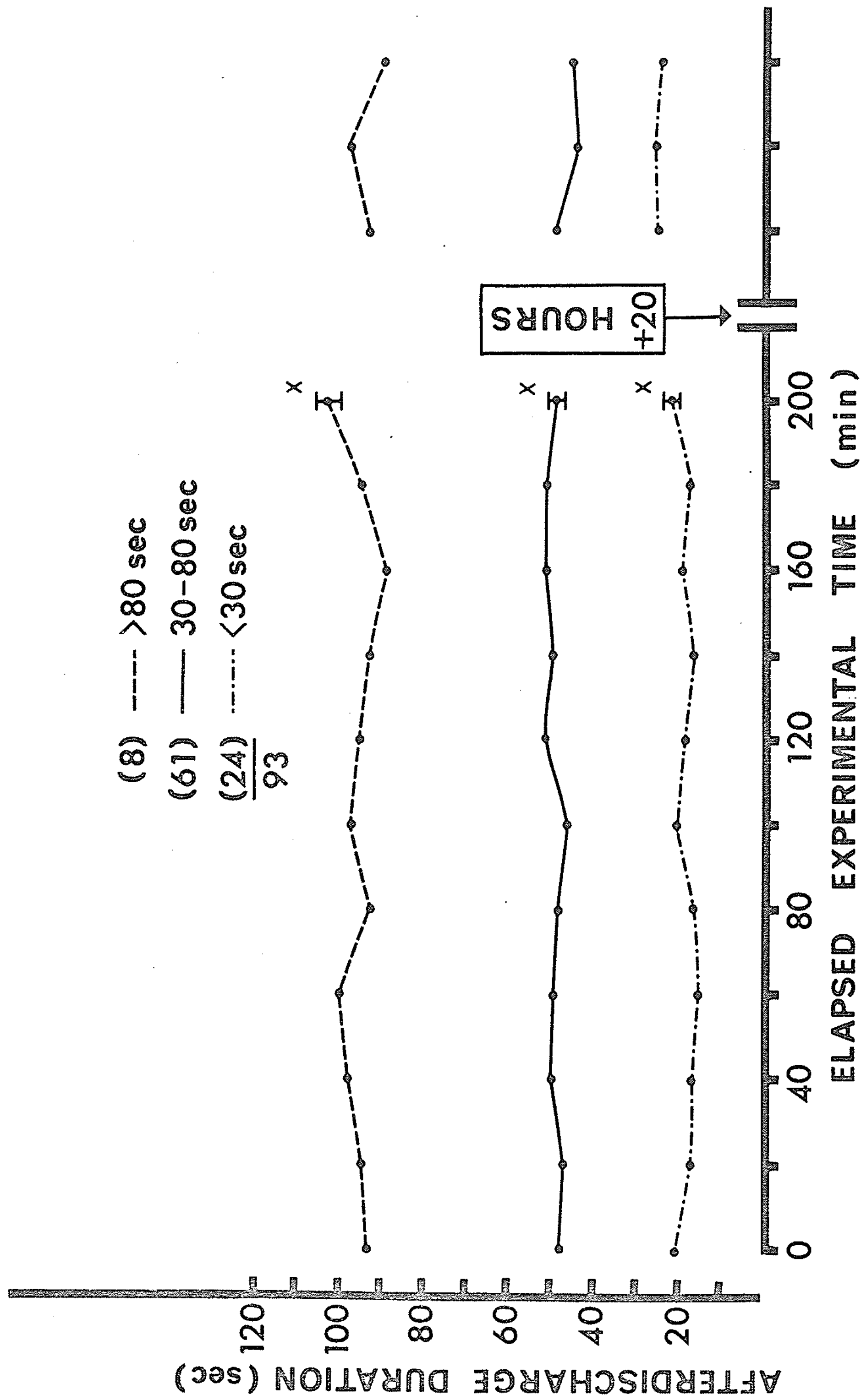


Figure 14.

Figure 15. Effect of different interstimulus intervals on EAD duration elicited in chronically isolated cortical slabs. When evoked every 15 or 20 min EAD durations were relatively constant during 2 hours of testing, and longer (not shown). The duration of successive EADs elicited at 10 min intervals (or less) declined in duration rapidly and were soon significantly different from EAD durations elicited at 15 or 20 min intervals. The mean EAD duration for each point was calculated from the results of experiments on 6 cats in each group. X refers to the pooled S.E. of all mean EAD durations in each group of cats.

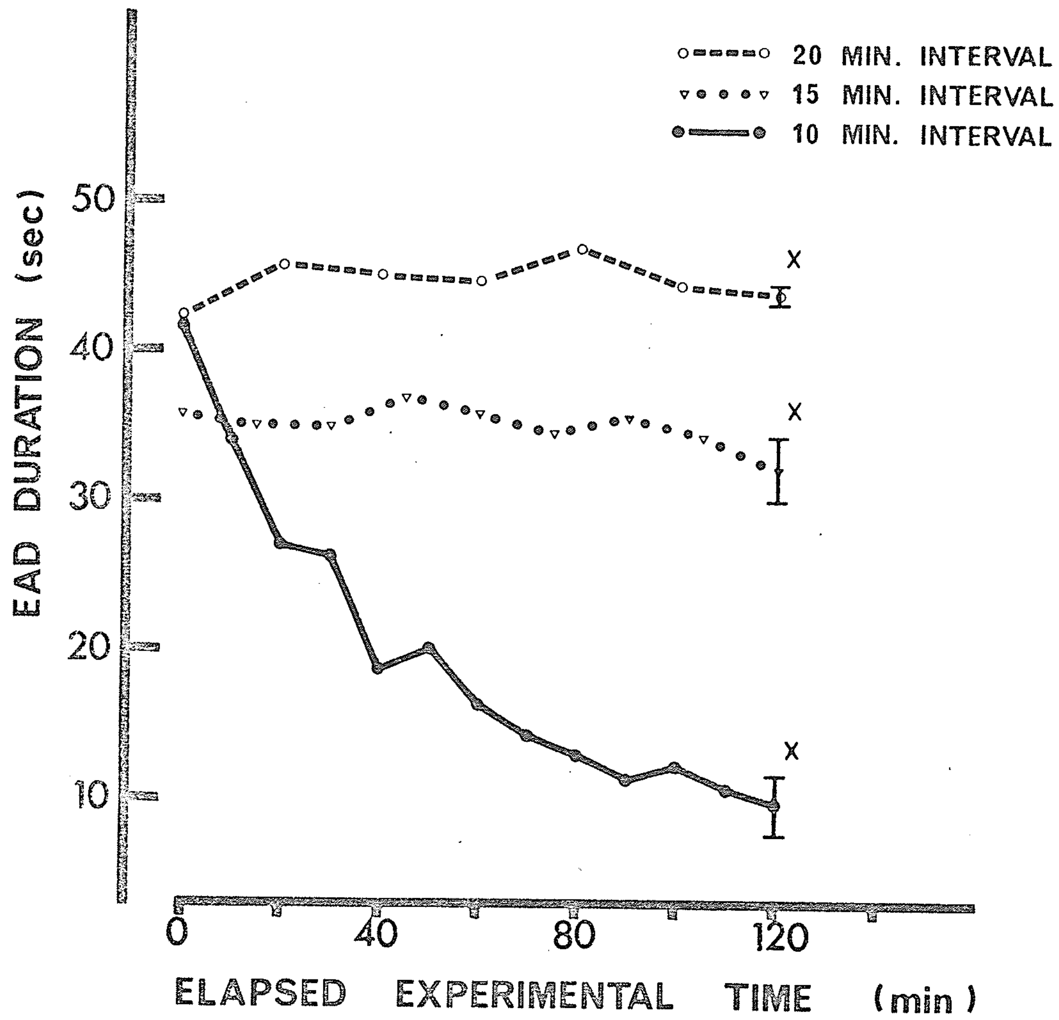


Figure 15.

though the initial responses may have been 40 sec or more (Fig. 15). For this reason the interstimulus interval of 20 min was adhered to in all experiments. The stability of the responses was also evident when injections of saline were administered at regular intervals during control experiments (Fig. 16).

e. Effects of solvent vehicles on EAD duration

Since it became necessary to use solvent vehicles other than normal saline or distilled water to dissolve some of the drugs used in this study, the effect of the different vehicles on EAD duration was studied (Fig. 16). Wherever possible, saline ($<$ 1 cc/kg) was the vehicle of choice. As seen in Fig. 16, injection of increasing volumes of saline (0.5 - 2.5 cc/kg) in 6 cats does not influence EAD duration. Injecting the same volume of distilled water (not shown) in 6 cats was, likewise, without significant effect on EAD duration. Another solvent vehicle not having any significant effects on EAD duration was that used to solubilize the agent haloperidol.

Using the diphenylhydantoin vehicle it was found that doses of 2.5 cc/kg or more, caused significant reductions in EAD duration. This was also observed when using propylene glycol, a major component of this vehicle. Propylene glycol alone was used to solubilize phenoxybenzamine (50 mg/ml). Whenever injecting either of these two "depressant" vehicles, the final concentration was adjusted so as to keep injection volume considerably less than 1 cc/kg, usually 0.5 cc/kg.

On the other hand, administration of the diazepam vehicle in amounts above 1.0 cc/kg in cats, caused highly significant increases in EAD duration. This was a unique situation and was reflected in the results of the study with diazepam. Other experiments revealed

Effect of Solvent Vehicles on EAD duration

Figure 16. Effect of some solvent vehicles on EAD duration. X refers to the pooled S.E. for all the mean EAD durations in each group calculated from the results of experiments on 6 cats in each group. Note comparative stability of EAD duration when administering N-saline (or distilled water, not shown). Injections indicated by arrows.

Figure 16A. Effect of small amounts of ethyl alcohol on EAD duration. Injections of ethyl alcohol, 20 mg/kg and 40 mg/kg indicated by arrows. X refers to pooled S.E. for all the means calculated from the results of experiments with 4 cats. * = $P < 0.05$.

As in all cases stimulus interval is 20 min, as indicated by data points. Mean difference in EAD duration from control is shown in seconds (sec) along the ordinate. Elapsed experimental time in minutes (min) is shown along the abcissa. Time of injection and dose administered is indicated by arrows. X refers to a pooled estimate of the S.E. for all the mean differences calculated for each study. This is the convention that is used in all subsequent graphs showing the results of the drug studies.

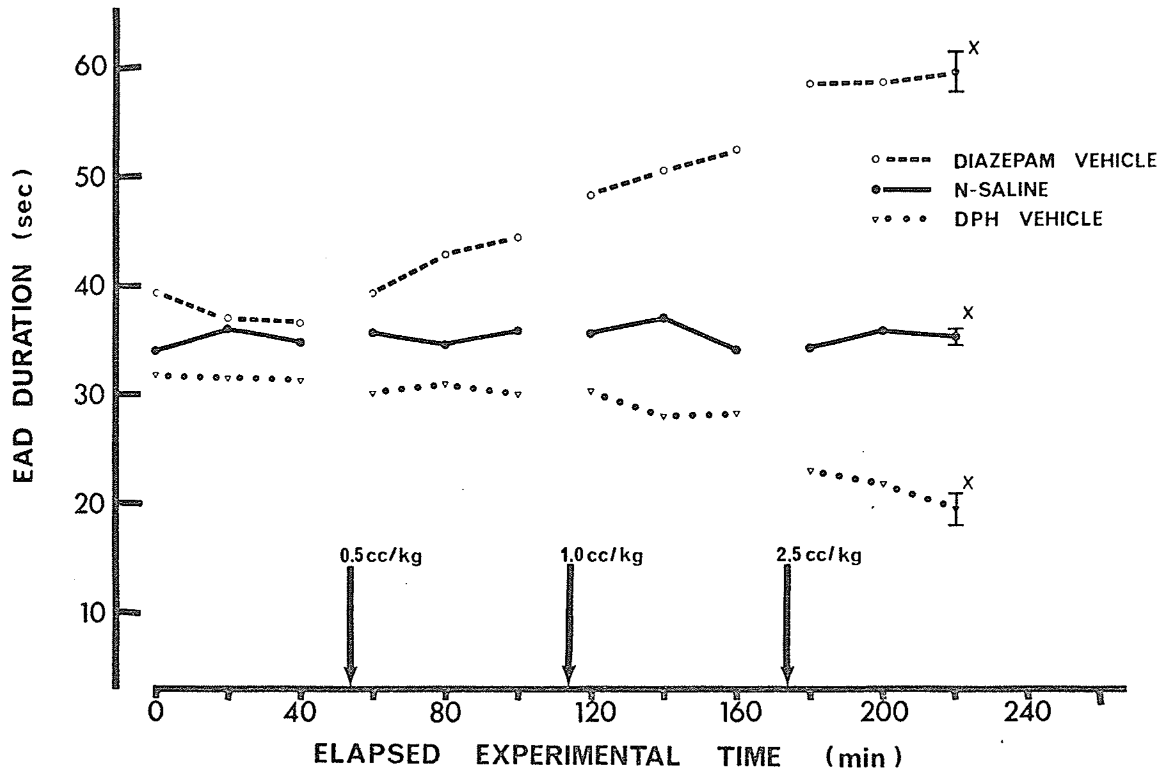


Figure 16.

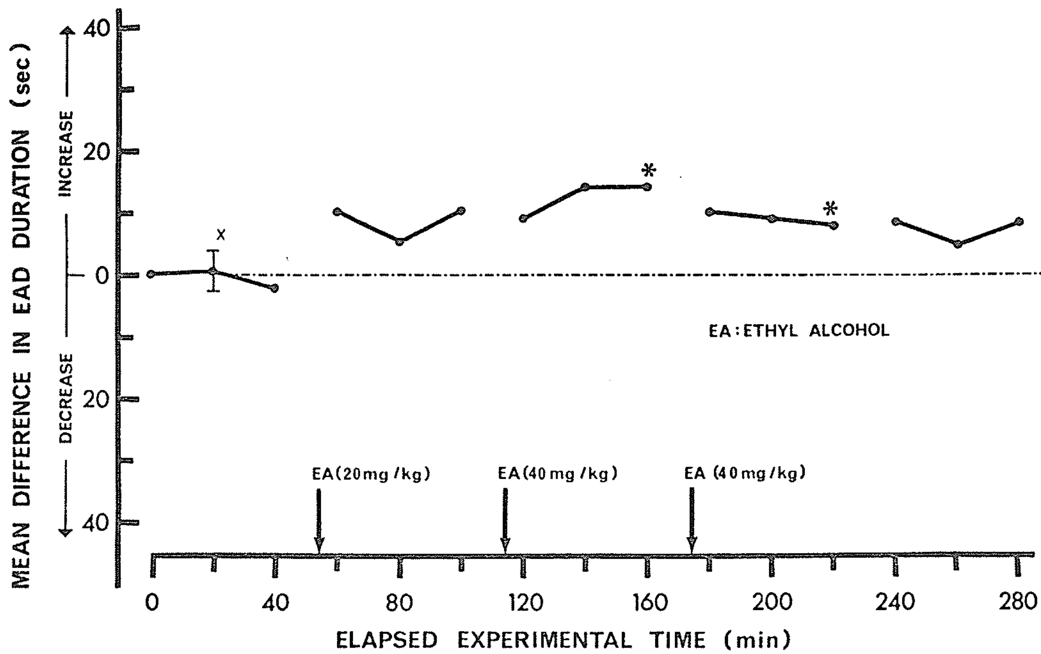


Figure 16A.

that alcohol caused increases in EAD duration (Fig. 16A). Alcohol was a minor component of the DPH vehicle.

The effect on EAD duration of increasing amounts of alcohol was examined in 4 cats (Fig. 16A). Small doses (20-40 mg/kg) of this substance caused small, but significant ($p < 0.05$) increases in EAD duration of about 10 sec. Greater amounts of ethanol (100 - 250 mg/kg) did not appear to change EAD duration in 2 cats. In this case there was a tendency to reduce EAD duration approximately 5 sec.

f. General observations

No study was made of thresholds since it was found that in the majority of experiments there was little or no change in this parameter. Stille and Sayers (1970) have reported that sensorimotor cortex appears to have the highest thresholds for eliciting EADs, which may explain why there is little change of this parameter in this region. As there was a considerable range of EAD patterns, no attempt was made to analyse the patterns of the response observed on electrographic records, other than to note major changes. No doubt, some of the differences in the recorded patterns were probably due to changes in orientation and position of the electrode tips at the cortical surface.

C. Discussion

a. Neuronal re-organization

There is no doubt that significant cytological re-organization has occurred in chronically isolated cortex (Rutledge *et al.*, 1969; Weisman, 1969; Krnjevic *et al.*, 1970). This does not mean that such cortex is an inert mass of tissue, for many recognizable elements still remain (Weisman, 1969). The greatest changes appear to have taken place in the lower (layer V) regions of the cortical slab. Adequate circu-

lation appears to be maintained (Hirsch et al., 1969) and may even be increased (Gottesfeld et al., 1971) due to the shrinkage of tissular, but not vascular elements in the isolated slab. In acute experiments, Ingvar (1955a) observed that electrical silence of the isolated cortex results shortly after compromising the local cerebral circulation, and returns after restoring adequate cerebral circulation.

Section of specific afferent fibres and collateral axons of the large pyramidal cells with resultant degeneration most likely contributed to the very significant disappearance of these cells in layer V. It would seem that the small cells having relatively short processes are far removed from any direct and indirect effects of the isolation and survive. More than adequate circulation of blood supplying the isolated region is in evidence (Hirsch et al., 1969; Gottesfeld et al., 1971; Fig. 10A).

A recent report (Krnjevic et al., 1970) has shown that small isolated cortical slabs undergo more severe degenerative changes than relatively large slabs, as evaluated by histological methods. In agreement with their findings there are no large pyramidal cells in layer V, but in my histological results (Fig. 9A; 9B; 10B; 11B) we do see small pyramidal cells in this and other regions of the chronically isolated slabs. Dendritic structures are reasonably well preserved (Fig. 10B; 11A; 11B) and it is to be presumed that there are extensive synaptic interconnections among the remaining cortical elements.

The hyperexcitability of chronically isolated neurons is well-established (Sharpless and Halpern, 1962; Echlin and Battista, 1963; Halpern and Ward, 1969; Krnjevic et al., 1970). It is not well established what is the cellular basis for this observed hyperexcitability.

Some pyramidal cells with apical and basilar dendrites remain. Thus, there are elements present with connecting processes probably responsible for the spreading of neuronal hyperactivity. As the work by Colonnier (1968) and Szentagothai (1969) has suggested, there appear to be interconnections between neighbouring pyramidal cells by means of stellate interneurons. These authors also demonstrated that when the cerebral cortex is undercut, the synapses believed to be involved in excitation are preserved along with many of the elements present in normal cortex. Thus, probably pyramidal cell collaterals connect with the basilar dendrites of adjacent pyramidal cells forming a positive feedback mechanism that favours repetitive discharges under the appropriate conditions. In any case, all of the changes e.g. degeneration, astrocytosis, gliosis, shrinkage etc. that have been reported for chronically isolated cortex (Reiffenstein, 1964; Rutledge et al., 1969; Weisman, 1969) each probably contribute to the observed electrophysiological phenomena. Rutledge (1969) considers that the loss of axo-dendritic synapses is most likely concerned with this excitatory activity. This is in agreement with earlier electrophysiological studies (Garner and French, 1958).

Inhibitory mechanisms appear to be substantially preserved in chronically isolated cortex (Krnjevic, 1970a). Colonnier (1966) and Szentagothai (1965) have described well preserved intracortical inhibitory type synapses in chronically isolated cortex. Thus, it appears that adequate cortical elements are preserved and that these probably include synaptic junctions. Rutledge (1969; Rutledge et al., 1969) has shown that regular daily stimulation somehow preserves morphological and functional units in partially isolated cerebral cortex. Other work in

their laboratory (Duncan et al., 1968) shows that chronic isolation leads to lowered AChE levels and decreased numbers of dendritic spines. These two morphological changes might well be the most important factors responsible for the development of the prolonged EAD in isolated slab. Ferguson and Jasper (1971) have recorded what they tentatively considered to resemble inhibitory interneurons in undercut cortex. These cells were detected < 1.3 mm below the cortical surface, showing repetitive firing during the latter stages, or after epileptiform spikes recorded at the surface. Much further work is required to elucidate the effects of prolonged deafferentation on many other cortical elements which no doubt are also involved.

Ward (1961; 1969) has studied the effects of alumina cream lesions to the cortex and he proposes chronic depolarization of apical dendrites as a mechanism for epileptogenesis. Studies in his laboratory (Westrum et al., 1965) suggested to these authors that the disappearance of dendritic spines correlates well with the development of epileptiform activity in such regions of the cortex. As the studies of Rutledge (1969) and Weisman (1969) show that extensive and similar deformation occurs in chronically isolated cortex, it seems that the neurons remaining in isolated slabs of cerebral cortex bear some similarity to hyperexcitable neurons in alumina foci and that there are probably some basic epileptogenic mechanisms that these two experimental preparations have in common. This aspect has been discussed previously (section III. D).

b. Development of the prolonged afterdischarge

Epileptiform activity has been recorded in the vicinity of apparently isolated epileptogenic cortex overlying cerebral tumours in

patients with epilepsy (Henry and Scoville, 1952). Earlier work has established that there is a close similarity between the patterns of the afterdischarge evoked by direct repetitive stimulation of the cerebral cortex, and the forms of electrical activity accompanying various types of clinical epilepsy (Penfield and Jasper, 1954; Gangloff and Monnier, 1957; Strobos and Spudis, 1960; Vastola and Rosen, 1960). Thus, there appears to be a common basis for the convulsive activity which may be recorded from idiopathic and different types of induced cortical epileptogenic foci, including isolated regions of cerebral cortex.

Chronic isolation, either total (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962) or partial (Echlin and Battista, 1963) of a mass of cerebral cortex leads to changes in the paroxysmal or epileptiform electrical activity which may be elicited locally from the isolated region by direct electrical stimulation. The durations of EADs increase with increasing periods of isolation, the greatest increase occurring in the tonic phase (Halpern and Ward, 1969) which now predominates. This response can be elicited on acutely-isolated cortex but thresholds are higher and durations of EADs are considerably shorter (Burns, 1951; Grafstein and Sastry, 1957). In addition, the spread of EADs in chronic cortical slabs is enhanced (Sanders and Pinsky, 1967), apparently by a process of facilitation which activate neurons and by spatial summation of electrical activity.

Intermittent stimulation, administered at least once weekly, prevents the development of prolonged EADs in chronically-isolated slabs of cat's cerebral cortex. Regular daily stimulation has been reported to reduce the apparent "supersensitivity" of chronically-

isolated cortex (Rutledge *et al.*, 1967) as well as preserving neuronal elements (Rutledge *et al.*, 1969). Moreover, regular sessions of stimulation on deafferented cortex forestall the appearance of paroxysmal epileptiform electrical activity in the chronically-isolated cortical slab (Sharpless and Halpern, 1962; Rutledge *et al.*, 1967). Some evidence of this tendency is seen in acute experiments reported in this thesis (Fig. 15), since stimulation at intervals of less than 15 min appears to result in significant "fatigue" of the EAD on the chronically-isolated cortical slab, also observed by Halpern (1961). The elicited EADs become even shorter in duration than those resulting during the course of 1-2 stimulating-recording sessions weekly, wherein a stimulus train is delivered every 15 - 20 min. Probably, the fatigue and subsequent decrease in EAD duration, seen when stimulating at 10 min intervals or less, are part of the same general phenomena.

c. Cellular mechanisms involved in the generation of an EAD

The electrical activity of cerebral cells recorded with microelectrodes has been the subject of numerous investigations (section IV.A.c; IV.B.c). Reports dealing with unitary cell activity in the cerebral cortex during EAD activity are numerous (e.g. Bremer, 1958; Gerin, 1960; Morrell, 1961; Ward, 1961; Halpern and Ward, 1969; Krnjevic *et al.*, 1970). In the majority of these studies an attempt was made to correlate single unit activity at specified cortical depths with epileptiform activity as seen in the ECoG. As many different patterns of cell firing have been reported (Halpern and Ward, 1969) there is still no general agreement on these events and their relation to the EAD recorded on the cerebral cortex.

It is apparent that the *in vivo* isolated cerebral cortex is

relatively free of the modulating influences present in normal cortex (compare electrical activity on intact cortex to electrical activity on isolated cortex Fig. 12A, 12B). On the basis of his early experiments Burns (1958) postulated that the EAD results from the re-excitation of reverberating circuits of cortical neurons. The study of Pinsky and Burns (1962) and other studies (Gerin, 1960; Goldring *et al.*, 1961) appear to support the hypothesis that the EAD results from differential repolarization of the neurons at the focus (Pinsky and Burns, 1962). That is, there is a difference in the rates which superficial and deep layers of the cortex repolarize following stimulation. Within given layers of the cortex the discharge of aggregates is somehow synchronized, perhaps synaptically. Pinsky and Burns (1962) consider that a minimum density of cortical neurons must be excited before a focus for an EAD is created.

Thus, the cortical EAD appears to represent an extreme form of facilitation. It is not clear what the role of synaptic structures in this facilitation might be. It appears that a negative DC shift occurs in the basal layers of the cortex and facilitates discharge of the pyramidal cells in this layer (Pinsky, 1963). A discharge of pyramidal cells over recurrent collaterals and other connections which would augment this radially oriented negative shift in the basal layers, has been proposed by Gloor *et al.*, (1964) as a possible positive feedback mechanism. Seizure activity proceeds at optimal levels as long as there is not excessive depolarization of pyramidal cells, in which case the seizure discharge is arrested. Thus, Gloor *et al.*, (1964) have proposed a negative feedback system that stabilizes seizure activity by virtue of partial inactivation of inhibitory neurons. The crucial

dependence of the self-sustained EAD upon the existence of a transcortical potential gradient with deep negativity, originally described in isolated cortex by Pinsky (1963), has been confirmed by Ferguson and Jasper (1971) for the ACh-evoked EAD in partially isolated cortex.

Ferguson and Jasper (1971) have recorded simultaneously from the cortical surface and from various cortical depths and revealed that there is a differential rate of repolarization between dendrites and soma when EADs were evoked by topically applied ACh. The sudden negative DC shift from the cortical surface and from the depth showed that sudden depolarization occurs to reach a maximum in 20 - 30 msec in layer V and in layers I and II. Occasionally there was a delay of a few msec for maximal depolarization to occur in layer V. Cells in layer V repolarized about twice as rapidly as layer I-II elements, probably superficial dendrites. In some experiments, this difference produced a steeper gradient of positive to negative potential difference between the more superficial layers and the deeper layers than was observed during the initial stages of the EAD. These authors (Ferguson and Jasper, 1971) speculated that this enhancement of differential repolarization between the somata and dendrites might be due to the action of inhibitory type synapses concentrated on the somatic membrane (Colonnier, 1968). Such a mechanism might conceivably be involved in the intermittent "bursting" paroxysms observed during the clonic phase of the EAD. Ferguson and Jasper (1971) also showed that the secondary oscillations superimposed upon the sustained depolarization during an EAD appeared to act back or gate the discharge of neurons in layer V. The more rapid surface positive phase of these oscillations was associated with excessive depolarization, often sufficient to block spike discharges.

These spikes were seen to re-appear soon during the repolarization phase, only to be blocked again during the longer lasting hyper-polarization represented at the surface by a slow negative wave. Once the secondary oscillations are interrupted, the rapid repetitive firing is resumed, suggesting that inhibition is somehow associated with the secondary phenomena which do not themselves change the steady level of the negative DC shift, i.e. sustained depolarization. Similar electro-cortical phenomena have been observed during penicillin-induced parox-ysmal discharges by others (Gumnit et al., 1970).

Similar findings, i.e. maximal negativity in layer V, have been reported for paroxysmal discharges provoked in the depth of penicillin-induced cortical focus (Gumnit et al., 1970). Therefore, the similar behaviour of neurons in various models suggests that this sudden massive depolarization of the dendritic and cellular components of pyramidal cells is apparently a general phenomenon in epileptic neurons. It must be emphasized that the hypersynchronous discharging of many such neurons is most likely achieved by a mechanism of intra-cortical synaptic circuits incorporating interneurons. This mechanism appears to be responsible for the large (500 μV) spikes of the EAD.

Smaller secondary oscillations in the EAD appear to originate from the more superficial layers of the cortex. These superficial, most likely dendritic elements, repolarize slower than the deeply situated pyramidal cells (Ferguson and Jasper, 1971). These discharges are associated with excessive depolarization and subsequent spike gener-ation, and it is suggested by these authors that this excessive depolar-ization is responsible for the interruption of spiking observed during the clonic phase of the EAD, which agrees with the hypothesis postulated

by Gloor et al., (1964).

Sanders (Sanders and Pinsky, 1967; Sanders and Gravlin, 1968) considers that the EAD results from activity in neuronal networks not involved in the generation of DCRs. Moreover, this author believes that the DCR represents a synaptic event, whereas the EAD is not the result of sequential synaptic events but the result of synchronously firing neurons. However, it is difficult to conceive the synchronized and prolonged firing pattern observed in an EAD without involving synaptic elements for the maintenance and spread of this synchronization. It has been shown that EADs could be prolonged for many minutes by relatively weak stimuli applied to the isolated slab at 0.5 - 2 Hz during an ongoing EAD (Sanders and Pinsky, 1967). These authors were unable to record EAD responses 10 mm beyond the point of stimulation, which might be explained by the fact that these investigators prepared "silent" slabs according to the method of Burns (1951). The present thesis does not provide any data on this point, but the observation that EADs elicited on undercut cortex will spread long distances into intact cortex (Echlin and Battista, 1963) indicates that EADs have the capability of spreading relatively long distances in the cortex.

If as Sanders proposed, the EAD does not involve synaptic events, then treatment with pharmacological agents which are known to modify synaptic activity, should not demonstrate interactions and relationships with putative transmitter agents. This conclusion appears invalid in view of the sensitivity of the EAD to varied pharmacological agents as shown in this thesis and the intra-cellular studies of Prince and Futamachi (1968, section IV A.c.).

The manner in which EAD duration was sustained for longer periods by weak stimuli (Sanders and Pinsky, 1967) raises the question as to the mode of termination of the EAD. These authors mention that these same weak stimuli were also effective in producing a PBR. Thus, there is the possibility that this treatment may somehow inactivate or block an inhibitory mechanism normally involved in the termination of afterdischarges by a synaptic mechanism. Pharmacological intervention would also be expected to prolong epileptiform activity. Whether this prolonged activity reflects a regenerative mechanism (Smith and Purpura, 1960) involving synapses for the maintenance of epileptiform responses or a mechanism of differential repolarization (Pinsky and Burns, 1962) is a difficult question to answer on the basis of the available evidence. Simultaneous study of both these aspects will throw more light on the subject.

Superimposed and rhythmic interruption of EADs elicited in isolated cortex has been described (Jasper, 1969; Ferguson and Jasper, 1971). It appeared that this pattern might be due to periodic waves of inhibition controlling otherwise sustained discharging of pyramidal cells. It can be suggested that perhaps collateral elements of these cells may be in contact with inhibitory structures which gradually and intermittently build up sufficient inhibitory influences. Such a mechanism favours the presence in the cortex of discrete but interconnected inhibitory structures, acting through synapses.

The fact that there might be discretely located cortical structures capable of opposing the activity of neurons in other regions of the cortex provides the basis for many types of modulatory effects. For example, epileptiform cortical hyperactivity may result from the

depression or removal of inhibitory influences normally exerted on pyramidal cells by intracortical inhibitory interneurons (Phillips, 1959). Such a possibility may explain why some drugs favour (Brooks and Asanuma, 1965) EADs and the hyperexcitability of neurons following prolonged deafferentation (Ward, 1969). The preliminary work of Holubar et al., (1967) suggests that there are deeply situated, predominantly excitatory, pyramidal cells, and more superficially located, smaller, stellate, apparently inhibitory interneurons. Some inhibitory type cells were found in lower layers of the cortex and presumably these are more directly involved in mediating inhibitory effects. Others have studied the laminar distribution of potentials in the cortex during EADs (Gumnit et al., 1970; Ferguson and Jasper, 1971) and proposed the concept of spatial organization of excitatory and inhibitory structure, i.e. excitatory neurons in layer V and inhibitory elements more superficially in layers II, III, IV.

It is possible that the failure of adequate synaptic transmission within mutually interconnected aggregates of epileptogenic neurons is a primary factor in the generation of the EAD in neuronally isolated cortex. The report by Ferguson and Jasper (1971) that paroxysms evoked by topically applied ACh are initiated by a sudden surface negative DC shift upon which are superimposed characteristic repetitive epileptiform spikes suggests that perhaps a sudden arrest of inhibitory activity may be responsible for the abrupt increase in negativity. This increase in negativity is most marked in layer V and most likely caused by the excessive and synchronized firing of pyramidal cells. As the inhibitory cells are apparently more superficial, they may be non-specifically blocked by the topically applied ACh, whereas

the pyramidal cell bodies are relatively remote and little affected by this treatment. Strong inhibitory drive at the major apical trunks of dendrites or nearby by axodendritic or axosomatic synapses could prevent excitatory effects from extending out into the terminal portions of the dendrites, as has been shown for the Nwave DCR (Ochs and Clark, 1968).

Sawa et al., (1963) proposed that the epileptiform activity observed after repetitive stimulation of the cortex is likely due to a decrease in the available quantity of some inhibitory synaptic transmitter. Thus, when efficiency of the inhibitory mechanism is further reduced, e.g. following de-afferentation, isolated cortical slabs would respond to stimulation with relatively prolonged EADs. Treatment with the appropriate pharmacological agents should help to correct this deficiency in cortical function. Consideration for different synapses subserving different electrophysiological functions has been discussed extensively (Humphrey, 1968; Creutzfeldt et al., 1969). Undoubtedly, the diffuse nature of chronic epileptic foci and the possible shifting relationships between surface slow potentials and unit activity, and their uncertain behaviour during epileptogenesis may require a more flexible categorization than most investigators are willing to accept. Watanabe and Creutzfeldt (1966) have detected EPSPs and IPSPs in chronically isolated cortex following surface stimulation. Further definitive microelectrode studies are needed in this area (see section IV. B.d.).

Of course, facilitation might also be involved in the production of EADs. This facilitation could also be achieved by means of synaptic potentials through a positive feedback system. Maintenance

of seizure discharges in the hippocampal cortex of the cat has been explained in terms of a process of post-tetanic potentiation which is initiated by repetitive stimulation (Gloor et al., 1964). To cover various levels of activity it is also necessary to propose various inter-related feedback mechanisms in this scheme.

In his dissertation, Brock (1967) on the basis of micro-electrode studies on acute and chronic cortical slabs, was able to show that a shell of potential gradient radiates a distance of 1.5 mm from the point of stimulation and that "the burst-burst delays" recorded are highly suggestive of a mechanism of recirculation of electrical activity as long as the EAD is ongoing. These findings on isolated cortex are in accord with those reported by Gumnit et al., (1970) for paroxysms on intact cortex.

d. Stability of EADs

Some investigators (Rech and Domino, 1960; Maiti and Domino, 1961; Halpern, 1961) have reported encountering much difficulty with the stability and reproducibility of EAD duration in their experiments on isolated cortex. As shown in the present thesis (Fig. 15) the interstimulus interval seems to be an important factor in EAD stability. The earlier workers adopted differing stimulus delivery schedules of from 1 - 10 min which may explain part of the variability of the response that plagued their studies. Certainly, this is a most compromising factor when considering their drug studies. In addition, the number of stimulus presentations at 20 min intervals during a given testing session in our laboratory is invariably less than twenty. The greater number of stimulus trains delivered (20 - 40) in the study by Halpern (1961) and apparently in other work (Rech and Domino, 1960;

Maiti and Domino, 1961) far exceeds the demands imposed in our experiments and may help to explain the considerable element of fatigue reported in the earlier studies. On the other hand, some other, presently unknown, difference in technique may be responsible for the differences reported. In my experience, the EAD duration provides a consistent and useful measure of electrophysiological phenomena on isolated cortex.

Halpern (1961) reported that thresholds also increase during this stage of apparent fatigue. This might have occurred in our experiments, but would have to be confined to a small range, since our arbitrary increase of threshold by 10 percent does not appear to provide much of a range for detecting changes in threshold. Thus, if there were changes in threshold in our experiments they were of a small order ($< 10\%$). My experimental preparation is quite similar in most respects to that developed by Halpern (1961; Sharpless and Halpern, 1962). Major differences are the surgical isolation technique, electrode assembly and the experimental design, all of which probably account for the discrepancy regarding the influences of fatigue. Straw and Mitchell (1966) have shown that preparations bearing chronically implanted electrodes compared to animals prepared acutely, produce stable EAD responses.

Another explanation may be that some background factors, e.g. peripheral stimulation of the animal, modified the experimental seizures. Such stimulation of any part of experimental animals has been shown to increase seizure expression and even overcome the effects of treatment with DPH (Louis *et al.*, 1971). More likely an explanation is the selection of preparations with EAD durations falling into a range

around 45 sec. Although this meant extra animals the results seem to be that stability of the response is ensured. The animals studied in my thesis were, apparently, in excellent condition.

D. Resume

Although the evoked afterdischarges involve various intracortical neuronal circuits, which may be independent circuits on their own, it is apparent that in normal cortex this activity is modified by various extracortical influences. Confining the response to an isolated region, therefore, facilitates analysis of electrophysiological phenomena of purely cortical origin. On the same basis, the isolated cortex would prove to be a suitable preparation for the investigation of the effects of pharmacological agents on cortical neurons and intracortical connections.

Thus, it is apparent that in chronically-isolated cortex there is a tendency for prolonged epileptiform activity. Whether this is due to an increase in number of efficiency of excitatory elements, or a decrease in number or efficiency of inhibitory elements, or both, is not presently discernible. It would seem that loss of inhibitory structures and/or a decrease in their efficiency would provide a more ready explanation for the enhanced paroxysmal and elicited epileptiform afterdischarges routinely observed in deafferented cortex.

X. ACETYLCHOLINE

Effects of Cholinergic Drugs and their Antagonists on EADs Elicited in Chronically Neuronally Isolated Slabs of Cerebral Cortex

A. Effects of Methylatropine on EAD Duration

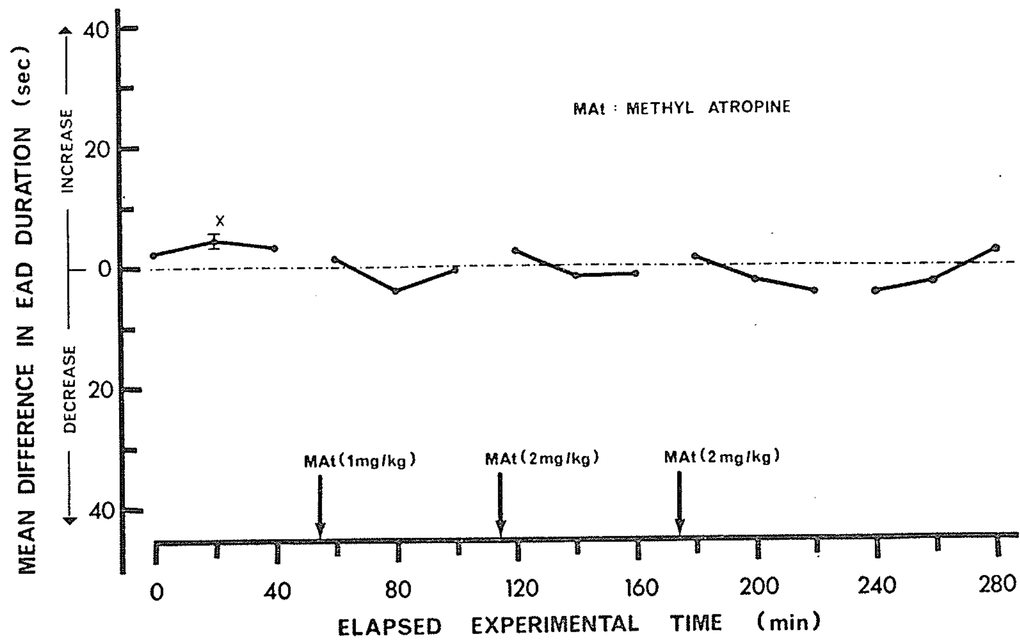
Prior to commencing experiments with cholinergic drugs, it was decided to protect the animals against the marked peripheral effects of these agents. Methylatropine, a form of atropine incorporating a charged quaternary ammonium group in the molecule, does not cross the blood-brain barrier (Paul-David *et al.*, 1960) and does not alter control EAD durations unless injected intracerebrally (Bernard *et al.*, 1968).

In order to determine whether methylatropine has any effect on cortical afterdischarge in my experimental preparation, methylatropine nitrate was administered intraperitoneally (IP) to 8 cats (Fig.17). It is clear from the results (Fig. 17) that there were no significant effects on EAD duration resulting from the injection of methylatropine 1 mg/kg and 2 mg/kg. The final level of drug in these experiments was probably 5 mg/kg as there would certainly be cumulation of drug during the period of time (120 min) taken up by this series of injections. No significant changes in the duration of afterdischarges could be detected up to 6 hours after the last injection.

In all subsequent experiments in which cholinergic drugs were to be administered, the cats were routinely pretreated with methylatropine nitrate 4 mg/kg to protect them from the effects of peripheral cholinergic stimulation.

Figure 17. Example of lack of effect of methyl atropine nitrate (MAt) on EAD duration elicited in chronically isolated cortical slabs. Mean difference in EAD duration in sec is shown along the ordinate. Data points on this and all other graphs indicate time of stimulation, at 20 min intervals. Arrows indicate time of injection of drug. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 8 cats.

Figure 18. Examples of lack of effect of dl-muscarine iodide (MSc) on EAD duration. Experiments were conducted on animals pretreated with methyl atropine (MAt) 4 mg/kg. Muscarine was tested at doses of 0.01 mg/kg and 0.02 mg/kg. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.



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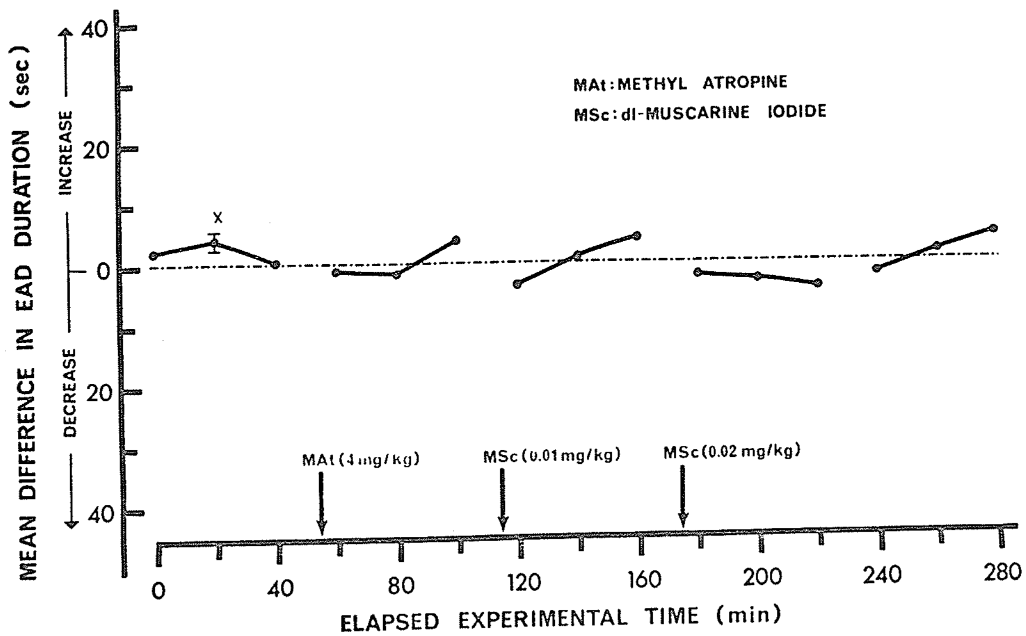


Figure 18.

B. Effects of Some Cholinergic Drugs on EAD Duration

a. Cholinergic drugs not distributed into cerebral tissues

In these three groups of experiments dl-muscarine iodide (Fig. 18) carbamylcholine chloride (carbachol, Fig. 19) and acetyl-β-methylcholine (methacholine, Fig. 20) were studied. Pretreatment with methylatropine 4 mg/kg was sufficient to protect the animals from the peripheral actions of these cholinergic drugs, e.g. salivation, emesis, diarrhea and the observed general discomfort.

From the results of experiments in 4 cats (Fig. 18) it is apparent that muscarine in doses of 0.01 or 0.02 mg/kg caused no significant change in EAD duration.

The administration of carbachol at doses of 0.1 mg/kg or 0.4 mg/kg (Fig. 19) in 4 cats also resulted in no significant change in EAD duration over the testing period. There was probably a slight degree of accumulation of the drug with these successive injections.

Injections of increasing doses of methacholine 1 mg/kg, 1.5 mg/kg and 2.5 mg/kg in 6 cats (Fig. 20) did not cause any significant change in EAD duration. Cumulation of methacholine most likely occurred so that the final dose probably was somewhere near 5 mg/kg.

Thus, from the results of 14 experiments, the cholinergic drugs muscarine, carbachol and methacholine do not, on their own, modify EAD duration at the doses administered. The most likely explanation for this lack of effect on the cerebral cortex is that all of these agents possess a charged quaternary nitrogen group in their molecules, which precludes their penetration into cerebral tissues (Goodman and Gilman, 1970).

Figure 19. Effect of carbamylcholine chloride (carbachol, CL) on EAD duration. Cats were pretreated with methyl-atropine 4 mg/kg, and then received carbachol 0.1 mg/kg and 0.4 mg/kg. Time of injection indicated by arrows. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. Note lack of significant change in EAD duration.

Figure 20. Effect of acetyl-β-methyl choline chloride (methacholine, MC) on EAD duration. Methyl atropine 4 mg/kg was injected as a pretreatment. Time of injection indicated by arrows. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments in 6 cats. Note lack of any significant change in EAD duration.

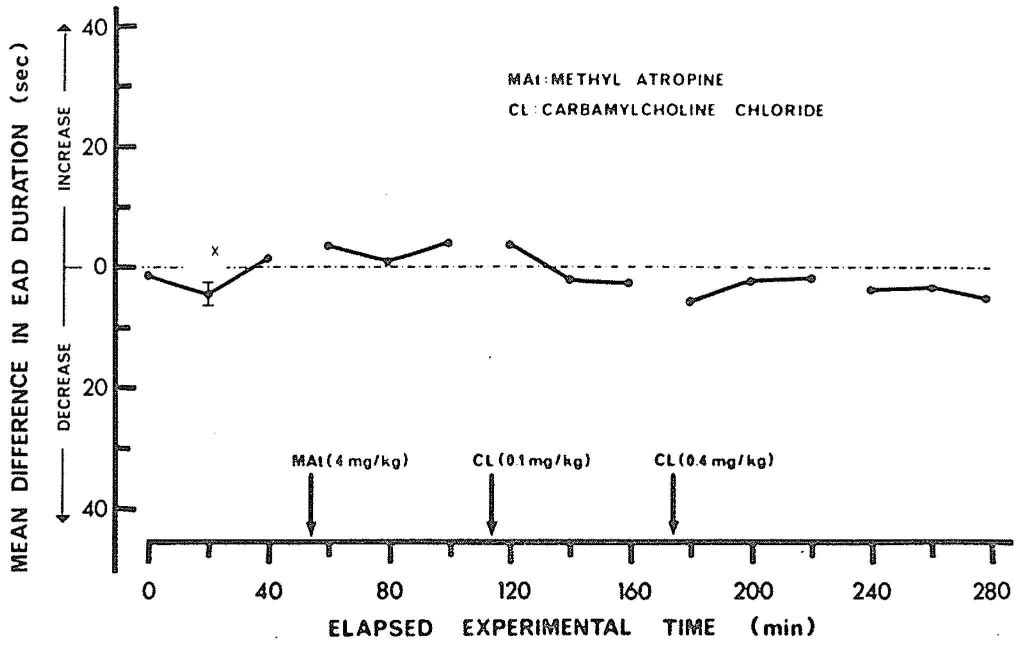


Figure 19.

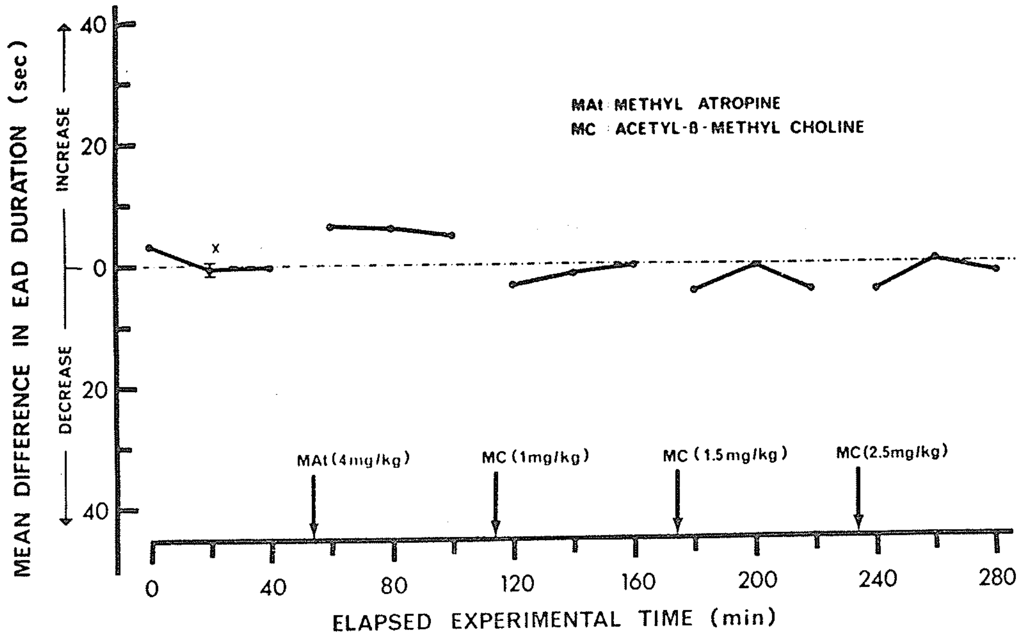


Figure 20.

b. Ganglionic stimulant drugs

Two tropane derivatives, 4-(m-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343), and N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602) have ganglionic stimulant properties that are stronger than their actions on peripheral cholinergic junctions (Goodman and Gilman, 1970). These drugs are known to stimulate ganglia by activation of muscarinic sites (Jaramillo and Volle, 1967). In two groups of 3 cats each, McN-A-343 and AHR-602 were tested for their effect on EAD duration. The results of these experiments are summarized in Table 1.

Table 1. Effects of Ganglionic Stimulant Drugs on EAD Duration

SALINE-CONTROL			TREATMENT - 1
Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	Mean Difference in EAD Duration (sec) ± S.E. (N)
3.8 ± 1.1 (3)	McN-A-343	0.5 mg/kg	2.5 ± 0.9 (3)
1.6 ± 1.0 (3)	AHR-602	0.5 mg/kg	2.4 ± 0.7 (3)

N = number of animals

Neither McN-A-343 or AHR-602 at doses of 0.5 mg/kg altered afterdischarge duration significantly. This lack of effect on EAD duration is probably best explained by the exclusion of these drugs from the brain following systemic administration, by virtue of their possessing charged nitrogen atoms in their molecules (Burger, 1970). The animals in these experiments were not protected with methylatropine and some peripheral effects, e.g. salivation, were observed.

C. Effects of Centrally-Active Cholinergic and Anticholinergic Drugs on EAD Duration

a. Cholinergic drugs

The cholinergic drugs arecoline, physostigmine (eserine), pilocarpine and oxotremorine are known to cross the blood-brain barrier in significant amounts and possess direct cerebral actions (Goodman and Gilman, 1970). In these series of experiments, methylatropine was used as a pretreatment, except in two sets of experiments in which the effects of low doses of arecoline (Fig. 22) and low doses of eserine (Fig. 24) were tested.

In 8 cats arecoline 0.5 mg/kg (Fig. 21) caused a highly significant ($P < 0.01$) decrease in EAD duration (EAD duration was decreased by approximately 18 sec). A second injection of arecoline 0.5 mg/kg, 60 min later, produced the same result. Increasing the dose, 60 min later, to 1.5 mg/kg produced the same highly significant ($P < 0.01$) mean decrease in EAD duration. There was no significant difference between the three responses to arecoline and there is probably negligible cumulation of this drug under the present dosage schedule.

Administration of 0.1 mg/kg arecoline (Fig. 22) in 4 cats resulted in a small, but significant ($P < 0.05$) reduction in EAD duration (EAD duration was decreased by 5-7 sec). Doses of arecoline of 0.03 mg/kg, 0.02 mg/kg or 0.05 mg/kg did not produce any significant differences in EAD duration. These animals were not pretreated with methylatropine, because at these low doses of arecoline, the animals showed only slight signs of peripheral cholinergic stimulation.

Eserine was tested in 10 cats (Fig. 23) and a highly significant ($P < 0.01$) mean decrease in EAD duration was produced by all doses

Figure 21. Effect of arecoline hydrochloride (Ar) on EAD duration. X refers to pooled standard error for all the mean differences calculated from the results of experiments on 8 cats. Methylatropine (MAt) 4 mg/kg was administered as a pretreatment. Time of injection indicated by arrows. Note immediate significant decrease in EAD duration after injection of arecoline.
** = $P < 0.01$.

Figure 22. Effect of low doses of arecoline hydrochloride (Ar) on EAD duration. This group of animals was not pretreated with methylatropine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. Time of injection indicated by arrows. Note that there are no significant changes in EAD duration at doses of arecoline less than 0.1 mg/kg.
** = $P < 0.01$.

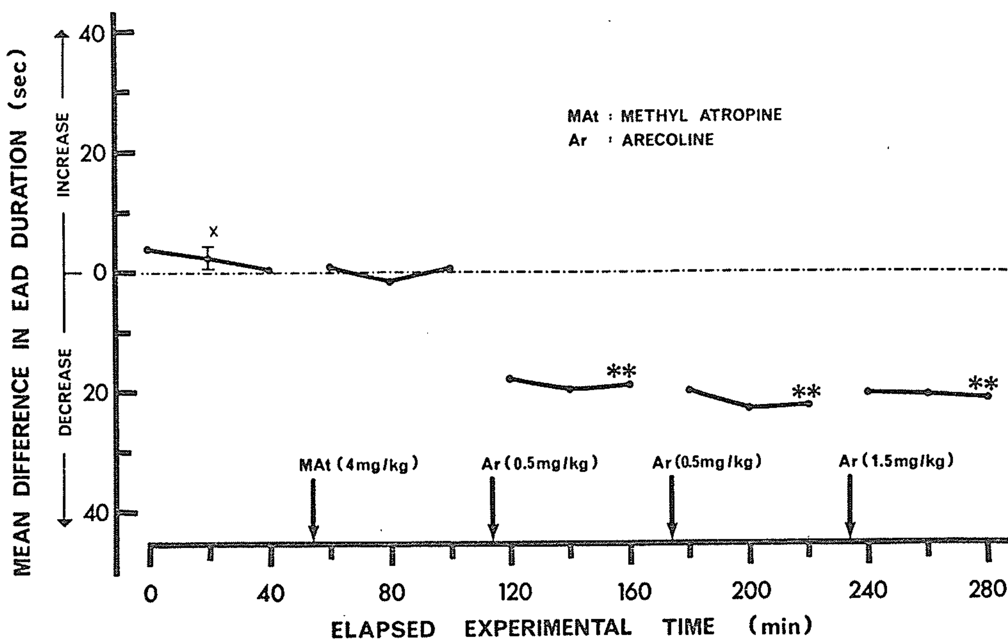


Figure 21.

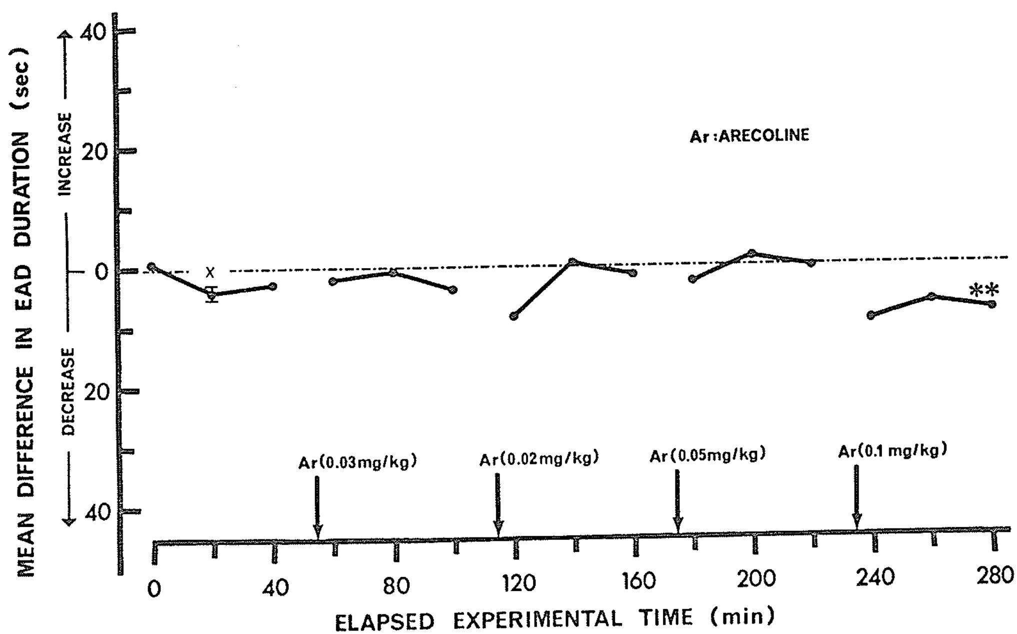


Figure 22.

tested. The first injection of eserine, 0.5 mg/kg produced a decrease in EAD duration of about 10 sec while the next injection of eserine 0.5 mg/kg decreased EAD duration approximately 15 sec. The third injection of eserine 1 mg/kg lowered EAD duration further to a final mean decrease of approximately 20 sec. Thus, there appears to be some cumulative effects following the 3 injections of eserine within one hour.

Administration of low doses of eserine (Fig. 24) 0.03 mg/kg or 0.02 mg/kg to 5 cats (not pretreated with methylatropine) did not modify control EAD responses. Following an injection of eserine 0.1 mg/kg there was a highly significant ($P < 0.01$) mean decrease in EAD duration of 5-7 sec.

Two sets of experiments were performed to test the effects on EAD duration of two centrally active cholinomimetics, pilocarpine (Fig. 25) and oxotremorine (Fig. 26) both agents having relatively specific muscarinic actions (Goodman and Gilman, 1970).

In 10 cats pilocarpine 1 mg/kg produced a highly significant ($P < 0.01$) mean decrease in EAD duration. Doses of pilocarpine 3 mg/kg caused a further decrease in duration to produce a mean decrease in EAD duration of 10-12 sec. The final dose of pilocarpine 4 mg/kg reduced EAD duration to about 15 sec blow control levels.

Injection of oxotremorine in 5 cats (Fig. 26) also produced a highly significant ($P < 0.01$) decrease in EAD duration at all doses tested. A complication in these experiments was the appearance, except at the lowest dose of oxotremorine, of definite signs of CNS stimulation resembling a rage reaction. Oxotremorine 0.01 mg/kg produced a mean decrease of about 5 sec, while the next injection of 0.09 mg/kg reduced

Figure 23. Effect of eserine (Es, physostigmine salicylate) on EAD duration. Methylatropine (MAt) 4 mg/kg was administered as a pretreatment. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 10 cats. Note immediate decrease in EAD duration after injection of eserine.
** = $P < 0.01$.

Figure 24. Effect of low doses of eserine (Es) on EAD duration. This group of animals was not pretreated with methylatropine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Time of injection indicated by arrows. Note lack of significant change after doses of eserine less than 0.1 mg/kg.
* = $P < 0.05$; ** = $P < 0.01$.

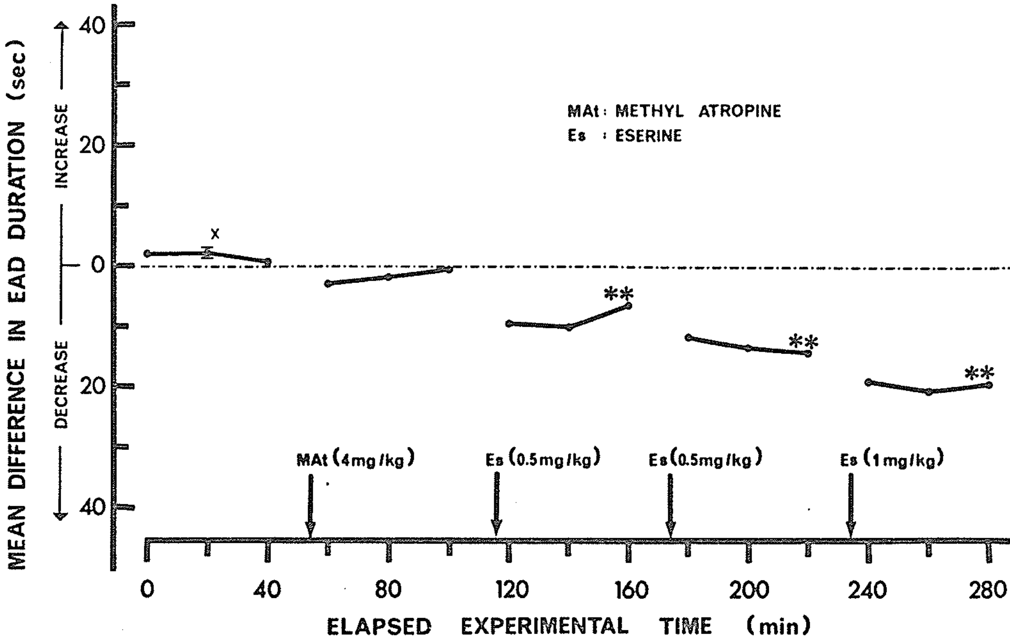


Figure 23.

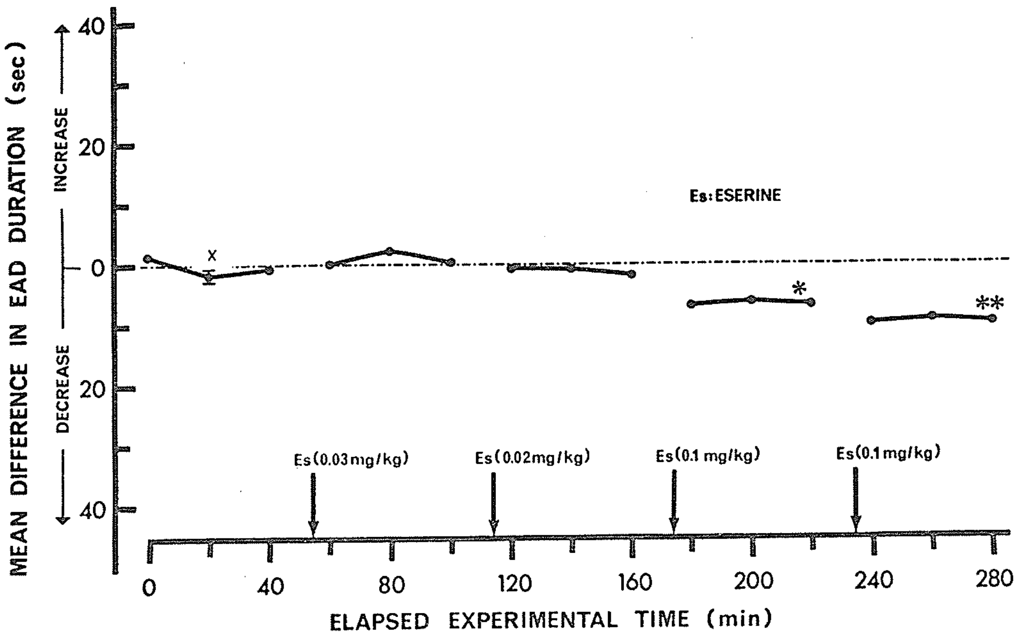


Figure 24.

Figure 25. Effect of pilocarpine hydrochloride (Pc) on EAD duration. Methyl atropine (MAt) 4 mg/kg was injected as a previous treatment. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 10 cats. Note immediate action of pilocarpine after injection.
** = $P < 0.01$.

Figure 26. Effect of oxotremorine sesquifumarate (OT) on EAD duration. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Methyl atropine 4 mg/kg was injected as a pretreatment.
** = $P < 0.01$.

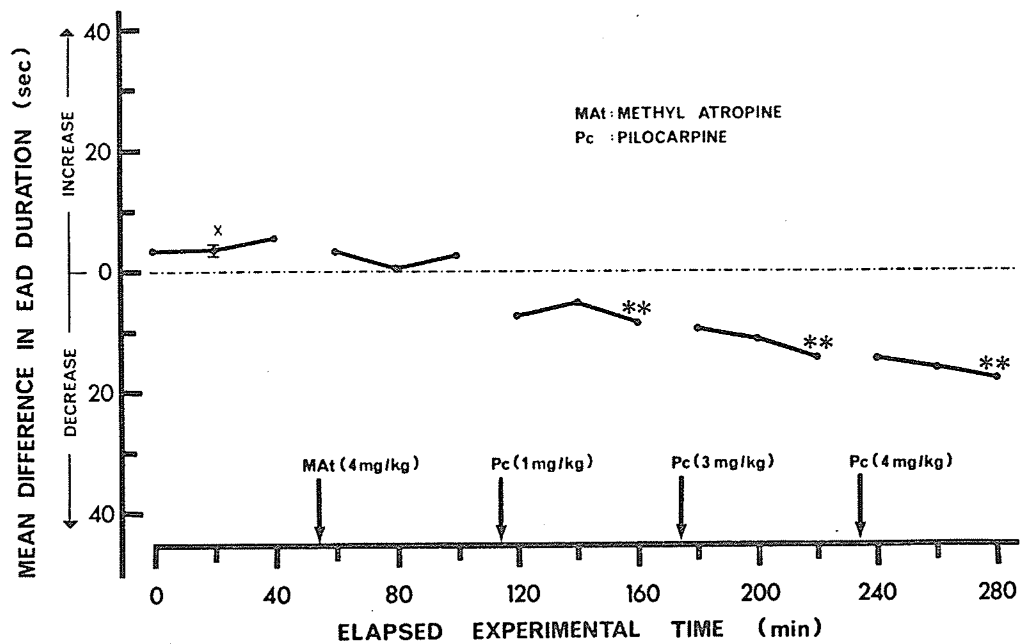


Figure 25.

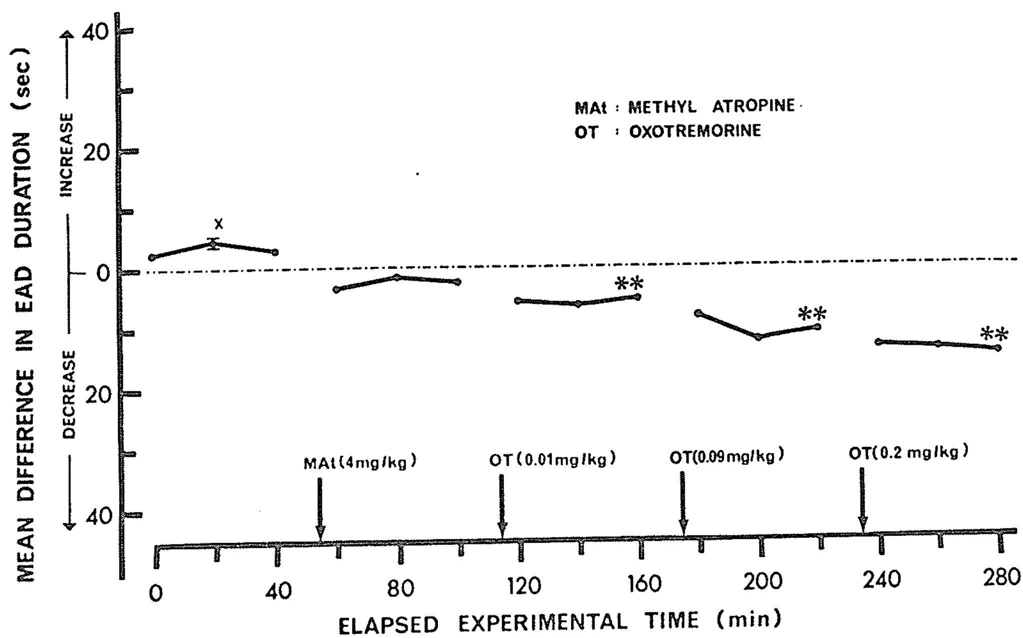


Figure 26.

EAD duration to about 10-12 sec below control responses. A final dose of oxotremorine 0.2 mg/kg decreased EAD duration further to about 15 sec less than control responses. There was probably some cumulation of oxotremorine with this schedule of drug administration.

Thus, based on the results of 29 experiments, the cholinergic drugs arecoline, eserine, pilocarpine and oxotremorine, all cause highly significant reductions in EAD duration.

b. Anticholinergic drugs

The antimuscarinic drugs atropine sulfate and scopolamine hydrochloride (hyoscine) are known to have potent central effects (Goodman and Gilman, 1970).

Atropine sulfate (Fig. 27) produced a highly significant ($P < 0.01$) increase in EAD duration at all doses tested. Methylatropine 4 mg/kg was injected as a control in 15 cats (Fig. 27) and did not produce any change in EAD duration. Administration of atropine 1 mg/kg to these same animals produced a mean increase in EAD duration of 5 sec. A further dose of atropine 2 mg/kg increased EAD duration to about 15 sec above control levels. Another injection of atropine 2 mg/kg (Fig. 27) produced a total mean increase of about 20 sec in EAD duration. There was probably a significant amount of cumulation of atropine following the successive doses of drug over a period of 2 hours (final cumulated dose 5 mg/kg).

The effects of low doses of atropine were studied in 6 cats (Fig. 28). Following an injection of atropine 0.05 mg/kg there was no change in EAD duration, whereas after the next injection of 0.05 mg/kg there was a highly significant ($P < 0.01$) increase of 5 sec in EAD duration. Following the next injection of atropine 0.1 mg/kg there was a

Figure 27. Effect of atropine sulfate (At) on EAD duration. Methyl atropine (MAt) 4 mg/kg was injected as a control. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 15 cats. Note immediate significant increase in EAD duration after injection of atropine.
** = $P < 0.01$.

Figure 28. Effect of low doses of atropine sulfate (At) on EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. Note that most doses (0.05 mg/kg - 0.3 mg/kg) tested increased EAD duration significantly.
* = $P < 0.05$; ** = $P < 0.01$.

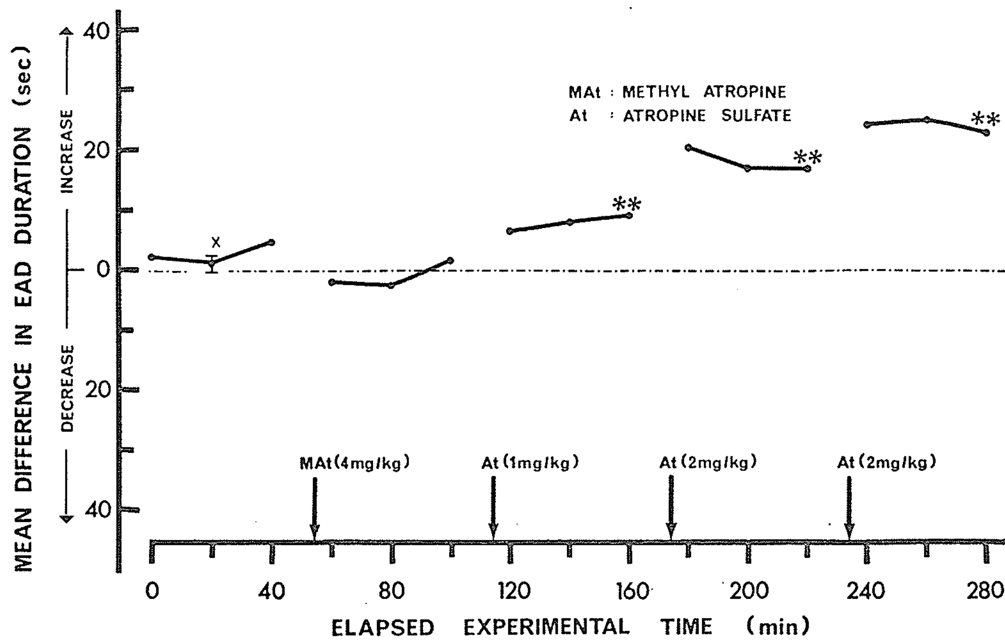


Figure 27.

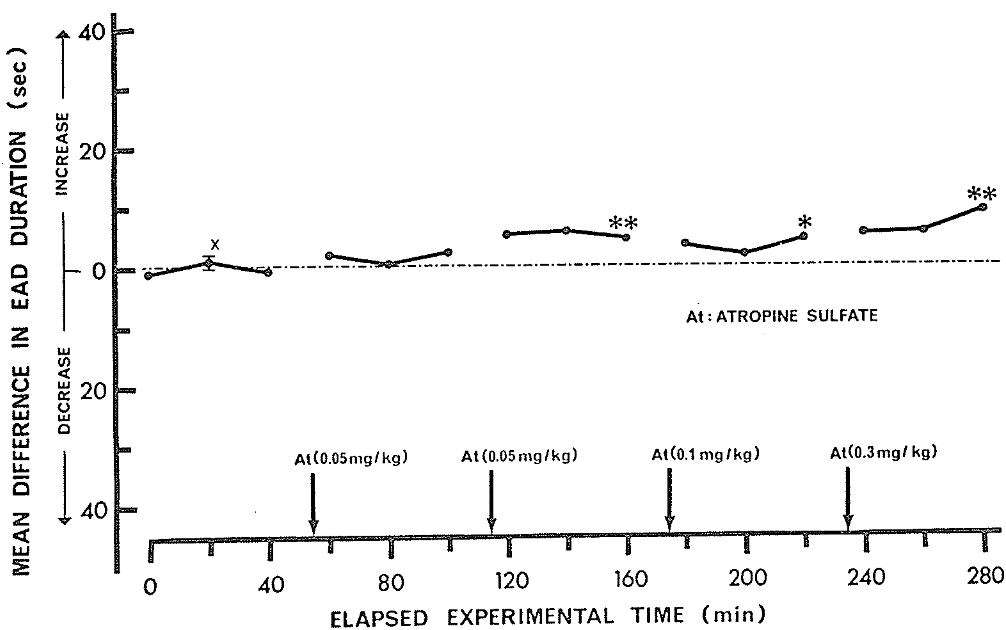


Figure 28.

Figure 29. Effect of scopolamine hydrochloride (Sc) on EAD duration in the isolated cortex. X refers to pooled S.E. for all the mean differences calculated from results of experiments on 8 cats. Note immediate increases in EAD duration.
** = $P < 0.01$.

Figure 30. Effect of low doses of scopolamine hydrochloride (Sc) on EAD duration. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note the immediate increases in EAD duration.
** = $P < 0.01$.

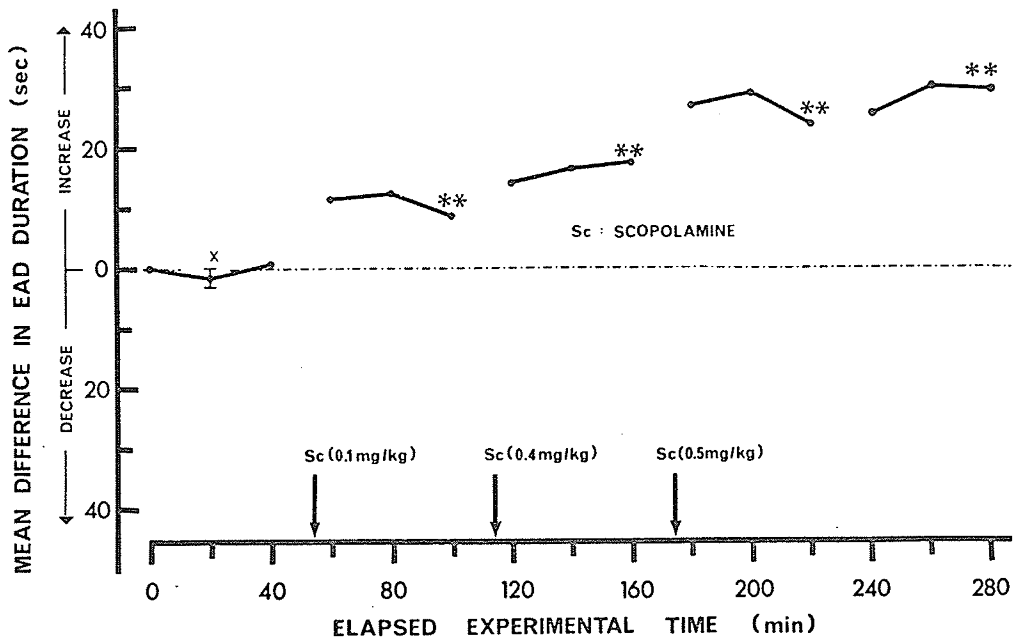


Figure 29.

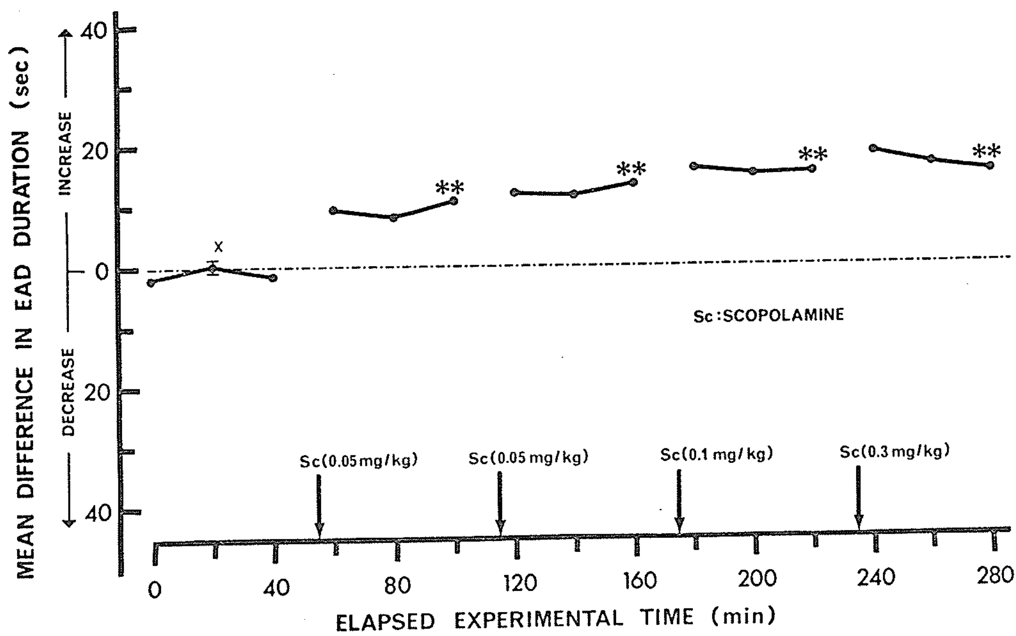


Figure 30.

small but significant ($P < 0.05$) increase in EAD duration of less than 5 sec. The final injection of atropine 0.3 mg/kg caused a highly significant ($P < 0.01$) increase of 5-8sec.

Scopolamine was studied in 8 cats (Fig. 29) and the resultant increase in EAD duration from control was, in each case, highly significant ($P < 0.01$). Injection of 0.1 mg/kg produced an increase of about 12 sec. The next injection of scopolamine 0.4 mg/kg elevated EAD duration further to approximately 15 sec while the last dose of 0.5 mg/kg resulted in a maximal increase of approximately 30 sec. It appears that there was cumulation of the drug with succeeding doses.

Low doses of scopolamine were also administered and it is clear from the results on 5 cats (Fig. 30) that scopolamine, even at low doses, is a potent agent for increasing EAD duration. After doses of 0.05 mg/kg, 0.1 mg/kg and 0.3 mg/kg there is a highly significant ($P < 0.01$) increase of EAD duration from 10 to 20 sec above control levels. As with higher doses of scopolamine (Fig. 29), there appears to be cumulation of scopolamine with these low doses (Fig. 30).

No overt behavioural effects were noticed after injections of scopolamine 0.5 mg/kg whereas at 1 mg/kg (cumulated dose) scopolamine usually produced signs of mild CNS stimulation. These usually consisted of fixed, randon, staring movements and in 2 cats an apparent state of hallucination. All cats receiving antimuscarinic drugs displayed widely dilated pupils and were slightly restless.

The effects of two anti-parkinson agents, trihexyphenidyl (Benzhexol) in 3 cats, and procyclindine (Kemadrin in 2 cats, were evaluated using afterdischarge duration as a parameter. The results of these studies are given in Table 2.

Table 2. Effects of Atropinic Anti-Parkinson Drugs on EAD Duration

SALINE-CONTROL			TREATMENT - 1
Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	Mean Difference in EAD Duration (sec) ± S.E. (N)
3.4 ± 0.6 (3)	Trihyexyphenidyl	1 mg/kg	13.1 ± 1.7 (3)*
4.2 ± 1.3 (2)	Procyclidine	1.5 mg/kg	14.6 ± 2.5 (2)*

N = number of animals
* = $P < 0.05$

Thus, anti-parkinson drugs with atropinic activity prolong EAD duration significantly ($P < 0.05$) by about 10 sec.

D. Effects of Cholinergic and Anticholinergic Drugs on EAD Duration

At this point it if reasonably clear that cholinergic drugs with direct central actions decrease EAD duration, whereas centrally-active anticholinergic drugs increase EAD duration. The next step in this thesis was the investigation of the effects of either of these types of drugs on the activity of the other type, in order to study in this experimental preparation the interactions between these mutually pharmacologically antagonistic classes of compounds.

In 5 cats (Fig. 31) atropine 3 mg/kg increased EAD duration very significantly ($P < 0.01$) by about 20 sec. This dose of atropine was followed by eserine 0.5 mg/kg which produced no further change. A second injection of eserine 0.5 mg/kg (cumulated dose 1 mg/kg) decreased the EAD duration (that had been increased previously by atropine) almost back to control levels (Fig. 31).

In another 6 cats (Fig. 32) injection of atropine 5 mg/kg

Figure 31. Interaction between cholinergic agonists and antagonists upon EAD duration in the isolated cortex. At = atropine sulfate 3 mg/kg; Es = eserine (physostigmine salicylate) 0.5 mg/kg. _X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note lack of change in EAD duration after first injection of eserine. ** = $P < 0.01$.

Figure 32. Interaction between cholinergic antagonists and agonists upon EAD duration. At = atropine sulfate 5 mg/kg and 3 mg/kg Pc = pilocarpine hydrochloride 4 mg/kg. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. Note lack of significant changes in EAD duration after first injection of pilocarpine. ** = $P < 0.01$.

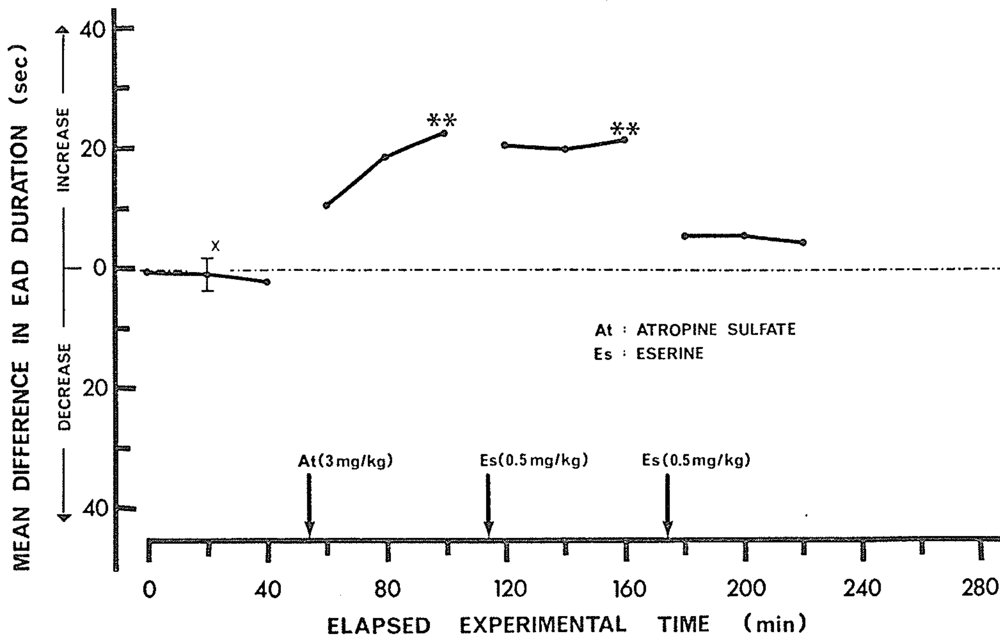


Figure 31.

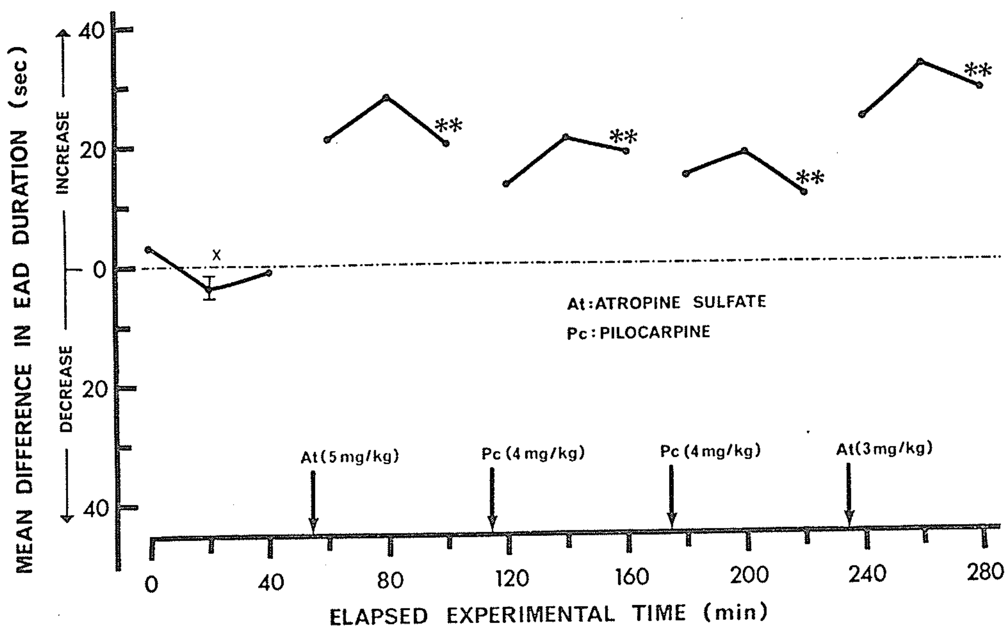


Figure 32.

resulted in a very significant ($P < 0.01$) increase in EAD duration (about 25 sec; Treatment-1). Following this treatment, pilocarpine 4 mg/kg was injected, and this resulted in a small (about 5 sec), but not significant, reduction in EAD duration. A second dose of pilocarpine 4 mg/kg reduced EAD duration further, but EAD duration still remained at about 15 sec above control levels. This EAD duration was very significantly ($P < 0.01$) longer than control EAD duration but also significantly ($P < 0.05$) different from the duration of EADs elicited after injection of atropine (Treatment-1). A final dose of atropine 3 mg/kg reversed this small decrease in the atropine augmented EAD duration caused by pilocarpine and returned it to a level about 25 sec above control, which was highly significantly ($P < 0.01$) different from control. The durations of EADs elicited after this last dose of atropine were also significantly ($P < 0.05$) greater than those elicited after the administration of pilocarpine.

Thus, on the basis of 11 experiments, atropine is able to antagonize the effects of cholinergic drugs upon EAD duration and cholinergic drugs antagonize atropine effects on EAD duration.

In the next two series of experiments, the cholinergic drugs pilocarpine (Fig. 33) or arecoline (Fig. 34) were administered 60 min before injecting atropine. The animals in these experiments were pretreated with methylatropine 4 mg/kg.

Intraperitoneal administration of pilocarpine 4 mg/kg (Fig. 33) in 5 cats decreased EAD duration very significantly ($P < 0.01$) to about 15 sec below control. The next injection consisted of atropine 3 mg/kg which reversed the decrease in EAD duration produced by

Figure 33. Interaction between a cholinergic agonist and antagonist upon EAD duration. MAt = methyl atropine nitrate 4 mg/kg (pre-treatment) Pc = pilocarpine hydrochloride 4 mg/kg; At = atropine sulfate 3 mg/kg. X refers to pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

Figure 34. Interaction between cholinergic agonists and antagonists upon EAD duration. MAt = methyl atropine nitrate 4 mg/kg (pre-treatment) Ar = arecoline hydrochloride 1 mg/kg; At = atropine sulfate 3 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
** = $P < 0.01$.

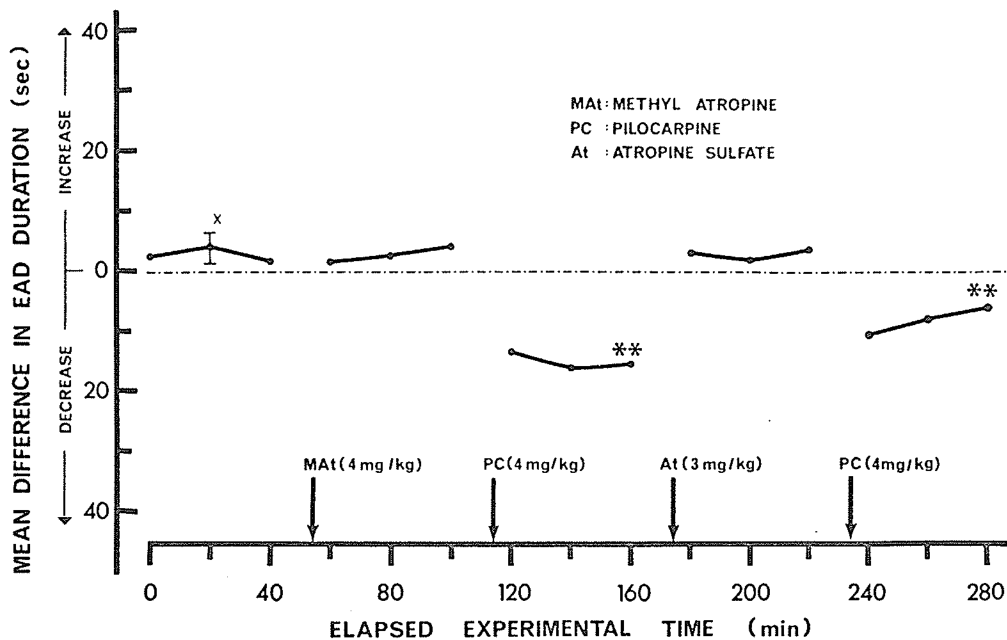


Figure 33.

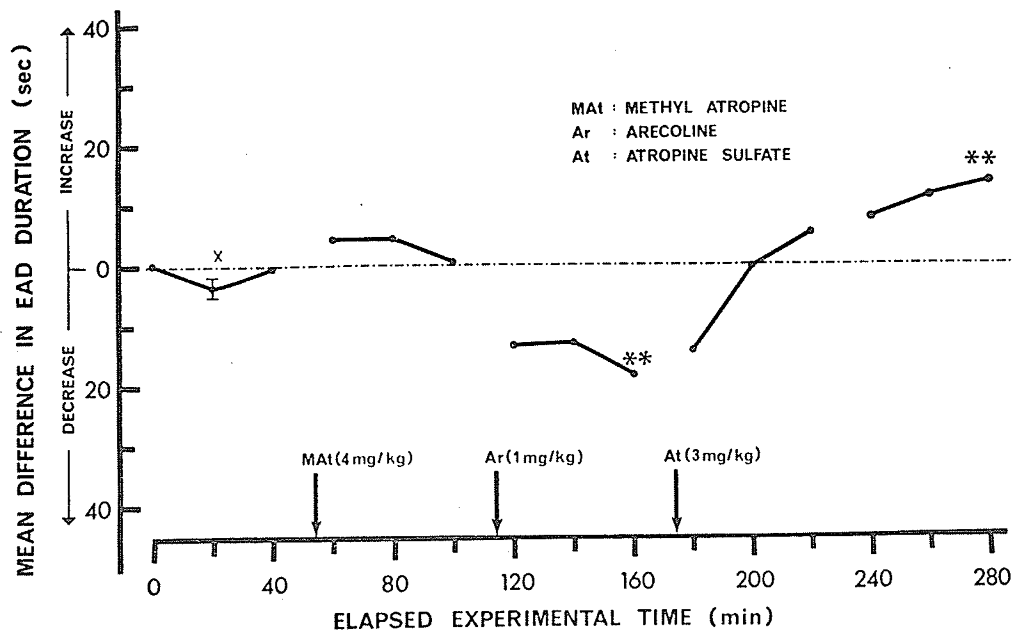


Figure 34.

pilocarpine, returning it to control levels, a change that resulted in a highly significant difference when comparing it to the previous responses decreased from control by pilocarpine. The final injection in these experiments, pilocarpine 4 mg/kg caused a smaller (< 10 sec) decrease in EAD from control values, which was, however, still highly significant ($P < 0.01$).

Another group of 6 cats (Fig. 34) received arecoline 1 mg/kg which very significantly ($P < 0.01$) reduced EAD duration by about 15 sec. When this treatment was followed by the injection of atropine 3 mg/kg, EAD duration returned to control levels, which represented a highly significant change (Fig. 34). Monitoring the response for 60 min, it can be seen that the EAD duration becomes very significantly ($P < 0.01$) increasingly longer, going by about 10 sec beyond control levels. Thus, pretreatment with cholinergic agents also is able to antagonize, at least partially, the effect of a subsequent dose of an antimuscarinic drug. Using afterdischarge duration as a parameter it appears that cholinergic and anticholinergic drugs are mutually antagonistic in their effects in EAD duration in the chronically isolated cortical slab.

E. Effects of Nicotinic or Nicotinic Blocking Drugs on EAD Duration

Since the results, thus far, show that cholinergic drugs decrease, and anticholinergic drugs increase EAD duration, it seemed reasonable to investigate further whether nicotinic receptors are involved in the pharmacological control of this response.

a. Nicotinic agonists

In 5 cats (Fig. 35) nicotine sulfate was injected at various

increasing doses and no significant effect of this agent on EAD duration was observed. Neither 0.2 mg/kg, 0.6 mg/kg or 0.8 mg/kg changed the response. These doses of nicotine were reasonably well tolerated. In 1 cat there was emesis and diarrhea. All cats ultimately showed pupillary dilatation. In 3 cats there was some initial evidence of slight central and respiratory stimulation at the highest 0.8 mg/kg dose of nicotine.

Four cats were treated with lobeline sulfate (Fig. 36). Neither 2.5 mg/kg nor 7.5 mg/kg produced any significant alteration in EAD duration. Other than emesis in one cat after 7.5 mg/kg of lobeline, no noticeable side-effects due to this agent were observed.

Thus, on the basis of results from experiments on 9 cats, it is apparent that nicotinic drugs, at the doses tested, lack significant actions upon afterdischarge duration.

b. Nicotinic blocking agents

Many drugs known to block peripheral nicotinic receptors are not capable of penetrating into the CNS because they have a highly charged quaternary nitrogen atom (Goodman and Gilman, 1970). Two drugs having pharmacological nicotinic blocking action and distributing appreciably into cerebral tissue, as well, are mecamylamine and dihydro-β-erythroidine (Goodman and Gilman, 1970).

In 6 cats mecamylamine (Fig. 37) tested at 3 doses did not cause any significant change in EAD duration from control. The injection of mecamylamine 0.5 mg/kg on two occasions caused no change. If there was cumulation of drug, this does not appear to have influenced the response. In any case, a final injection of mecamylamine 1.5 mg/kg

Figure 35. Effect of nicotine sulfate (NS) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.

Figure 36. Effect of lobeline sulfate (LS) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.

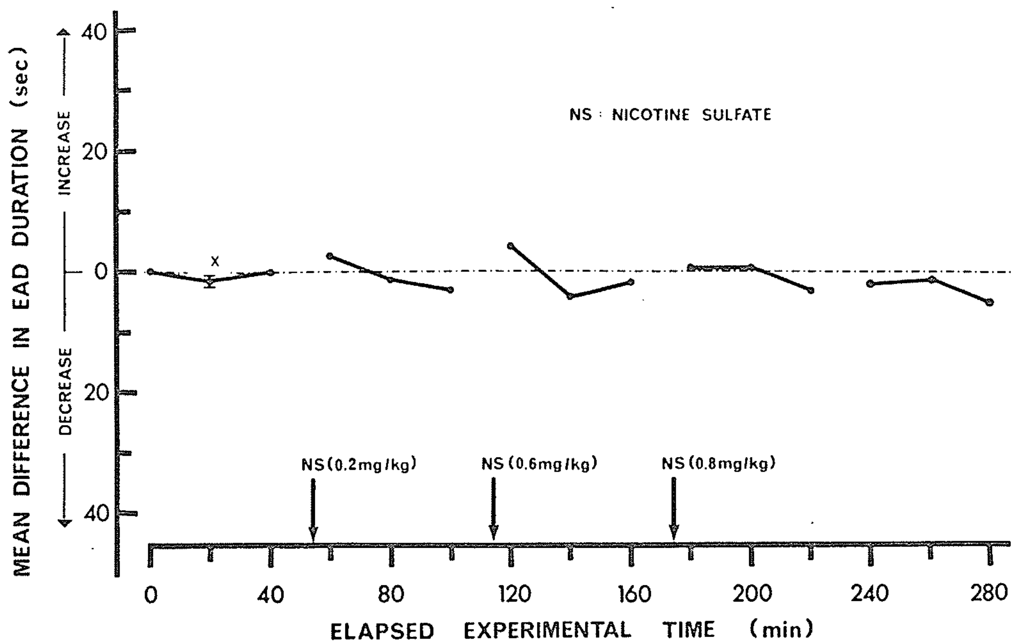


Figure 35.

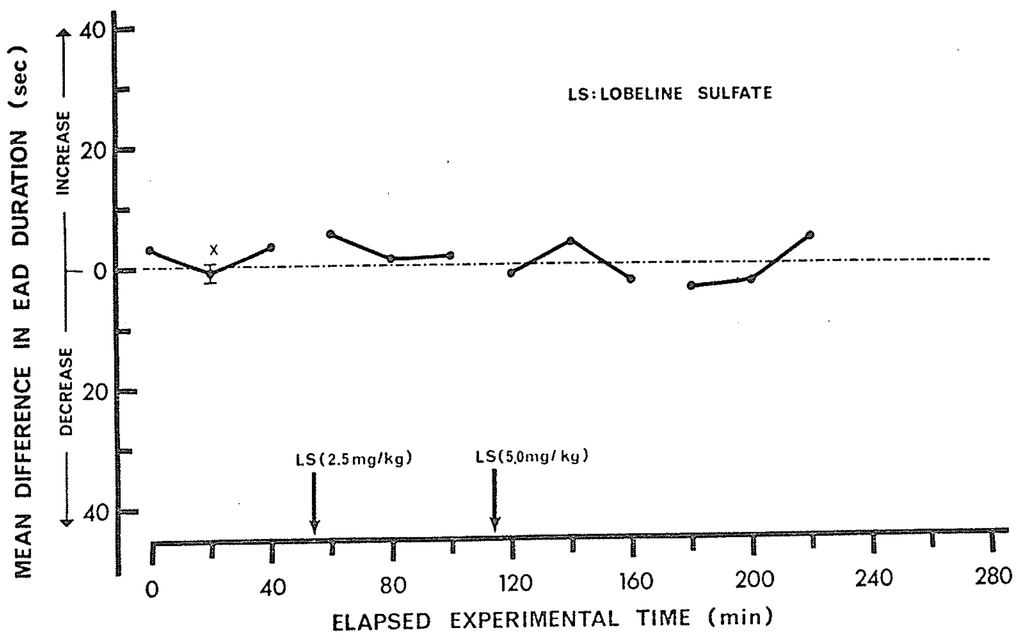
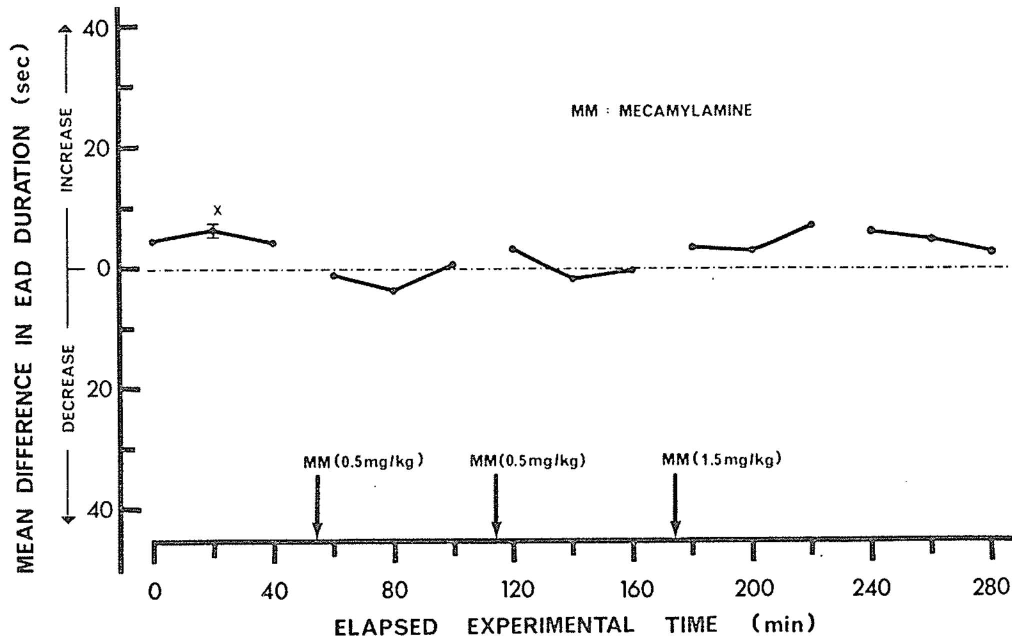


Figure 36.

Figure 37. Effect on an anti-nicotinic agent, mecamylamine hydrochloride (MM), upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. Note lack of significant effect at all three doses tested.

Figure 38. Effect of an anti-nicotinic agent, dihydro-β-erythroidine hydrobromide (BE) on EAD duration. X refers to the pooled S.E. for all the mean differences from the results of experiments on 5 cats. Note lack of significant effect at each of three doses tested.



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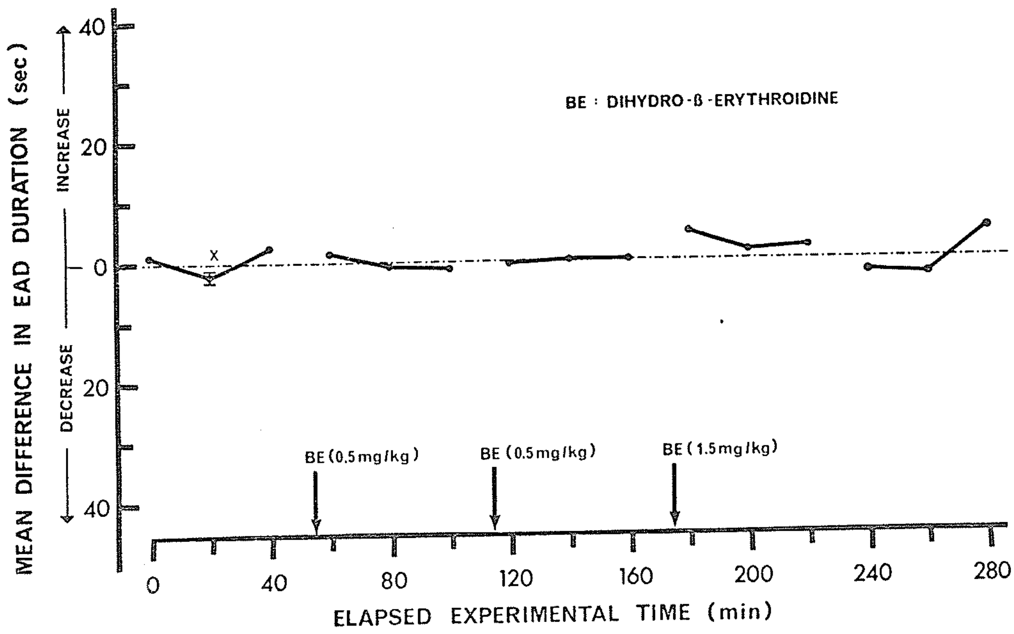


Figure 33.

produced no change in EAD duration.

Dihydro-β-erythroidine was tested in 5 cats (Fig. 38). In order of treatment, the doses administered IP were 0.5 mg/kg, 0.5 mg/kg and 1.5 mg/kg. No change in EAD duration was observed following any of these treatments. Again, it is not clear whether there was any cumulation of drug following this series of drug injections.

Thus, the centrally active drugs mecamylamine and dihydro-β-erythroidine, which are known to compete for nicotinic receptors in the periphery, and penetrate the blood-brain barrier (Goodman and Gilman, 1970), do not modify EAD duration in the chronically isolated cortical slab.

F. Effects of Muscarinic and Nicotinic Drugs and Their Antagonists on EAD Duration

To better evaluate the apparent lack of effect on EAD duration of nicotinic agonist and antagonist drugs, experiments were designed in which muscarinic and nicotinic drugs and their antagonists were tested at the same time. These studies were performed to help to determine whether the action upon EAD duration produced by the cholinergic agents used was purely muscarinic or whether it had some nicotinic component.

In 5 experiments cats were pretreated with methylatropine 4 mg/kg (Fig. 39), 10 min after which mecamylamine 1 mg/kg was injected. The next injection in this experiment was oxotremorine 0.1 mg/kg which caused a highly significant ($P < 0.01$) decrease in EAD duration. The next treatment, scopolamine, 1 mg/kg reversed this decrease and ultimately caused a significant ($P < 0.05$) increase in EAD duration. Test-

ing further revealed that this increase in EAD duration became very significantly ($P < 0.01$) greater than the duration of the control EADs and greater than EAD duration after oxotremorine.

Administration of dihydro-β-erythroidine 1 mg/kg 10 min after pretreatment with methylatropine 4 mg/kg (Fig. 40), in another 5 cats, did not result in any significant change in EAD duration. This treatment was followed by the injection of eserine 0.5 mg/kg which produced a highly significant ($P < 0.01$) reduction in EAD duration (about 18 sec shorter than control). The next injection consisted of atropine 5 mg/kg which reversed the previous decrease in EAD duration produced by eserine and returned EAD duration to control levels.

Thus, on the basis of 9 experiments, the nicotinic blocking drugs mecamylamine or dihydro-β-erythroidine do not modify the subsequent effects of muscarinic or of antimuscarinic drugs upon the duration of EADs.

The remaining studies were done to investigate whether nicotine sulfate would modify the response to subsequently administered muscarinic or antimuscarinic drugs.

Administration of nicotine sulfate 0.8 mg/kg (Fig. 41) in 5 cats caused no change in control EAD duration. This injection was followed by the injection of pilocarpine (4 mg/kg), which produced a highly significant ($P < 0.01$) reduction in EAD duration (almost 20 sec shorter than control). This reduction was reversed by the injection of scopolamine 1 mg/kg.

Another group of 5 cats was treated with atropine 3 mg/kg (Fig. 42), which resulted in a highly significant ($P < 0.01$) increase

Figure 39. Lack of nicotinic component in cholinergic agonist-antagonist interaction on EAD duration. MAt = methyl atropine nitrate 4 mg/kg (pretreatment); MM = mecamylamine hydrochloride 1 mg/kg; OT = oxotremorine sesquifumarate 0.1 mg/kg; Sc = scopolamine hydrochloride 1 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
* = $P < 0.05$; ** = $P < 0.01$.

Figure 40. Lack of nicotinic component in the action of eserine upon EAD duration. MAt = methyl atropine nitrate 4 mg/kg (pretreatment); BE = dihydro-β-erythroidine hydrobromide 1 mg/kg; ES = eserine (physostigmine salicylate) 0.5 mg/kg; At = atropine sulfate 5 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

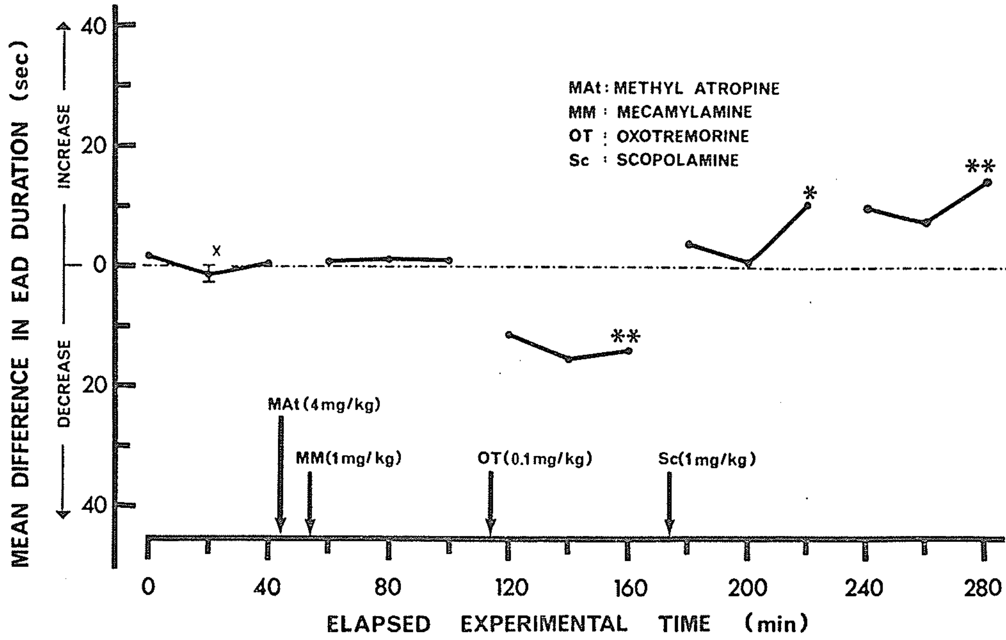


Figure 39.

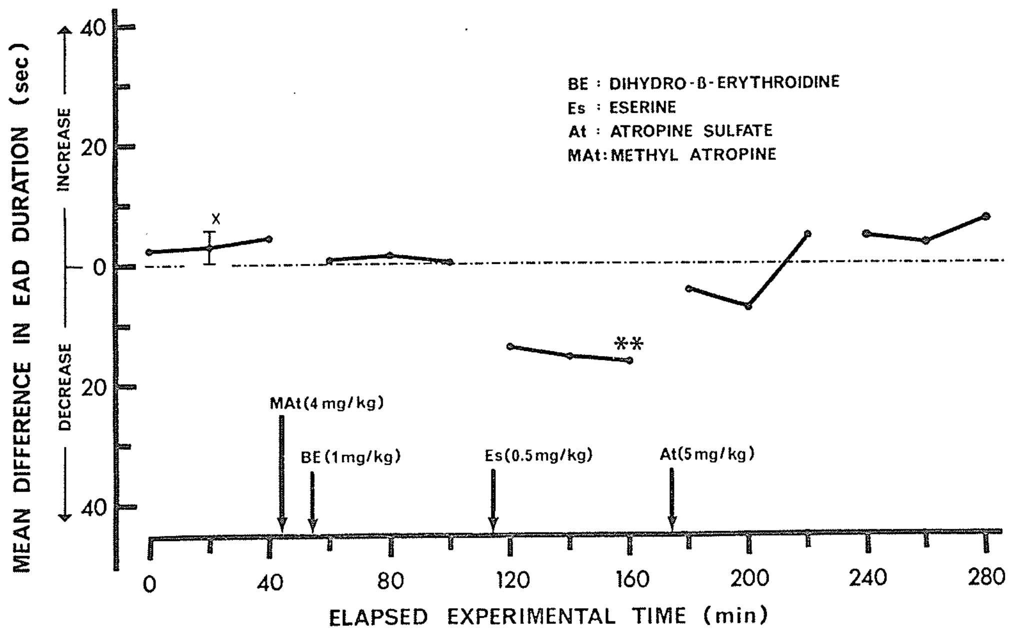


Figure 40.

Figure 41. Lack of effect of nicotine on cholinergic agonist-antagonist interaction upon EAD duration. MAt = methyl atropine nitrate 4 mg/kg (pretreatment); NS = nicotine sulfate 0.8 mg/kg; PC = pilocarpine hydrochloride 4 mg/kg; Sc = scopolamine hydrochloride 1 mg/kg. X refers to pooled S.E. for all of the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

Figure 42. Lack of effect of nicotinic component in the action of atropine upon EAD duration. At = atropine sulfate 3 mg/kg. NS = nicotine sulfate 0.8 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

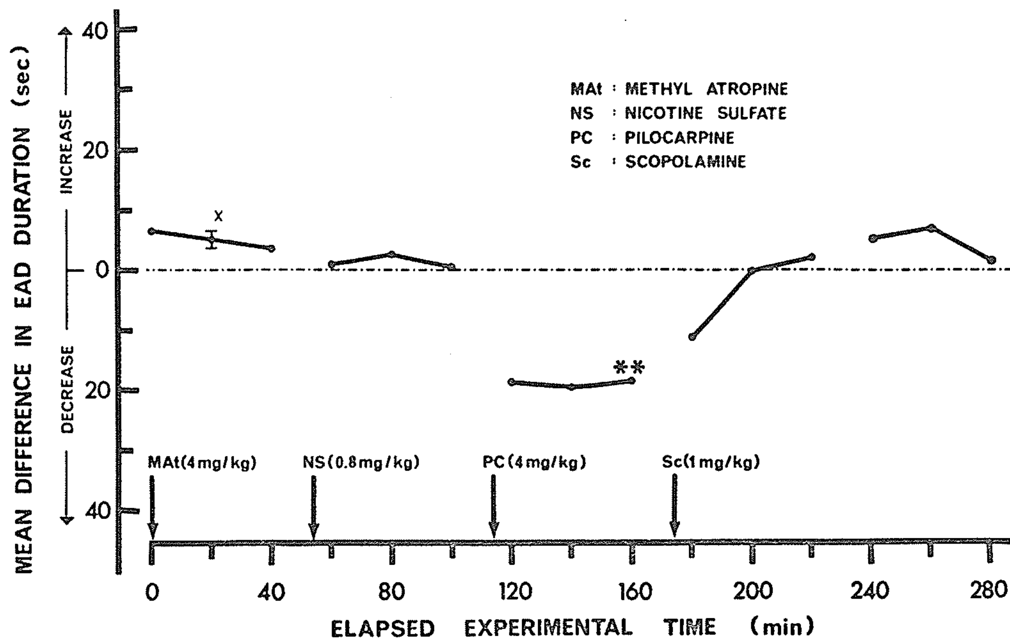


Figure 41.

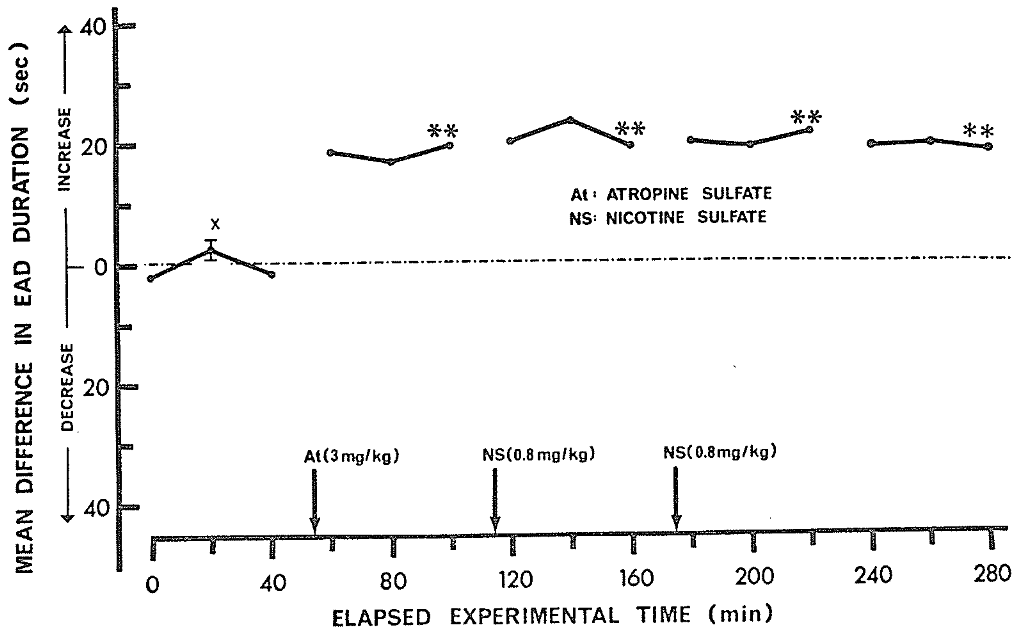


Figure 42.

(about 20 sec) in EAD duration. This treatment was followed by the injection of nicotine sulfate (0.8 mg/kg) which did not modify this already increased afterdischarge duration. Another subsequent injection of nicotine sulfate 0.8 mg/kg was also without any significant effect on the atropine-increased EAD durations. All of the EADs following administration of atropine remained very significantly ($P < 0.01$) longer than control responses.

Thus, in 9 experiments, nicotine did not modify the response to muscarinic and antimuscarinic drugs. If nicotine is acting in the CNS as it does in the periphery (Goodman and Gilman, 1970) then probably the first response 5 min after injection is during a phase of transient stimulation. In all probability, the next two responses were elicited during the phase of depressant or inhibitory activity of nicotine on the same structures (if they are indeed present in the cerebral cortex).

G. Resume

It is apparent that cholinergic drugs (Fig. 21-26) produce significant decreases in EAD duration. This action appears to be exerted on specifically muscarinic structures as nicotinic drugs (Fig. 35; 36) have no effect on EAD duration. Antimuscarinic agents (Fig. 27-30) on their own significantly prolong EAD duration, whereas anti-nicotinic agents (Fig. 37; 38) had no effect on EAD duration. Experiments with both muscarinic and antimuscarinic drugs (Fig. 31-34) show that these types of agents are mutually antagonistic with respect to EAD duration. Nicotinic and anti-nicotinic drugs (Fig. 39-42) did not alter the effects or the interactions on EAD duration of the muscarinic and antimuscarinic drugs.

H. Discussion

The results of studies with cholinergic agents presented in this dissertation, suggest that there are cholinoceptive sites in the cerebral cortex which are capable of limiting the duration of epileptiform afterdischarges, and that ACh has a role in the epileptiform activity. All the centrally active cholinomimetics administered (Fig. 21; 23; 26) reduced significantly the duration of afterdischarges. This must have been a direct action on the cerebral cortex, since cholinergic drugs with charged quaternary nitrogen groups (Fig. 18; 19; 20) and methyl atropine (Fig. 17), agents which do not penetrate into the CNS but that have considerable effects on the cardiovascular system, did not modify afterdischarge duration.

The anticholinergic drugs atropine and scopolamine significantly prolonged EAD duration (Fig. 27; 28; 29; 30), and are capable of blocking the action of the cholinergic drugs (Fig. 31; 32).

The fact that eserine modified EAD duration and that this action is blocked by atropine (Fig. 31) suggests that there is in the neuronally isolated slab of cerebral cortex an endogenous and continuous cholinergic tone. Moreover, the antagonism by eserine of the effects of atropine (Fig. 32) and the antagonism by atropine of the effects of eserine (Fig. 40) suggests that this action of cholinomimetics is exerted via central muscarinic and not nicotinic receptors. This apparent lack of nicotinic structures in the chronically isolated cortex is also borne out by lack of effect of nicotinic drugs (Fig. 35; 36) and of nicotinic receptor blocking drugs (Fig. 37; 38; 39; 40). In addition, treatment with nicotine (Fig. 41; 42) or with nicotinic antagonists (Fig. 39; 40) does not modify the response of the slab to

muscarinic or antimuscarinic drugs, or their interactions with each other.

Therefore, it appears that the cholinergic modulation of afterdischarge duration in the cerebral cortex is mediated by inhibitory muscarinic synapses. Interruption of this tonic mechanism surgically or pharmacologically results in the hyperexcitable behaviour of a population of cortical neurons. Thus, the cerebral cortex appears to be provided with a cholinergic mechanism capable of limiting the spread of epileptiform activity.

Stavraky (1961) has showed that destruction or ablation of one portion of the cerebrum apparently renders descending chains of neurons from that area supersensitive to topically applied ACh. Echlin and Battista (1963) have reported that there is an increased sensitivity of chronically isolated cortex to administered ACh and which is primarily responsible for its increased epileptiform tendency.

Infantellina (1955) studied the effects of ACh on isolated cortex and showed that low concentrations suppressed burst responses, while larger concentrations augmented spiking. Maiti and Domino (1961) showed that high doses of eserine decreased EAD duration. In contrast, low doses of eserine increased EAD duration. Gol and Kellaway (1963) have reported that "critical" concentrations of eserine changed paroxysmal activity of isolated cortex to more regular EEG rhythms, while higher concentrations elicited irregular discharges. Rosenberg and Echlin (1968) suggested that chronically isolated cortex is more permeable to ACh. Phillis and York (1968a) have shown that ACh applied mircoiontophoretically inhibits cortical neurons, probably acting through muscarinic and nicotinic receptors. Evoked responses in the

cerebral cortex have also been shown to be inhibited by cholinergic drugs (Malcom et al., 1967). More recently, Bernard et al., (1969) have reported that ACh or eserine topically applied to the cortex shortened or abolished EADs at the treated cortical site. This evidence suggests that inhibitory effects in the cortex due to cholinergic drugs are possible.

As discussed earlier (section VII.A.) it is quite likely that the epileptiform responses evoked in the cortex by topically applied cholinergic agents are part of a generalized response to relatively large amounts of drugs administered by this route. Thus, when a large variety of substances are tested topically on the cerebral cortex, usually in high concentrations, paroxysmal discharges and seizures are produced. It is probable that the modes of action of these substances at a specific level (neurons, membranes, enzymes, ions, etc) are widely different. Whatever their mechanism of action, these substances probably upset the balance or co-ordination between inhibitory and excitatory phenomena at cerebral synapses, leading to uncontrolled excitation.

Using a simplified approach to the problem, Neal (1967) has studied the effect of convulsant agents on the isolated guinea pig ileum since this preparation showed some properties of cholinergic neurons. He found that all convulsant agents tested decreased the release of ACh, as shown by a depressed twitch response. Thus, if convulsions do occur as a result of decreased ACh release as these findings by Neal suggest, it is possible to relate this to my findings that cholinergic drugs arrest excessive epileptiform activity in the cortex.

Ward (1961; 1969) has suggested that chronic deafferentation may be a mechanism for epileptogenesis in the cortex. This postulate is based on observations made on chronic alumina foci (Ward, 1961, Westrum _et al._, 1965) which showed that there is a disappearance of spines from the dendrites of pyramidal cells. Similar findings have been reported for samples of human epileptic cortex (Scheibel and Scheibel, 1968). Furthermore, the experimental foci (Westrum _et al._, 1965) showed an overall decrease in neuronal elements, increase in glia, and a less dense pattern of terminal arborizations in superficial layers of the cortex. Similar findings in chronically isolated cortex have been reported by Reiffenstein, (1964), Rutledge _et al._, (1967; 1969) and Weisman (1969). Sypert and Ward (1967) have correlated altered neuronal firing similar to EEG patterns obtained from epileptic patients, with structural changes in the cortical elements examined in epileptogenic foci.

There is histomorphological evidence (Colonnier, 1966; Gray, 1969; Szentagothai, 1969) that chronically isolated cortex retains synaptic structures apparently involved in inhibitory phenomena at axo-somatic junctions. Randic _et al._, (1964) reported that neurons in more superficial layers of the cortex (layers II-IV) are inhibited by ACh. This is in accord with later studies by Phillis and York (1967) who postulated the presence of an intracortical cholinergic inhibitory synapse (Phillis and York, 1968a).

Previously (section VII.A.), evidence indicating that significant reductions in specific AChE activity in isolated cortex occur with increasing periods of isolation was discussed. However, Tower (1955) observed increased AChE levels in cortical epileptogenic lesions, which

he regarded as a compensatory mechanism for a decreased "binding" of synthesized ACh in such tissue. Sastry (1956) has shown that there are significant reductions in slab ACh content several weeks after isolation. It is not clear whether ACh and AChE levels in this tissue are related and whether lowered AChE activity indicates a reduced cholinergic activity. If we attribute an inhibitory function to cortical ACh, any reduction in cholinergic activity should disrupt normal inhibitory control, which in turn would allow excitatory phenomena in the cortex to become exaggerated.

Output of ACh (Collier and Mitchell, 1967; Dudar and Szerb, 1969), AChE activity (Hebb *et al*., 1963; Rosenberg and Echlin, 1965; 1968; Duncan *et al*., 1968) and ACh content (Sastry, 1956) all show significant decreases in isolated cortex at a time when supersensitivity to electrical stimulation develops (Sharpless and Halpern, 1962). Apparently, these alterations in the cholinergic system reflect parallel changes in the level of available and functional ACh. Duncan *et al*., (1968) have demonstrated that long-term intermittent stimulation, with subthreshold stimuli, of chronically isolated cortex prevents the expected decrease in AChE content, and minimizes the appearance of increased cortical excitability. These findings suggest that there is an inverse relationship between the level of cholinergic activity and the epileptogenicity of isolated cortex. However, since there is also a concomittant reduction in neurons in isolated cortex a change in slab total ACh content might not result in significant changes in cholinergic activity at the unitary level, although Krnjevic *et al*., (1970) indicate that it is considerably compromised.

Clinical evidence (Williams, 1941) has been published showing

that small doses of eserine in patients with petit mal prevented petit mal attacks, while large doses caused an increase in the number and duration of seizures. Hyde et al., (1949) tested diisopropyl fluorophosphate (DFP) in the cat and found that neither topical administration nor intravenous administration of this potent cholinesterase inhibitor resulted in convulsive activity. Thus, there is some evidence not agreeing with the convulsant actions of cholinergic drugs as reported by many authors (section V.A.).

In other studies, atropinic agents have been administered and shown to augment epileptiform activity (Albertoni, 1882; Minvielle et al., 1954; Bernard et al., 1968; Vas et al., 1969). These results suggest, indirectly, that pharmacological interruption of a central cholinergic mechanism, apparently inhibitory, somehow results in a tendency for enhanced epileptic activity. Bernard et al., (1969) have demonstrated that after atropine was topically applied to the motor and sensorimotor cortex of non-anaesthetized rabbits, the locally elicited EAD was markedly prolonged, which agrees with my findings.

If there is such a cortical cholinergic inhibitory mechanism as is proposed in this thesis, its details are difficult to unify, since there is much evidence in the literature showing that administration of ACh onto cortical and other cerebral structures results in the production of epileptiform activity (Brenner and Merritt, 1949; Forster, 1951; Purpura, 1953; Guerrero-Figueroa et al., 1964; Baker and Benedict, 1970). Other recent studies (Sie et al., 1965; Celesia and Jasper, 1966) have demonstrated that superfusion of the cortical surface with a physiological solution containing neostigmine results in the appearance of local EADs in animals displaying an alert EEG pattern.

Funderburk and Case (1951) also found that an excess of ACh may cause convulsions. However, they also found that curare or penicillin topically applied induced spiking in the EEG. Atropine (1 mg/kg i.v.) increased the spiking caused by curare or penicillin, and decreased or abolished that due to ACh. On the other hand, eserine increased spiking due to the large doses of ACh, but it decreased the spiking activity due to curare and penicillin respectively. These authors concluded that the spiking and epileptiform activity elicited by topically applied ACh were an unspecific rather than a synaptic effect. Maiti and Domino (1961) reported that low doses of eserine, in contrast to large doses, prolong afterdischarge duration, a result that I was unable to duplicate.

Silvestrini and Longo (1959) have demonstrated that eserine administered intravenously blocked strychnine evoked cortical spiking, whereas when eserine was applied topically it increased the spiking causing repetitive discharges. These authors also found that topically applied scopolamine did not block this repetitive discharge, whereas systemically administered scopolamine increased the frequency of spiking. Antimuscarinic like effects on evoked potentials by strychnine have been reported (Walther, 1969). Besides showing some agreement with the results presented in this thesis, the study of Silvestrini and Longo indicates the uncertainty of the results obtained by topical application of substances to the cortex.

Tower (1960) has postulated that epileptogenic cortex is characterized by a derangement of cerebral acetylcholine metabolism. However, attempts to reproduce the results that led Tower to these conclusions met with failure (Pappius and Elliott, 1958). Therefore,

although ACh may elicit seizure discharges and increase the tendency for epileptiform activity, there is no definitive relationship between disturbances in ACh metabolism in the CNS and the induction of epileptiform afterdischarges.

Several authors (Reiffenstein, 1964; Sphelmann *et al.*, 1970; Reiffenstein, 1970; Krnjevic *et al.*, 1970) have questioned the apparent relationship of the increased excitability of chronically isolated cortex to ACh-supersensitivity. Moreover, Spehlmann *et al.*, (1971) have reported recently that they were unable to demonstrate differences in sensitivity to topically applied ACh between chronically isolated and intact contralateral cortex in cats. In addition, these authors (Spehlmann *et al.*, 1971) found that low, (and probably more physiological) concentrations of ACh abolished spontaneous EADs, and they concluded that the spike discharges produced by the higher doses of ACh were unspecific effects produced by this mediator at other than synaptic structures. Forster (1951) who conducted similar studies on intact cortex failed to show the convulsant effects of topical ACh.

Jasper (1969; Ferguson and Jasper, 1971) has renewed interest in ACh-activated EADs in cerebral cortex and showed that chronic isolation (8 days) increased the sensitivity of deafferented cortex towards ACh solutions applied topically. These authors also observed that there was a long lasting surface negative DC shift which accompanied the appearance of the ACh-evoked EAD. This relationship agrees with similar findings by others who have elicited EADs with convulsant agents and epicortical stimulation (O'Leary and Goldring, 1964; Gumnit *et al.*, 1970). Moreover, the ACh-evoked EAD has the same point of reversal of potential, 0.2 mm beneath the cortical surface, as does

the primary potential of the DCR. This suggests that the EAD itself results from excessive depolarization of neuronal membranes, primarily dendritic structures.

Ferguson and Jasper (1971) therefore considered that synaptic mechanisms or the activation of intracortical neuronal circuits were not essential for the self-sustained EAD and that ephaptic processes may synchronize neurons depolarized by ACh.

Ferguson and Jasper (1971) failed to show unit firing in isolated cortex that correlated with surface paroxysms resulting from ACh application. As discussed previously (section IV.B.) demonstration of this relationship between events in the depths of the cortex and surface phenomena has eluded many investigators. These authors (Ferguson and Jasper, 1971) did locate a few apparently inhibitory neurons below the cortical surface (< 1.3 mm). These units consistently fired at the end of the EAD potential shifts and continually during the recovery period. Perhaps these elements are the small stellate cells which are too small for routine detection with conventional microelectrodes. These elements appear to be acting through synaptic inputs to achieve inhibitory drive at the level of the large dendritic trunks, as has been proposed earlier (Ochs and Clark, 1968).

Gumnit *et al.*, (1970) have found that there are vertically oriented dipoles in experimental seizure foci relatively independent of neighbouring dipoles. This demonstration of functionally discrete vertical columns agrees with similar columns of synaptically constituted cortical elements shown histologically by Colonnier (1966). On this basis, Gumnit *et al.*, (1970) concluded that if ephaptic influences are present they would have to be restricted within the columns themselves.

Interaction between columns would probably result through synaptic mechanisms.

My results with cholinergic and anticholinergic drugs suggest strongly that ephaptic mechanisms probably have a negligible role in the cortex. Ephaptic transmission has been proposed by Ferguson and Jasper (1971) to be one mechanism responsible for the synchronous massive depolarization of a large population of neurons. They based this conclusion on their observation that synchronization also occurs in undercut cortex.

Armitage and Hall (1968; Armitage *et al.*, 1969) concluded from their experiments that nicotinic structures do not appear to make up a significant functional component in the cerebral cortex. These authors showed however, that injections of nicotine produced either increases or decreases in cortical ACh output with corresponding changes in electrocortical activity. Since these investigators studied the whole brain it is likely that preferential actions upon particular cerebral structures, e.g. reticulo-cortical, thalamo-cortical or hippocampal, were responsible for these discrepancies. Kawamura and Domino (1969) have compared muscarinic and nicotinic agonists in cats with the brain stem transected at various levels. Their results based on EEG activation responses, showed that nicotinic cholinergic neurons are primarily located in the midbrain reticular formation. Randic *et al.*, (1964) have reported that ACh depression of cortical neurons was specifically antagonized by atropine. In addition, Malcom *et al.*, 1967, have studied the evoked responses on the rat somatosensory cortex and found that only antimuscarinic agents blocked the cholinergic inhibitory effects, whereas nicotine and anti-nicotinic agents did not. Other

workers have also shown that the majority of cholinergically excited cells in the cerebral cortex are muscarinic in character (Sigg _et_ _al_., 1965; Szerb, 1965; Crawford and Curtis, 1966). Kawamura and Domino (1969) concluded that there are relatively few cholinergic neurons in the brain that respond to nicotine.

Even though it remains a problem to state with certainty the identity and location of cortical cholinergic inhibitory neurons (probably interneurons), Krnjevic _et_ _al_., (1966, 1966a) have suggested that stellate cells (Golgi II cells) situated in layers III and IV would fulfil this role as their axons spread horizontally and make synaptic contact in layers II, III and IV of the cortex. Other small pyramidal cells in layer II (Sholl, 1956) send axons to layer I where they are arranged parallel to the pia for several millimetres. This proposal is entirely hypothetical but may have support from the work published by Holubar _et_ _al_., (1967) who located, with intracellular microelectrodes, small inhibitory interneurons from 0.2 to 1.7 mm below the surface of the cortex.

The deeply situated pyramidal cells have been shown to produce prolonged repetitive discharges in response to microiontophoretically applied ACh (Krnjevic and Phillis, 1963). As the neurons inhibited by ACh appear to be situated more superficially in layers II-IV of the cortex, this spatial separation of neuron types might be an explanation for the excitatory effects observed by some workers and the inhibitory effects observed by others.

This raises the question of whether the higher doses of ACh which invariably produce seizure discharges (Infantellina 1955; Esplin and Zablocka, 1963) allow ACh to reach the deeper excitatory cells in

greater amounts. However, atropine should block this action of ACh but unfortunately this has not been tested in most studies (Krnjevic _et al._, 1970; Ferguson and Jasper, 1971; Spehlmann et al., 1971). In other experiments (Esplin and Zablocka, 1964) atropine was ineffective in blocking epileptiform spiking provoked by pilocarpine, suggesting a non-specific action for ACh.

It does not seem likely that in my experiments atropine is acting as a local anaesthetic. The doses of atropine administered which prolong EAD duration are perhaps somewhat high, but if atropine blocks axonal conduction, it would prove difficult to explain the enhanced electrical activity which would be dependent on optimal conduction. More likely, atropine is interrupting cholinergic linkages normally mediating inhibition in the cortex (MacIntosh, 1963; Dudar and Szerb, 1969).

Vas _et al._, (1969) have found that antimuscarinic drugs in high doses prolong the duration of cortical EADs significantly without altering the threshold current required to elicit the cortical EAD. In the clinic these authors were able to show that atropinic agents provoked the appearance of subclinical seizure discharges in the EEG. Since these drugs are known to depress the reticular activating system (Exley _et al._, 1958) Vas and his associates explained this effect in terms of the antimuscarinic agents causing a partial release of cerebral cortical activity from the influence of the reticular formation. A similar mechanism, (release from reticular inhibition by atropine) has been invoked by others to explain the increased cortical ACh output resulting after atropine treatment (MacIntosh, 1963; Dudar and Szerb, 1969). A similar conclusion was formulated by Polak (1971) who carried

out extensive in vitro studies on the influence of antimuscarinic agents on ACh metabolism in cortical slices from rat brain, i.e atropine stimulates in the cortex by removal of a restraining mechanism involving muscarinic receptors. Since deafferentation would also cause this effect, and antimuscarinic drugs prolong EAD duration in chronically isolated cortex, it is obvious that some cholinoceptive sites capable of more direct modulation of EAD duration probably remain in the cortex.

Hemicholinium-3, an inhibitor of acetylcholine synthesis was tested by Reiffenstein (1964) on the isolated cortex. This treatment resulted in a greater number of spontaneous and elicited EADs, which further suggests a modulator role for ACh in the cortex. Dren and Domino (1968) reported that intraventricular administration of hemicholium-3 in dogs produced high voltage slow waves in the neocortex. This antagonized the activation of this EEG pattern by nicotine but not by muscarinic drugs.

The results of this thesis suggest that EAD duration can be influenced significantly by intracortical cholinergic synapses. It seems reasonable to propose therefore, that there are intracortical synaptic circuits incorporating interneurons responsible for modulating inhibitory processes and directing these effects upon repetitively discharging pyramidal or other cells, or both.

Even though the prevalent school of thought has implicated ACh as a convulsant substance, mounting evidence and the results in this thesis strongly suggest that, at least in the cerebral cortex, ACh might have a specific role as an inhibitory transmitter substance, at least in the modulation of epileptiform afterdischarges.

Thus, in chronically deafferented cortex acetylcholine appears

to be a very likely choice as a neurohumoural substance capable of decreasing the epileptogenic tendency of such cortex. Impairment or interruption of ACh activity at a specifically muscarinic site results in an increased susceptibility for epileptiform discharges in the cerebral cortex. Whether or not other transmitter systems influence this ability of cholinergic drugs to limit EAD duration was examined in the following sections of this dissertation. Similar effects on the EEG by cholinergic and adrenergic drugs have been observed (Longo, 1962).

XI. CATECHOLAMINES

A. Effects of Adrenergic Drugs and Antagonists on EAD Duration

Since catecholamines are present and much investigated in the CNS, studying the effects of adrenergic agents appeared to be a logical extension for further drug studies relating to the susceptibility of afterdischarge duration to drugs. The choice of centrally-acting adrenergic agonists and antagonist agents is rather poor, as the classical direct-acting sympathomimetics used in studies on peripheral organs are very poorly distributed into cerebral tissues and their effects on CNS functions and responses are unclear. Antagonists for the postulated adrenergic receptor lack, for the most part, specificity, are somewhat toxic, and pertinent knowledge on their CNS effects and distribution, to a great extent, is scarce. Moreover, whether endogenous catecholamines have a physiological role in the CNS has not as yet been established. The more prominent gross central effects of sympathomimetics are stimulant, whereas the best studied local effects are depressant. The observed central effects of α-adrenergic blocking drugs appear to influence CNS activity by mechanisms other than blockade, as these central actions are poorly correlated with blocking ability as determined on peripheral structures.

No special treatment or precaution was adopted to protect the animals from the peripheral actions of sympathomimetic drugs. For each drug used, an attempt was made to administer doses that were tolerated and caused negligible side effects.

a. Adrenergic (sympathomimetic) drugs

The amphetamines are potent CNS stimulants and are routinely

used as sympathomimetics for studying adrenergic mechanisms in the CNS. Controversy surrounds the mechanism of central action of amphetamines as many different studies have shown that these drugs may or may not depend on endogeneous CNS noradrenaline stores for their action (Goodman and Gilman, 1970).

Injection of d-amphetamine 2.5 mg/kg (Fig. 43) to 10 cats resulted in a small (< 10 sec) but highly significant ($P < 0.01$) decrease in EAD duration. A second injection of d-amphetamine 2.5 mg/kg 60 min later caused a greater decrease in EAD duration, the difference from control was now about 15 sec and was highly significant ($P < 0.01$). This decrease in EAD duration was also significantly ($P < 0.05$) different from the EAD duration after the first dose of amphetamine. A third injection of this agent, 5 mg/kg, did not reduce EAD duration further. When tested several hours later, the EAD duration had increased slightly (about 5 sec) and 24 hours after drug administration it had returned to control values.

To another group of 7 cats, methamphetamine (Fig. 44) was administered in increasing doses, and highly significant ($P < 0.01$) reductions in afterdischarge duration were evident after each injection. Following the administration of methamphetamine 1 mg/kg a highly significant ($P < 0.01$) decrease in EAD duration (less than 10 sec) occurred. Injection of methamphetamine 1.5 mg/kg 60 min later resulted in a further decrease in EAD duration to approximately 15 sec below control values. The next injection of methamphetamine which was 2.5 mg/kg caused no further change in EAD duration, the mean decrease in EAD duration remaining at about 15 sec. The response remained at this

Figure 43. Effect of d-amphetamine sulfate (d-A) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 10 cats. Note immediate significant decrease in EAD duration after injection of amphetamine. ** = $P < 0.01$.

Figure 44. Effect of increasing doses of methamphetamine hydrochloride (MA) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 7 cats. Note immediate decrease in EAD duration after injection of methamphetamine. ** = $P < 0.01$.

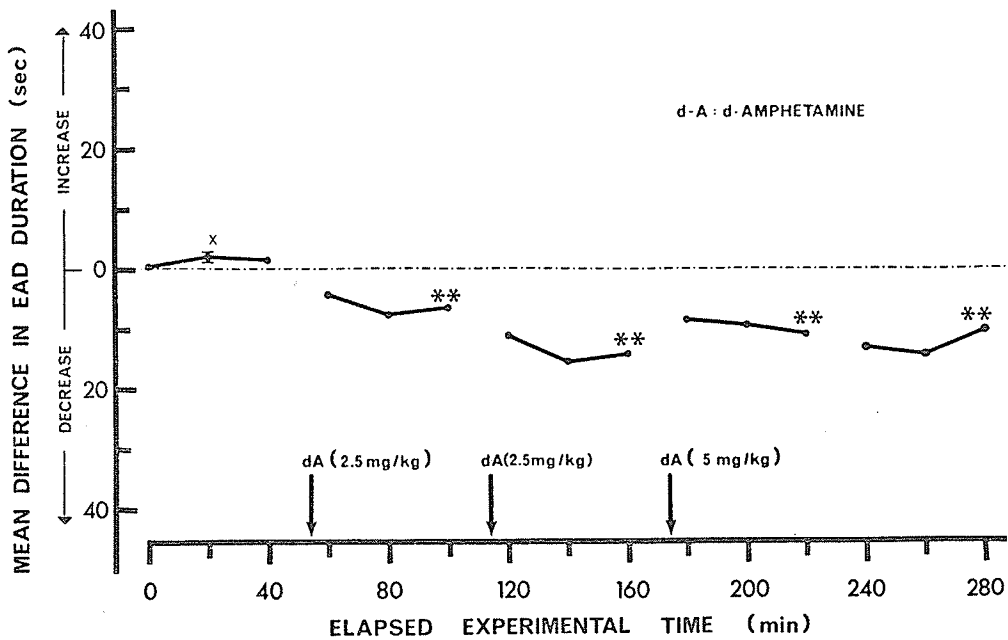


Figure 43.

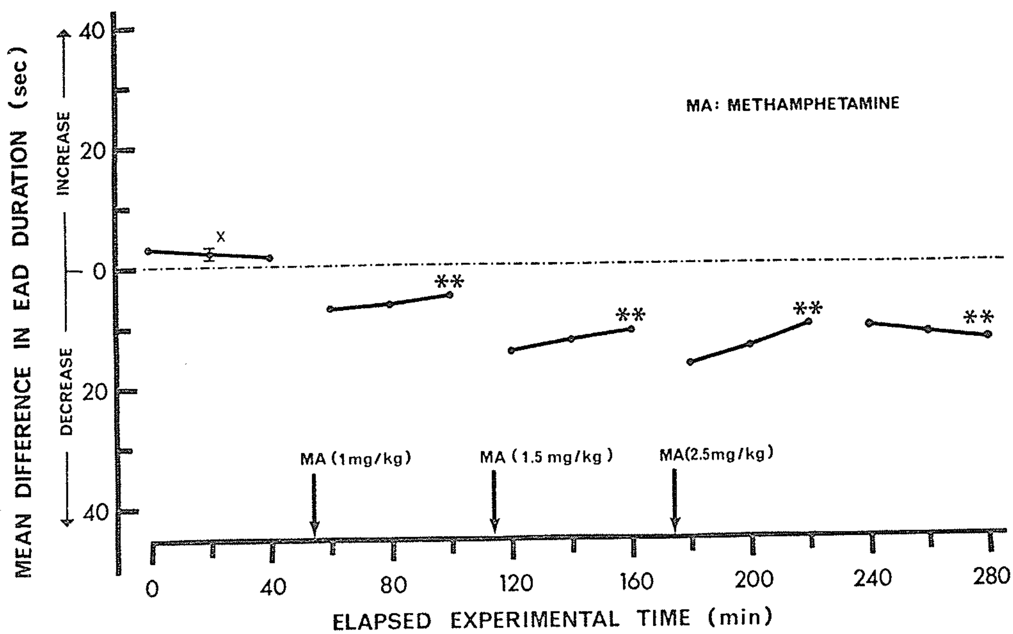


Figure 44.

level for several hours and had returned to control levels when tested 24 hours after drug administration. There appears to have been some cumulation of methamphetamine with this schedule of injections.

The peripheral actions of the amphetamines were evident less than 10 min after injection. These consisted of transient tachycardia, respiratory stimulation and some piloerection. These effects were more evident in cats treated with d-amphetamine than in cats injected with methamphetamine. The maximal doses of both agents produced definite signs of CNS stimulation as indicated by increased locomotor activity and apparent restlessness.

The injection of tyramine 5 mg/kg (Fig. 45) in 5 cats also caused highly significant ($P < 0.01$) reductions in EAD duration (18-20 sec of decrease). This effect was not evident until 60 min after the first injection. After 3 separate injections of tyramine 5 mg/kg at 60 min intervals, a maximal reduction in EAD duration of about 20 sec was produced. It is not clear whether tyramine is being accumulated following each injection of drug. The response had almost returned to control 3 hours after the last injection of tyramine.

Another group of 5 cats was treated with l-ephedrine (Fig. 46). The first injection of this agent decreased EAD duration (10 sec), and this change from control was significant ($P < 0.05$). Another dose of l-ephedrine 1 mg/kg reduced EAD duration another 10 sec and this decrease (total of 20 sec) was very significantly ($P < 0.01$) different from control. The last injection, of l-ephedrine which was 3 mg/kg decreased EAD durations by approximately 25 sec below control, which was a highly significant ($P < 0.01$) difference. Following this last

Figure 45. Effect of tyramine hydrochloride (TY) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note delay in effect of tyramine upon EAD duration.
** = $P < 0.01$.

Figure 46. Effect of l-ephedrine sulfate (l-E) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
* = $P < 0.05$; ** = $P < 0.01$.

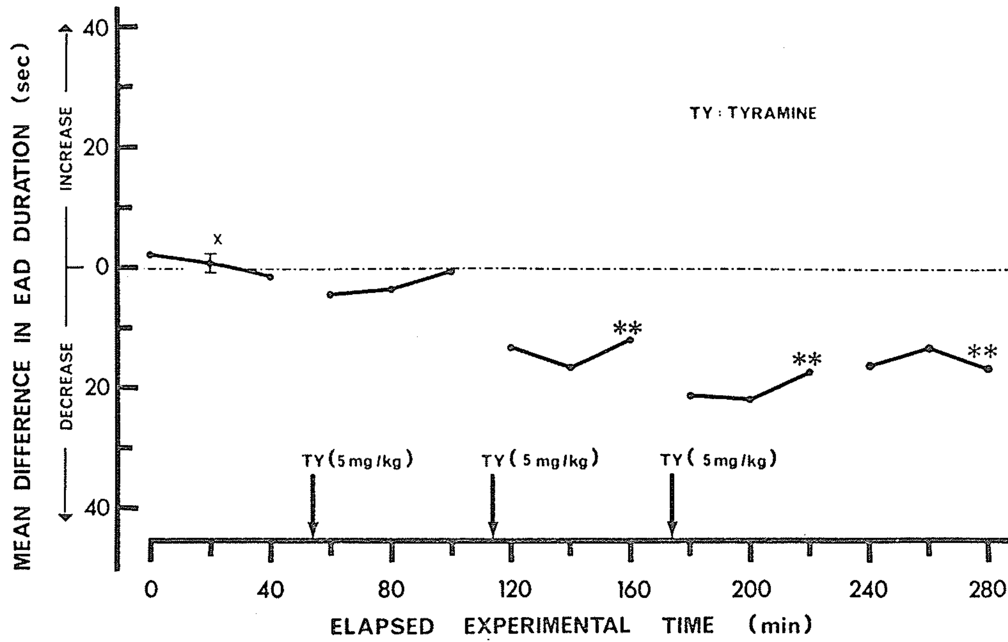


Figure 45.

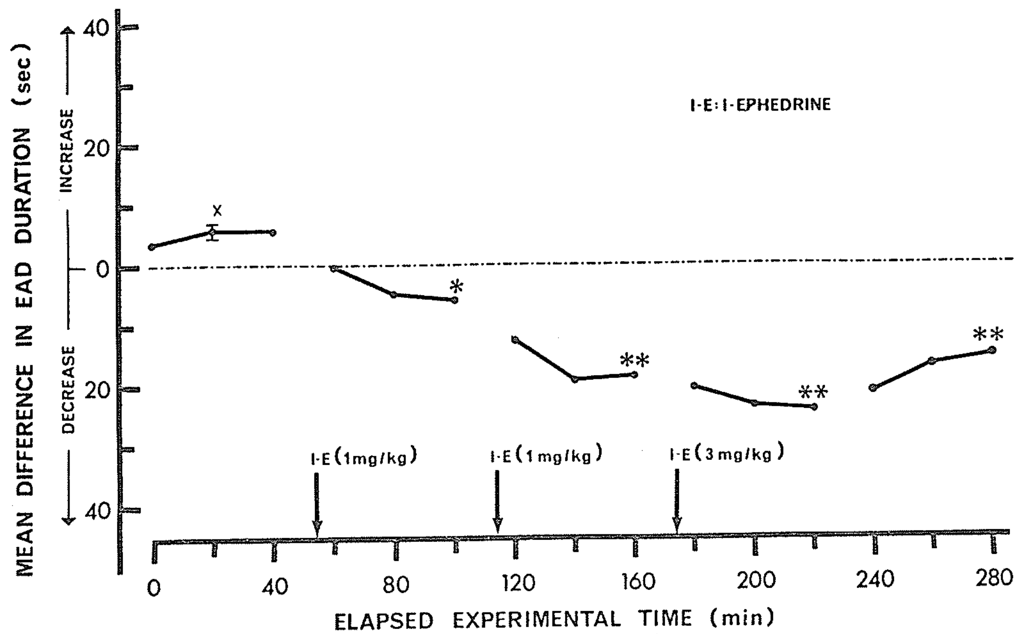


Figure 46.

treatment, EAD duration increased gradually, returning to control levels 4 hours later. As with the tyramine treatment, injections of l-ephedrine did not appear to result in as great a degree of CNS stimulation, but there were some behavioural signs of mild excitation.

The monoamine oxidase inhibiting agent pargyline was injected in 4 cats (Fig. 47). Following 2 injections of 0.5 mg/kg at a 60 min interval, no significant change in EAD duration was detected for two hours. EADs elicited after two hours showed a highly significant ($P < 0.01$) decrease in duration which persisted for up to 12 hours after drug injection. No behavioural side effects were noticed in pargyline treated animals during this period lasting 12 hours.

The sympathomimetic agent methoxamine has been reported to stimulate predominantly the α-adrenergic receptor (Goodman and Gilman, 1970). Increasing doses of this drug (1 mg/kg; 1.5 mg/kg and 2.5 mg/kg) were injected into 5 cats (Fig. 48), but no significant differences in EAD durations were found during these experiments. Negligible peripheral effects were observed.

Several other agents believed to act on α-adrenergic receptors were also tried. These were clonidine (Catapres^{R}) in 2 cats, phenylephrine in 3 cats, and dihyroxyphenylalanine (L-DOPA) in 3 cats (Goodman and Gilman, 1970). The results of these experiments are shown in Table 3.

Figure 47. Effect of a monoamine oxidase inhibitor pargyline hydrochloride (PG) 0.5 mg/kg upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. Note delay before decrease in EAD duration occurred.
** = $P < 0.01$.

Figure 48. Effect of methoxamine hydrochloride (MA) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note lack of any significant effect upon EAD duration at 3 dose levels tested.

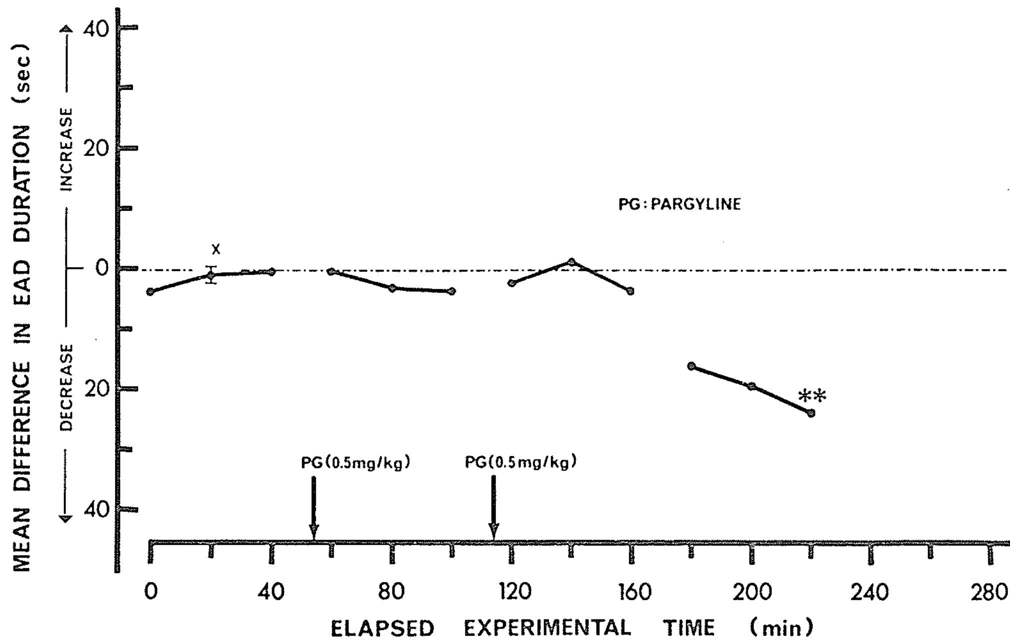


Figure 47.

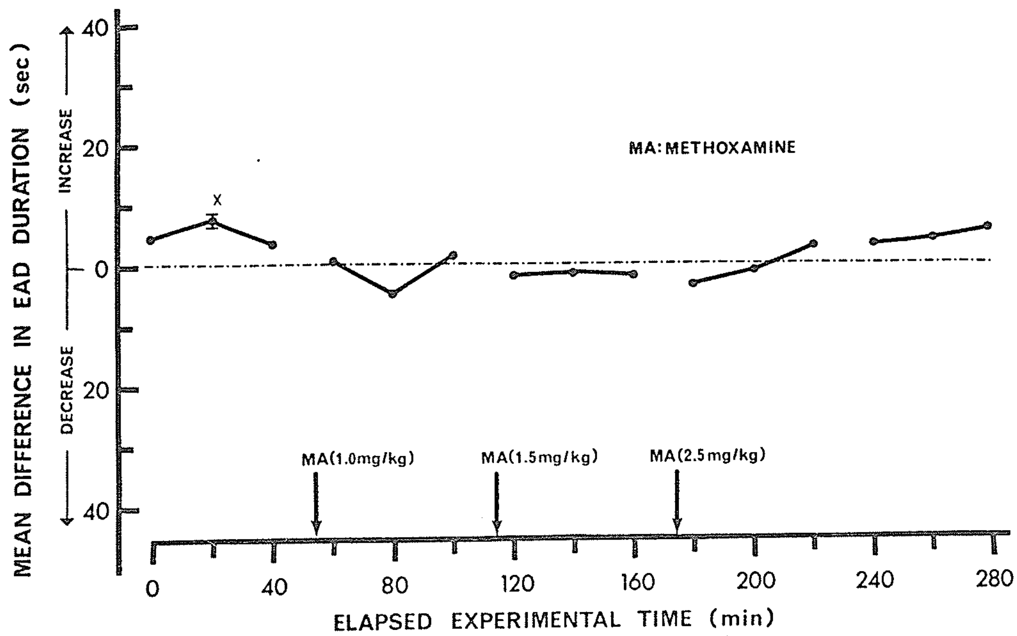


Figure 48.

Table 3. Effect on EAD Duration of Other Sympathomimetic Drugs

SALINE CONTROL			TREATMENT - 1
Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	Mean Difference in EAD Duration (sec) ± S.E. (N)
0.8 ± 1.9 (2)	clonidine	0.1 mg/kg	-3.9 ± 2.2 (2)
-2.7 ± 1.2 (3)	phenylephrine	2.5 mg/kg	-8.2 ± 2.6 (3)*
2.1 ± 0.7 (3)	L-DOPA	3 mg/kg	-6.6 ± 1.4 (3)*

N = number of animals
* = $P < 0.05$

The results from these experiments show there was a small but significant ($P < 0.05$) decrease in EAD duration produced by phenylephrine 2.5 mg/kg and L-DOPA at a dose of 3 mg/kg. More extensive testing with these agents was not carried out because there is insufficient knowledge regarding their precise role at adrenergic receptors (Goodman and Gilman, 1970).

b. Drugs blocking α-adrenergic receptors

Whether central adrenergic receptors, if present, bear any similarity to peripheral adrenergic structures has not been validated. Marked CNS effects have been reported following administration of α-adrenergic antagonists (Goodman and Gilman, 1970) but these do not appear to be related to their adrenergic blocking actions. Moreover, most of the commonly used blockers lack specificity in their antagonists actions.

Phentolamine was administered to 8 cats (Fig. 49). The doses administered at 60 min intervals were 2 mg/kg, 2 mg/kg and 4 mg/kg.

Over a period of 4 hours of elapsed experimental time, no significant changes in EAD duration were detected. Monitoring the response for longer periods of time in a few animals (3 cats) showed that no changes occurred for up to 8 hours. Peripherally, a marked relaxation of the nictitating membrane was observed within 10 min after injection. All the animals appeared to be slightly sedated at the maximal dose (probably 8 mg/kg due to cumulation).

The effect of phentolamine treatment on the response to d-amphetamine was tested in 6 cats (Fig. 50). Pretreatment with phentolamine 8 mg/kg did not alter afterdischarge duration. Injection of d-amphetamine 5 mg/kg after this was not immediately effective in reducing EAD duration, but gradually it decreased by about 7 sec below control, which was a significant ($P < 0.05$) change. During the next hour of testing the mean decrease was about 5 sec, and this decrease was not significant. Challenging with another dose of d-amphetamine 5 mg/kg two hours later in 2 cats revealed that d-amphetamine was capable of producing a reduction in EAD duration of 10-12 sec.

Phenoxybenzamine 5 mg/kg (Fig. 51) was studied in 7 cats. Three injections each of 5 mg/kg at 60 min intervals did not change EAD duration during 4 hours of testing. Testing further for up to 6 hours (3 cats) did not show any difference in afterdischarge duration. The animals appeared to be mildly sedated and their nictitating membranes were fully relaxed within 30 min after the first injection (5 mg/kg). The peripheral effects of phenoxybenzamine were present for up to 24 hours after injection.

In another group of 5 cats, phenoxybenzamine 5 mg/kg did not

Figure 49. Effect of phentolamine hydrochloride (PA) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 8 cats. Note lack of any significant effect after three injections of drug.

Figure 50. Partial blockade by an α-adrenergic receptor antagonist, phentolamine hydrochloride (PA), of the action upon EAD duration by d-amphetamine sulfate (d-A). X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. * = $P < 0.05$.

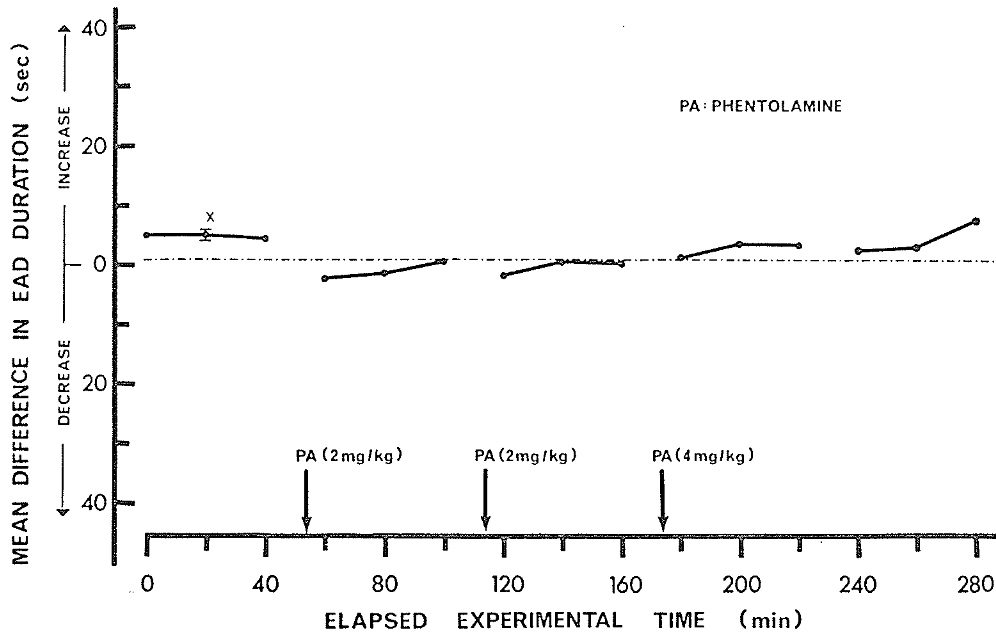


Figure 49.

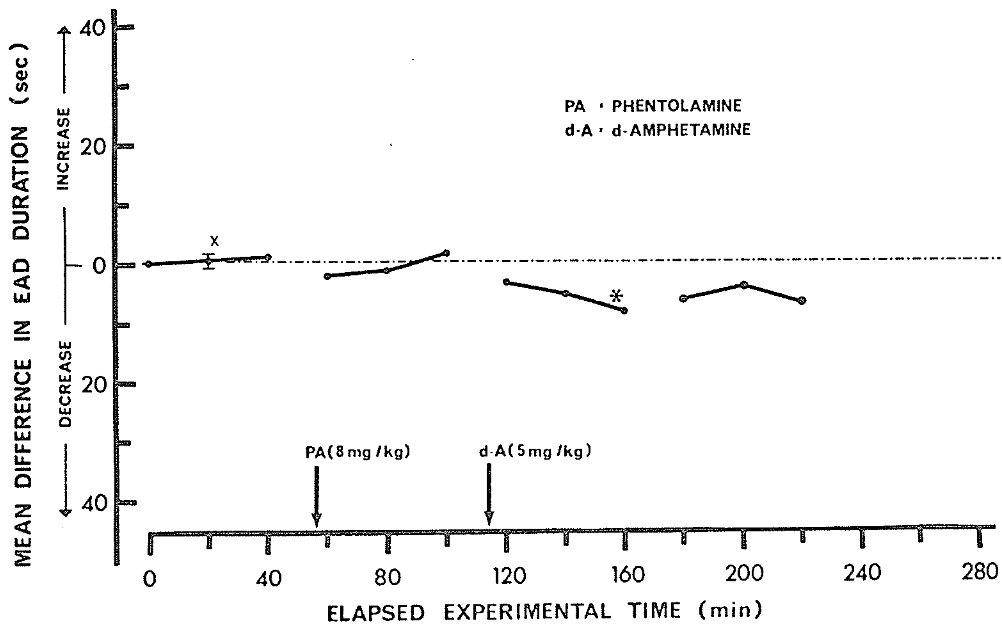


Figure 50.

Figure 51. Effect of phenoxybenzamine hydrochloride (POB) upon EAD duration in the isolated cortex. Note lack of any significant effect even after 3 injections of POB at a dose of 5 mg/kg each. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 7 cats.

Figure 52. Effect of an α-adrenergic receptor antagonist, phenoxybenzamine hydrochloride (POB) 5 mg/kg, on the action by meth-amphetamine hydrochloride (MA) 2.5 mg/kg upon EAD duration. Note significant decrease in EAD duration after injection of methamphetamine. X refers to the pooled S.E. for all the mean differences calculated from the results of experi-ments on 5 cats.
$* = P < 0.05$.

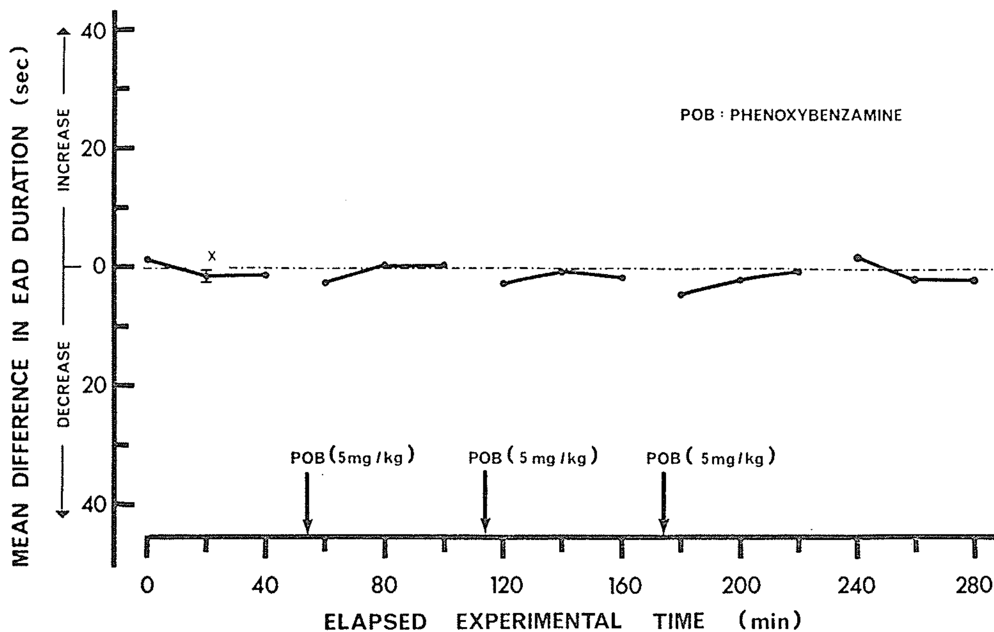


Figure 51.

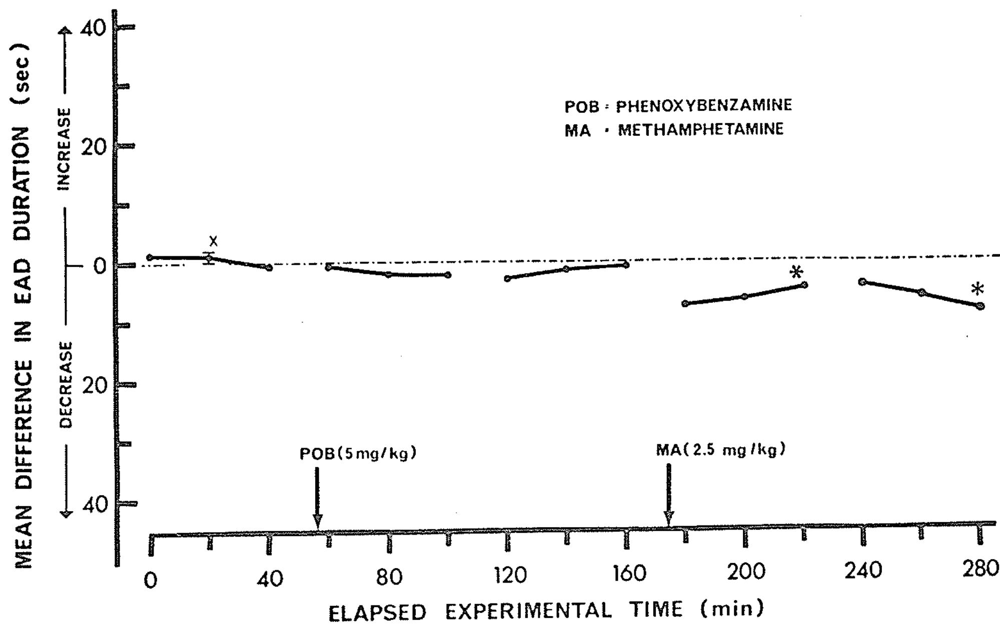


Figure 52.

change EAD duration during almost 2 hours of testing. Injection of d-amphetamine 5 mg/kg two hours after the phenoxybenzamine injection resulted in a small (5-7 sec) but significant ($P < 0.05$) reduction in EAD duration, which persisted for almost 2 hours of testing. Thus, amphetamine decreases afterdischarge duration following pretreatment with phenoxybenzamine, however, it appears that there has been some partial antagonism of this effect since, on its own (Fig. 43) d-amphetamine and methamphetamine (Fig. 44) caused much larger decreases in EAD duration.

Another α-adrenergic receptor blocking drug tested was tolazoline. In 6 cats (Fig. 53) three successive injections of 5 mg/kg at 60 min intervals did not result in any significant change in EAD duration during more than 3 hours of testing. These cats appeared to be drowsy and inactive after this course of injections, which probably resulted in a final dose of about 15 mg/kg due to cumulative effects.

Dibozane is a benzodioxan derivative having predominantly α-adrenergic blocking properties, (Goodman and Gilman, 1970). In 4 cats the injection of dibozane 0.5 mg/kg followed by 1.5 mg/kg 60 min later, did not result in any significant change in EAD duration. The last injection of dibozane was 3 mg/kg which caused a brief and significant ($P < 0.05$) increase in EAD duration of 10 sec above control. These cats were somewhat sedated and their nictitating membranes did not appear to relax fully.

A relatively new α-adrenergic blocking agent thymoxamine (Birmingham *et al.*, 1967) was also tested. This agent compares with phentolamine regarding effects on the cardiovascular system of the

Figure 53. Effect of tolazoline hydrochloride (TA) upon EAD duration. Note lack of any significant effect by this α-adrenergic blocking agent. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.

Figure 54. Effect of dibozane (DBZ), an adrenergic antagonist, upon EAD duration in the isolated cortex. Note brief significant increase in EAD duration after injection of dibozane 3 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.
$* = P < 0.05$.

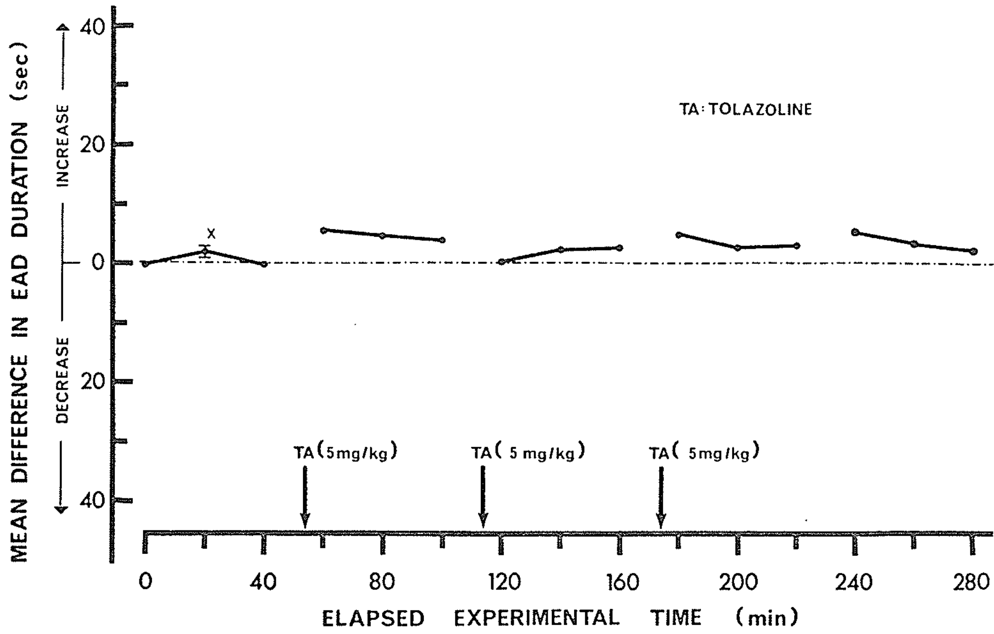


Figure 53.

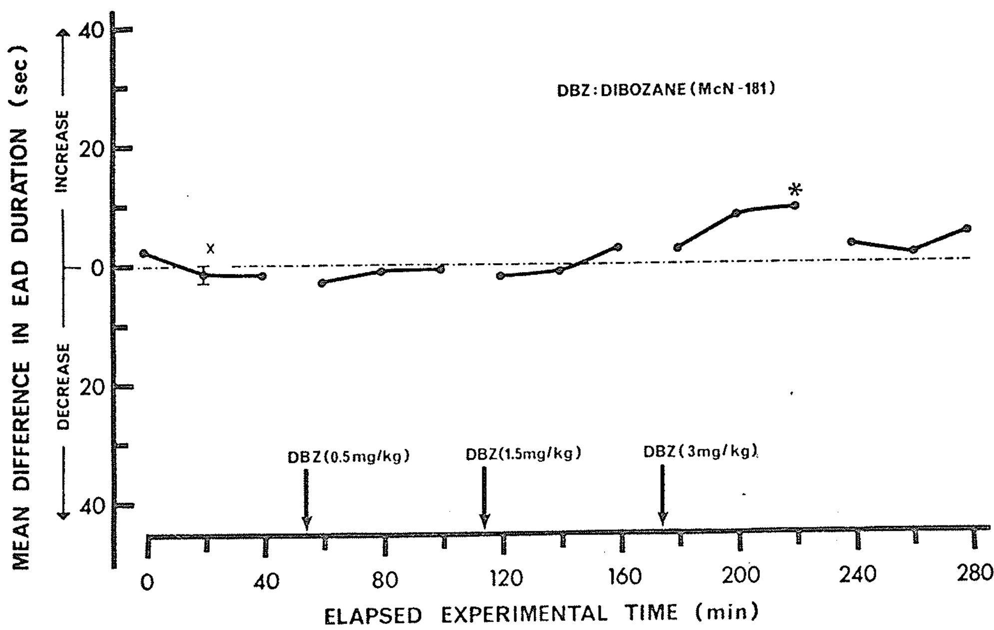


Figure 54.

cat. Thymoxamine at increasing doses from 1 mg/kg to 10 mg/kg in 3 cats did not alter EAD duration during 6 hours of testing in these experiments.

c. Psychotropic agents with adrenergic blocking properties

Many drugs are capable of antagonizing the response to adrenergic stimuli. However, such activity is not restricted to adrenergic structures and psychotropic drugs like chlorpromazine and haloperidol show, under the appropriate conditions, a wide spectrum of pharmacological blockade (Goodman and Gilman, 1970). In any event, their blocking actions have not been correlated, with their central tranquillizing action (Nickerson and Hollenberg, 1967).

Chlorpromazine was studied in 5 cats (Fig. 55). Shortly after injection of 2 mg/kg there was a significant ($P < 0.05$) increase in EAD duration. The next injection of the drug was 3 mg/kg and it caused a maximal increase in EAD duration of 15 sec which was very significantly ($P < 0.01$) different from control. Injection of chlorpromazine 10 mg/kg did not cause any further increase in afterdischarge duration. After this series of injections, EAD duration remained elevated above control by about 12 sec for another 6 hours (in 2 cats) and had returned practically to control levels 24 hours later (in 3 cats). The cats in this study were somewhat sedated and inactive following administration of chlorpromazine.

A butyrophenone derivative, haloperidol was studied in 4 cats. Injection of haloperidol 0.5 mg/kg (Treatment-1, Fig. 56) resulted in a very significant ($P < 0.01$) increase in EAD duration of 15-18 sec. Additional doses of this agent administered were 2 mg/kg

Figure 55. Effect of chlorpromazine hydrochloride (CPZ), a psychotropic drug with α-adrenergic blocking properties among its known blocking actions, upon EAD duration. Note increase in EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
* = $P < 0.05$; ** = $P < 0.01$.

Figure 56. Effect of haloperidol (HPL), a psychotropic drug with adrenoceptor blocking properties among its actions, upon EAD duration. Note increase in EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.
** = $P < 0.01$.

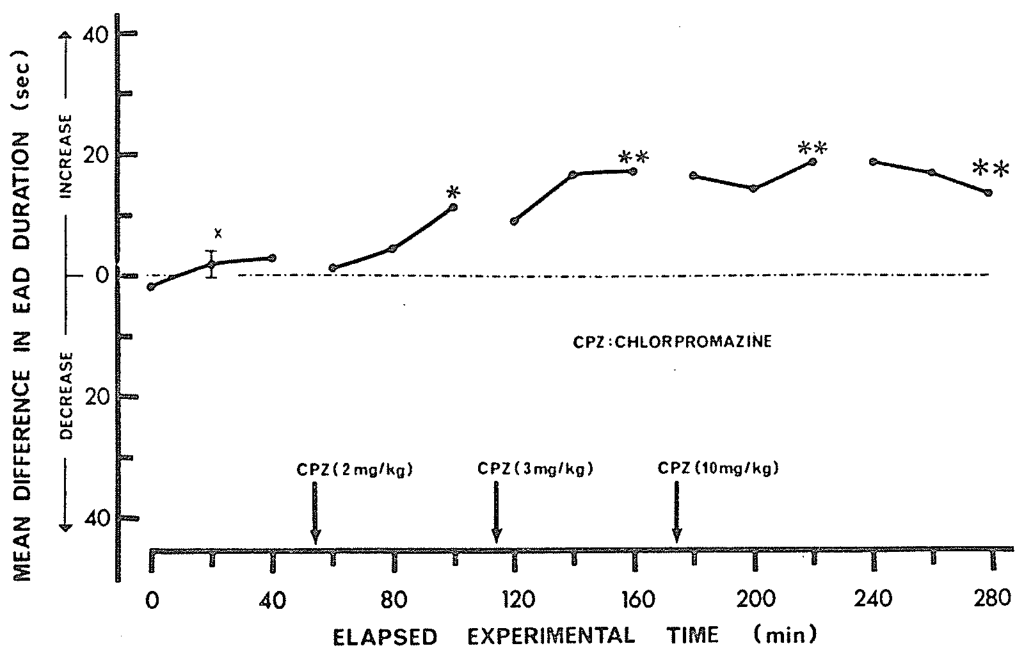


Figure 55.

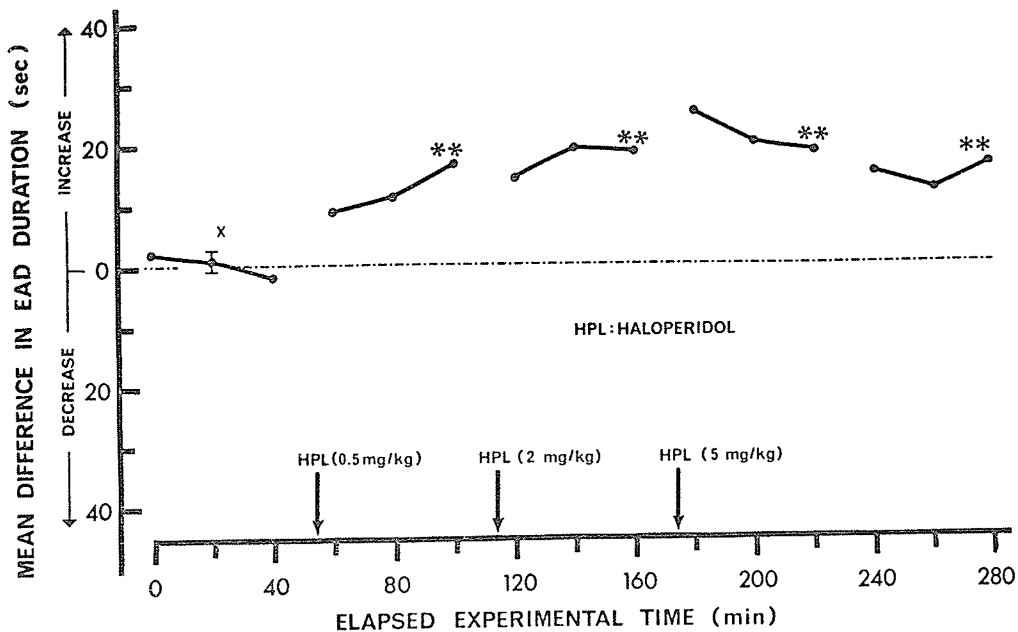


Figure 56.

and 5 mg/kg each producing a slightly greater increase in EAD duration (to approximately 20 sec above control). These increases in EAD duration persisted for 6 hours (in 2 cats) and had decreased only slightly 24 hours later (in 2 cats). The animals appeared to be less active and were sedated.

d. Agents lowering central stores of catecholamines

A recent approach to study the function of adrenergic mechanisms in the CNS has been to lower cerebral levels of catecholamines by inhibiting certain enzymes involved in the biosynthesis of these substances. Inhibition of dopamine-β-hydroxylase by disulfiram (Lippman, 1968) or FLA-63 (Svensson and Waldeck, 1969) results in depletion of cerebral noradrenaline and simultaneously decreased motor activity in mice. Inhibition of tyrosine hydroxylase by dl-α-methyltyrosine methylester results in selective depletion of brain catecholamines and concomittant disruption of the conditioned avoidance response in cats and rats (Hanson, 1965).

Preliminary experiments with disulfiram 50 mg/kg per day in 2 cats for 3 days resulted in diarrhea, anorexia, lethargy and ataxia. The treatment was discontinued at this point. Daily testing began 24 hours after the first injection. After 3 days of disulfiram a highly significant ($P < 0.01$) increase in EAD duration (about 20 sec) occurred. This was at the same time that the cats had profound peripheral and central symptoms. The results with FLA-63 at a dose of 15 mg/kg per day for 2 days were similar, except that the 2 cats tested did not appear as sedated as those treated with disulfiram. Afterdischarge duration in the FLA-63 treated cats increased significantly ($P < 0.05$) but only by about 8 sec.

Two other cats were treated with dl-α-methyltyrosine 100 mg/kg, one injection only, and studied 12 and 36 hours later. Soon after the injection the cats had emesis and became sedated. Their pupils became miotic and the cats became very lethargic. In one cat EAD duration was increased about 18 sec when tested 12 hours after the injection, and was only 10-12 sec above control at 36 hours after injection. The other cat, who also became ataxic, showed no difference in EAD duration at both times of testing. This animal died 3 days later.

On the basis of the variability of these results and the significant side effects encountered further experiments with these agents were not planned because it was felt that the results obtained were unreliable.

B. Interaction Between Adrenergic Drugs and Blockers, and Cholinergic and Anticholinergic Drugs

a. Effect of atropine on the action of methamphetamine on EAD duration

Injection of atropine 3 mg/kg in 7 cats (Fig. 57) produced a highly significant ($P < 0.01$) increase in EAD duration of approximately 25 sec. The administration of methamphetamine 2.5 mg/kg 60 min later resulted in no further significant change in EAD duration.

Another group of 6 cats was injected with methamphetamine 2.5 mg/kg (Fig. 58) which resulted in a decrease in EAD duration of more than 10 sec, which was highly significantly different from control. The next injection was atropine 3 mg/kg which immediately increased EAD duration to approximately 12 sec above control values. EAD durations were very significantly ($P < 0.01$) different from the control

Figure 57. Blockade by atropine sulfate (At) 3 mg/kg of the usual effect by methamphetamine hydrochloride (MA) 2.5 mg/kg upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 7 cats.
** = $P < 0.01$.

Figure 58. Reversal of the effect by methamphetamine hydrochloride (MA) 2.5 mg/kg upon EAD duration by atropine sulfate (At) 3 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
** = $P < 0.01$.

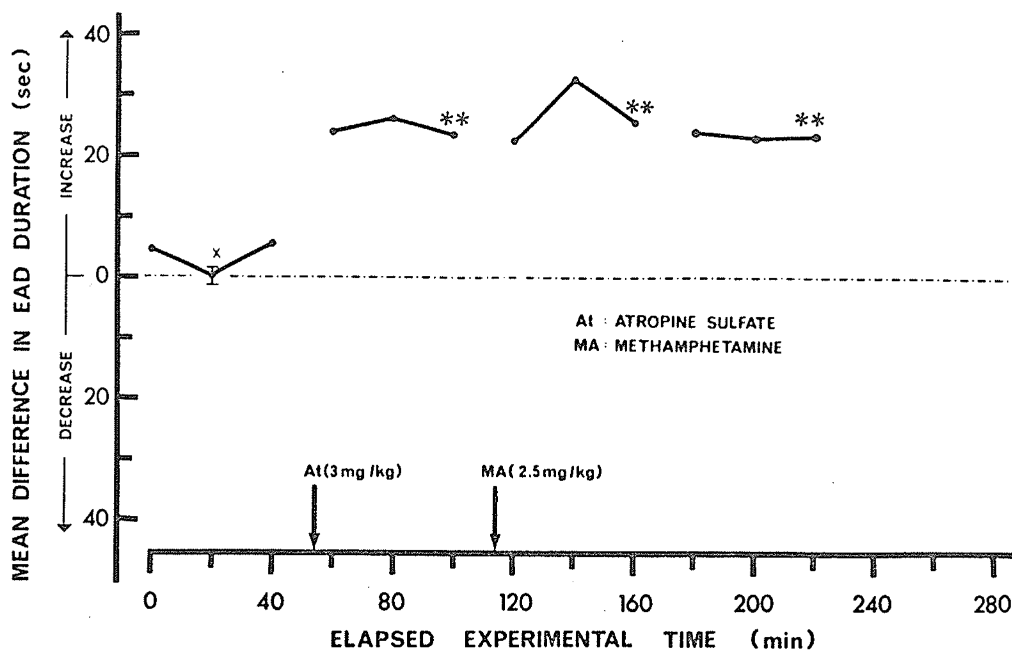


Figure 57.

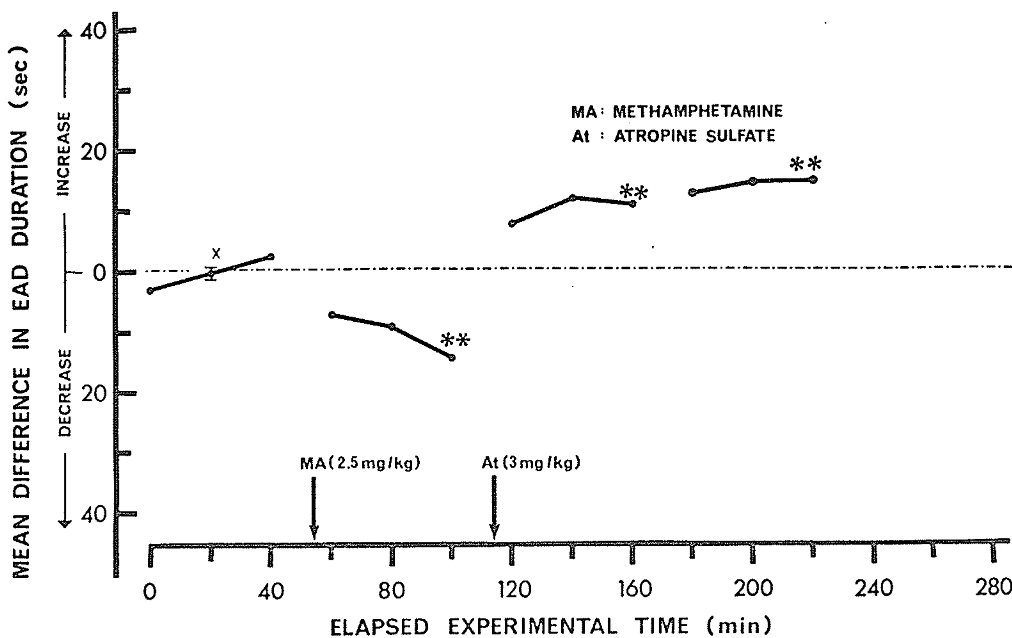


Figure 58.

and from the EAD durations after the injection of methamphetamine. Thus, atropine appears to have blocked and reversed the action of amphetamine, whereas amphetamine was not capable of altering the response to atropine.

In 5 experiments two doses of phentolamine 4 mg/kg (Fig. 59) were given 60 min apart. This treatment did not result in any change in EAD duration. When atropine 3 mg/kg was injected there was an immediate and highly significant ($P < 0.01$) increase in afterdischarge duration (almost 20 sec above control levels) which remained prolonged during almost 2 hours of testing.

Atropine 3 mg/kg (Fig. 60) in 6 cats resulted in the usual highly significant ($P < 0.01$) decrease in EAD duration of almost 20 sec. An injection of phentolamine 8 mg/kg following this atropine pretreatment, did not result in any further change in EAD duration during almost 3 hours of testing.

Thus, an α-adrenergic receptor blocking drug, phentolamine, does not modify the ability of atropine to increase EAD duration.

Phenoxybenzamine 5 mg/kg was injected twice, 60 min apart to 8 cats (Fig. 61), without significantly altering EAD duration. Ten minutes before injecting arecoline, a protecting dose of methylatropine 4 mg/kg was administered. The following dose of arecoline 1 mg/kg produced an immediate decrease in EAD duration of more than 10 sec, which was highly significantly ($P < 0.01$) different from control. This decrease gradually became smaller and 2 hours after the arecoline administration EAD duration had returned to control values.

Thus, on the basis of 8 experiments, the ability of cholin-

Figure 59. Lack of effect by an α-adrenoceptor antagonist phentolamine hydrochloride (PA) on the action by atropine sulfate (At) 3 mg/kg upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. ** = $P < 0.01$.

Figure 60. Lack of effect of phentolamine hydrochloride (PA) 8 mg/kg on the action by atropine sulfate (At) 3 mg/kg upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. ** = $P < 0.01$.

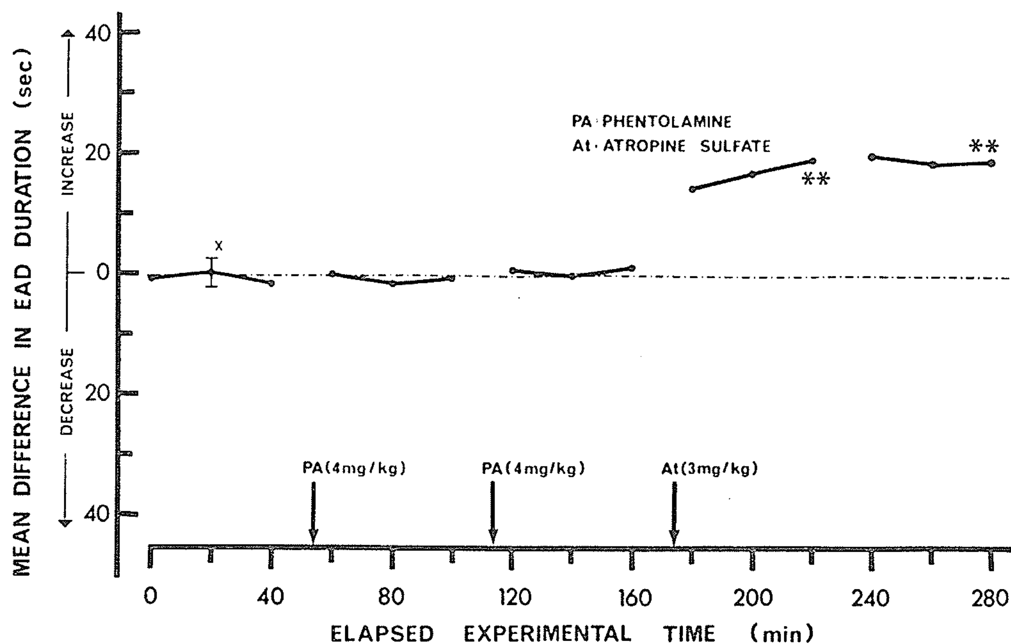


Figure 59.

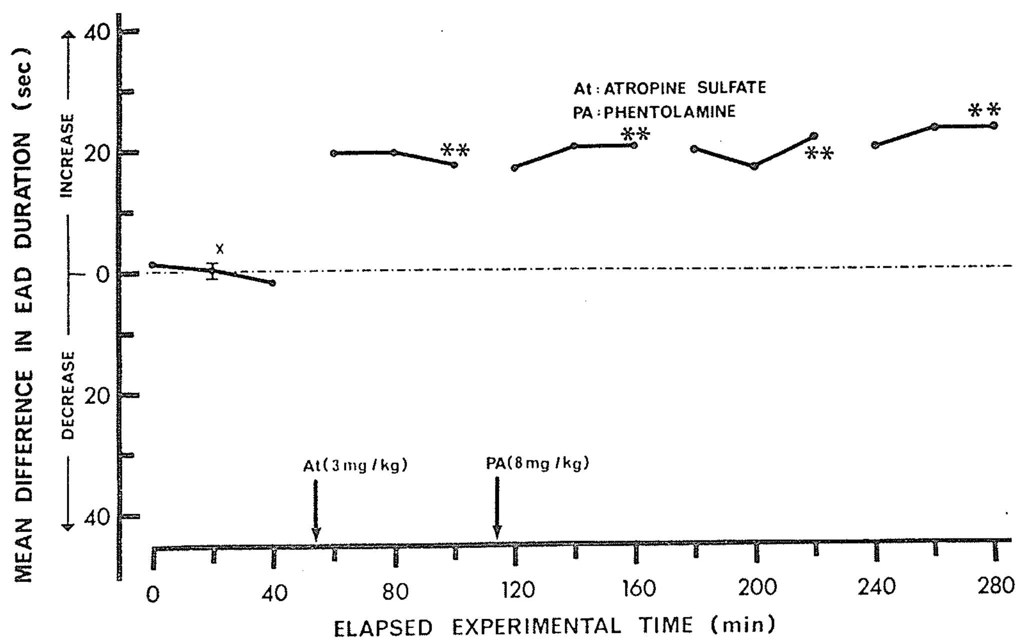
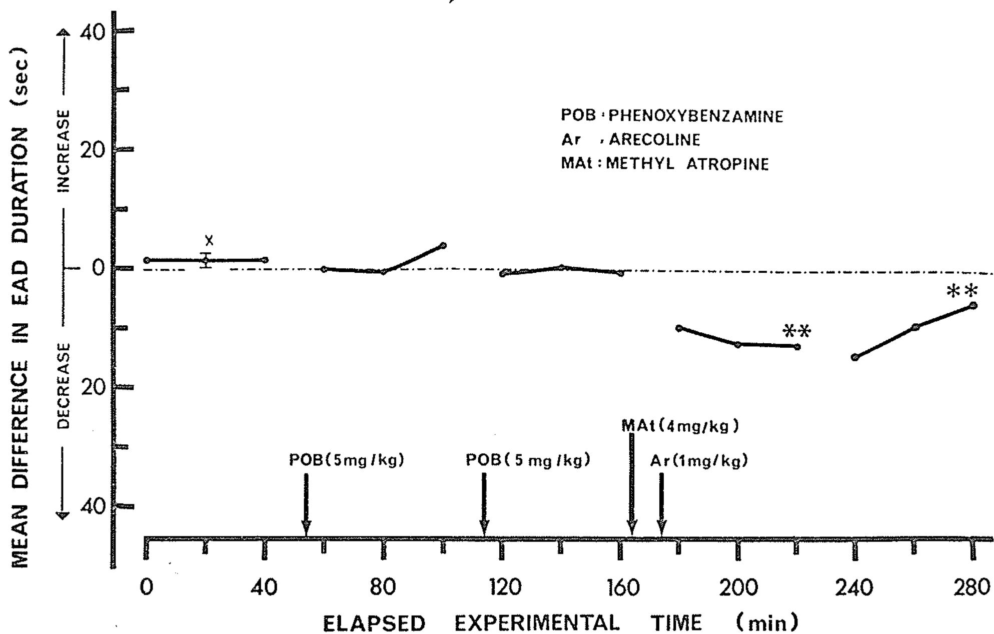


Figure 60.

Figure 61. Lack of effect of an α- adrenoceptor antagonist, phenoxybenzamine hydrochloride (POB), on the action by arecoline hydrochloride (Ar) upon EAD duration. MAt = methyl atropine nitrate 4 mg/kg as a pretreatment to protect against the peripheral actions of arecoline. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 8 cats.
** = $P < 0.01$.

Figure 62. Lack of effect of an α-adrenoceptor antagonist phentolamine hydrochloride (PA) 8 mg/kg, on the action of a monoamine oxidase inhibitor, pargyline hydrochloride (PG) 1 mg/kg, upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note delay in onset of action of pargyline.
** = $P < 0.01$.



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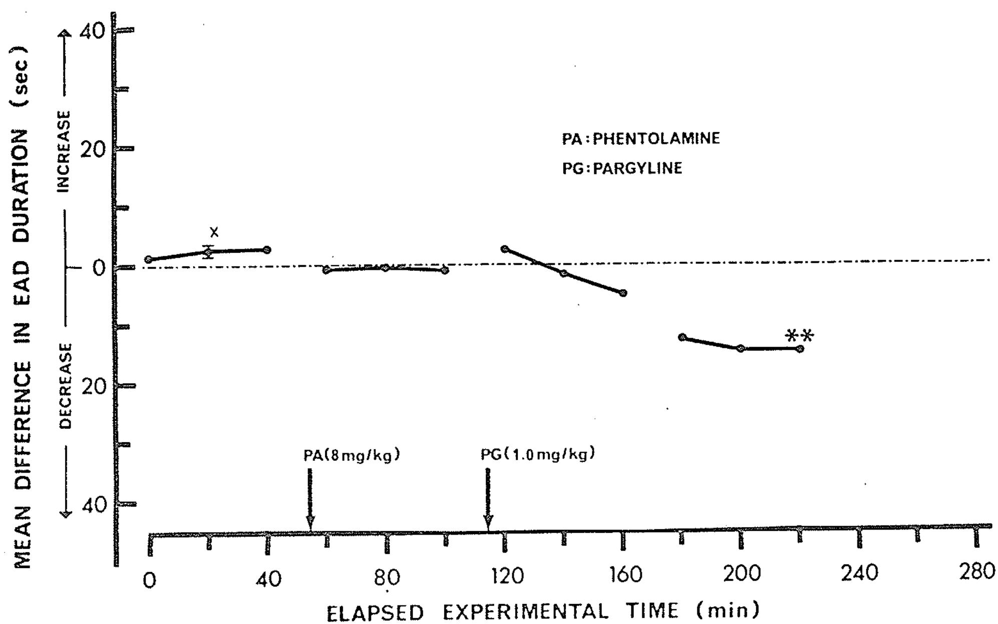


Figure 62.

ergic agents to decrease EAD duration is not prevented by α-adrenolytic agents.

The effect of monoamine oxidase inhibition in 5 cats pretreated with phentolamine was tested (Fig. 62). The first injection which was phentolamine 8 mg/kg produced no change in EAD duration. The second treatment was an injection of pargyline 1 mg/kg which resulted in a highly significant ($P < 0.01$) decrease in EAD duration (15 sec).

C. Resume

In summary, sympathomimetic drugs having direct central actions cause definite decreases in EAD duration (Fig. 43; Fig. 44; Fig. 45; Fig. 46). The majority of α-adrenergic blocking drugs tested did not modify afterdischarge duration (Fig. 49; Fig. 51; Fig. 53), except for one agent (Fig. 54) which caused a brief increase. Two agents having α-adrenergic receptor blocking properties in addition to their other central actions markedly increased EAD durations (Fig. 55; Fig. 56).

Pretreatment with α-adrenergic receptor antagonists prevented, to a significant extent, the action of amphetamine on EAD duration (Fig. 50; Fig. 52). However, increasing doses of the adrenergic agonist were capable of surmounting this blockade. The adrenergic drugs used are not able to overcome the action of anticholinergic drugs (Fig. 57), nor do they prevent it (Fig. 58). The α-adrenergic antagonists did not modify the action of anticholinergics (Fig. 59), nor did they reverse it (Fig. 60). Adrenolytic agents did not prevent the

action of cholinergic drugs (Fig. 61). Monoamine oxidase inhibition decreased EAD duration (Fig. 47) and this action was not blocked by pretreatment with an adrenergic blocker (Fig. 62).

D. Effects of β-Adrenergic Receptor Blocking Drugs on EAD Duration

The central actions of β-adrenergic receptor agonists and antagonists have been less studied than their α-adrenergic counterparts. Consequently, if little is known about the role of the α-adrenergic receptor in cerebral phenomena even less is known about the role, if any, of β-adrenergic receptors in CNS function. One review (Vaughan Williams, 1967) has suggested that β-adrenergic blocking drugs have direct actions on central neurons.

There have been reports that β-adrenergic receptor blocking drugs (pronethalol) influence the action of anticonvulsant drugs in electroshock studies (Mennear and Rudzik, 1966; Rudzik and Mennear, 1966a) and possess anticonvulsant activity (Murmann et al., 1966).

a. β-receptor stimulants

Injection of isoproterenol sulfate 0.01 mg/kg (Fig. 63) and 0.02 mg/kg in 4 cats did not cause any significant change in afterdischarge duration. Like many catecholamines, this agent probably penetrates poorly into cerebral tissues. Isoproterenol is the most specific and powerful β-adrenergic receptor stimulant known (Goodman and Gilman, 1970).

Another agent that was tried was dichloroisoproterenol, a β-adrenergic receptor blocking agent with appreciable intrinsic activity (Goodman and Gilman, 1970) i.e. it is a partial agonist. Dichloroisoproterenol was tested at 3 different doses in 5 cats (Fig. 64) but no

Figure 63. Effect of a β-adrenoceptor agonist dl-isoproterenol sulfate (ISO) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. Note lack of any significant effect on EAD duration.

Figure 64. Effect of a partial β-adrenoceptive agonist dichloroisoproterenol hydrochloride (DCI) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. Note lack of any significant change in EAD duration.

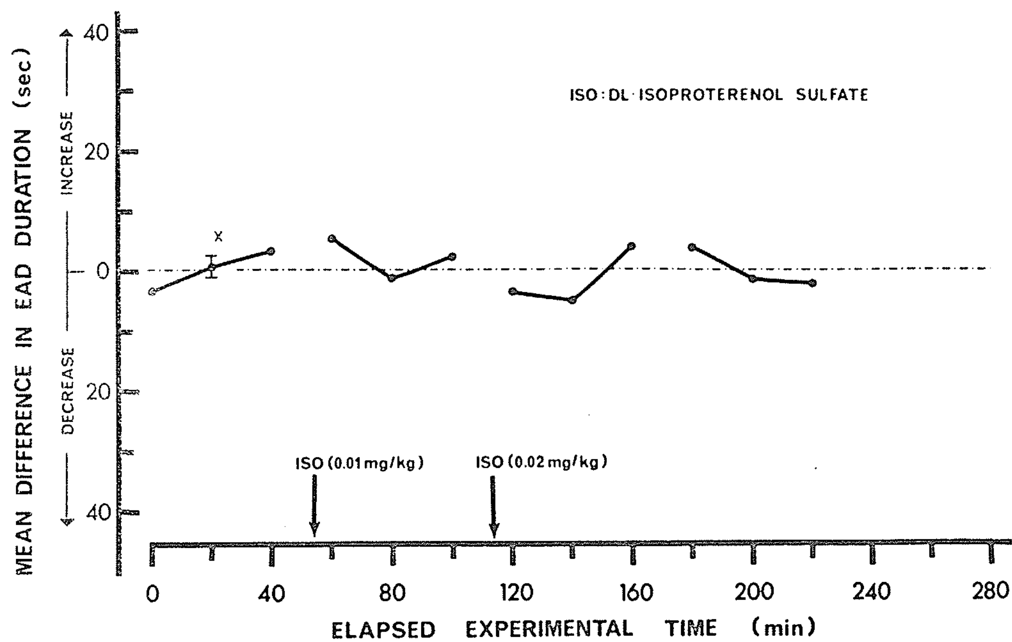


Figure 63.

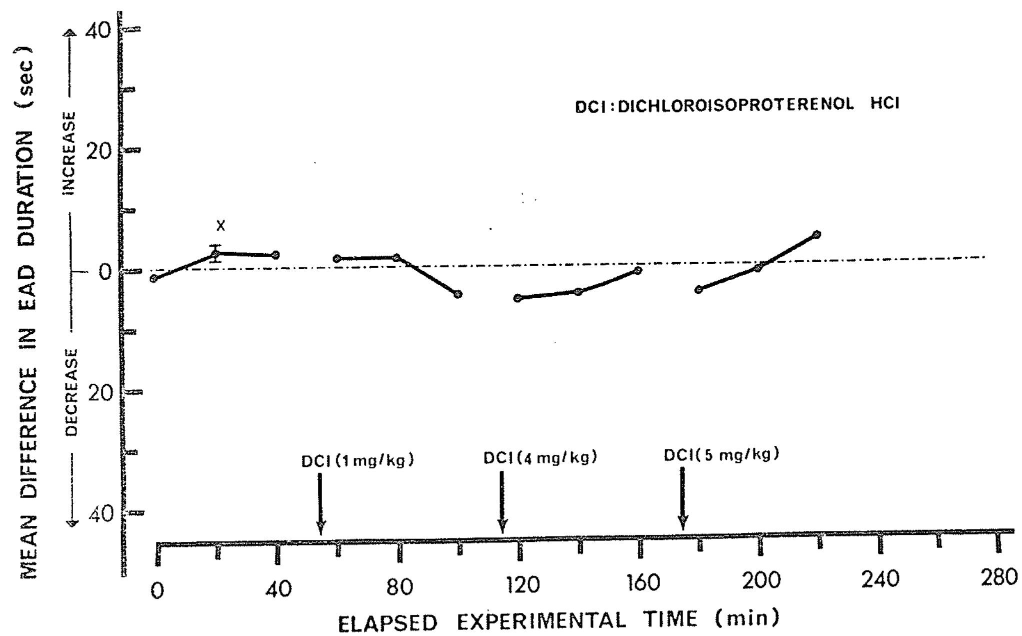


Figure 64.

significant change in EAD duration was detected. Neither 1 mg/kg, 4 mg/kg nor 5 mg/kg of this agent were effective in this regard. The response was monitored for 3 hours (in 2 cats) but no change was evident. It is not clear whether there was any cumulation of drug with each succeeding injection.

In 5 cats treatment with dichloroisoproterenol 5 mg/kg (Fig. 65) did not change EAD duration. The next injection in these experiments which was isoproterenol 0.03 mg/kg also produced no change in EAD duration.

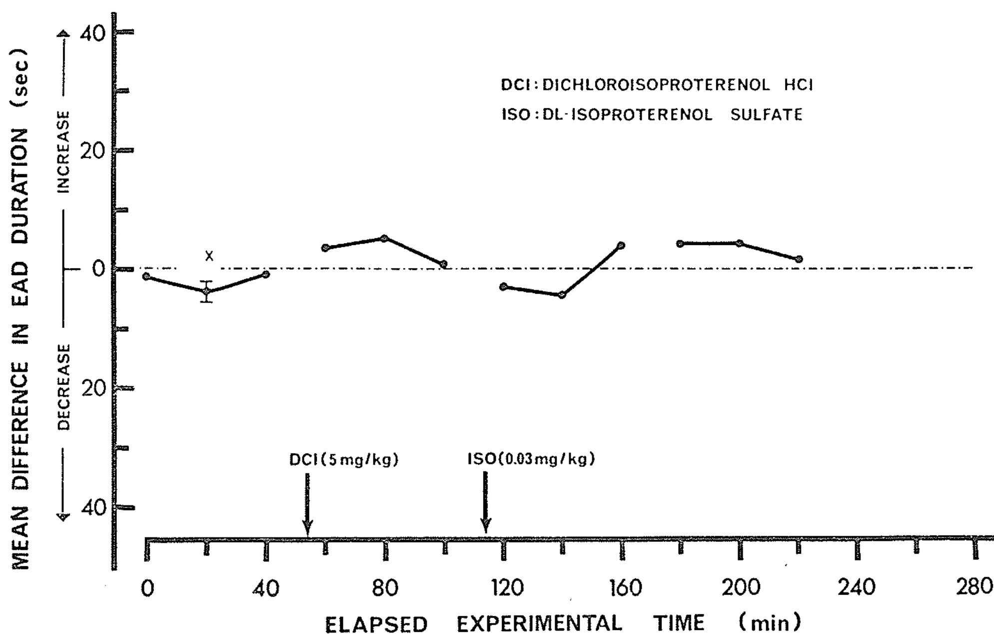
b. <u>β-adrenergic receptor blocking drugs</u>

Pronethalol is a β-receptor blocking agent that has some weak β-receptor stimulant properties (Goodman and Gilman, 1970). This drug was injected in increasing doses to 7 cats (Fig. 66). Injection of 2 mg/kg followed 50 min later by 3 mg/kg did not produce any change in EAD duration. A dose of pronethalol 5 mg/kg produced a decrease of almost 10 sec in EAD duration, which was highly significant ($P < 0.01$). The last injection of pronethalol which was 10 mg/kg produced a highly significant ($P < 0.01$) decrease in EAD duration of over 20 sec. It would appear that there was some drug cumulation with this dosage schedule. The only side effect observed was slight hyperventilation, seen only at the highest dose.

The next β-receptor blocker tried was dl-propranolol, an agent reported to have little or no β-receptor stimulant properties (Goodman and Gilman, 1970). An injection of dl-propranolol 2.5 mg/kg caused in 6 cats (Fig. 67) a significant ($P < 0.05$) increase in EAD duration of less than 10 sec. After the next injection of 2.5 mg/kg

Figure 65. Example of the lack of effect of a partial β-adrenoceptor agonist, dichlorisoproterenol sulfate (DCI) and a full β-adrenoceptor agonist, dl-isoproterenol sulfate (ISO) upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.

Figure 66. Effect of increasing doses of a β-adrenoceptor blocking agent, pronethalol (PN) upon EAD duration. X refers to the pooled S.E. of all the mean differences calculated from the results of experiments on 7 cats. Note lack of significant changes at lower doses of pronethalol
** = $P < 0.01$.



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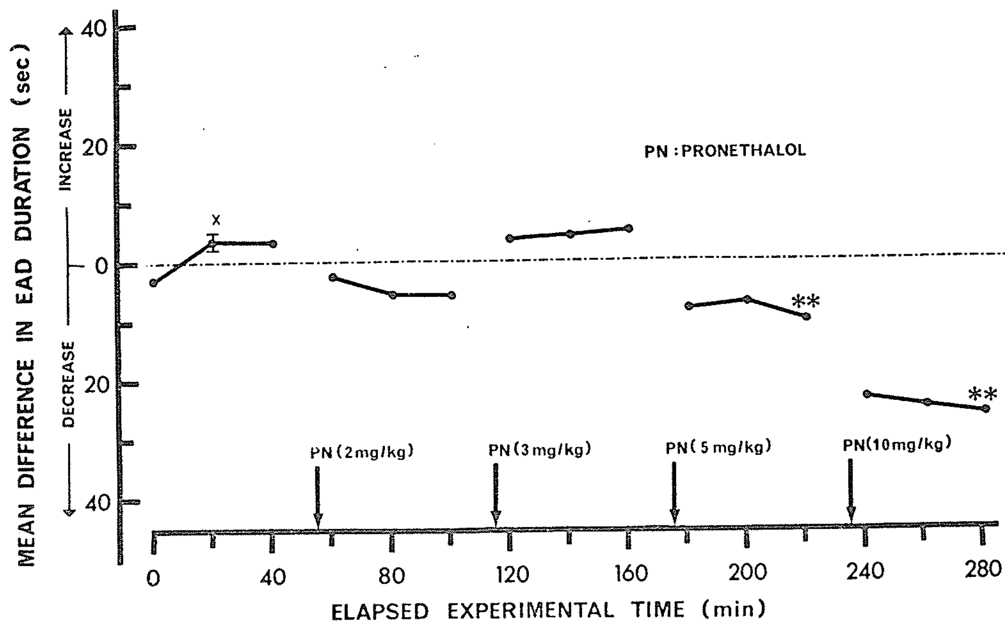


Figure 66.

Figure 67. Effect of increasing doses of β-adrenoceptor antagonist dl-propranolol (± PR) upon EAD duration. Note initial increase in EAD duration at low dose and subsequent decrease in EAD duration at higher doses. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. $* = P < 0.05$; $** = P < 0.01$.

Figure 68. Effect of a pharmacologically inactive isomer, d-propranolol (+ PR) upon EAD duration. Note progressive decrease in EAD duration at higher dose. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. $* = P < 0.05$; $** = P < 0.01$.

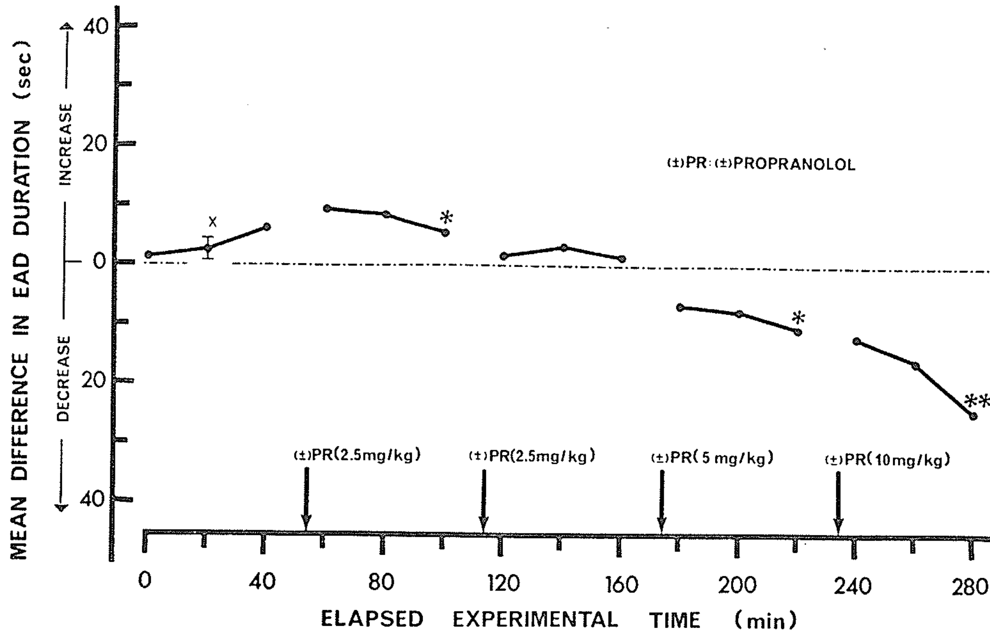


Figure 67.

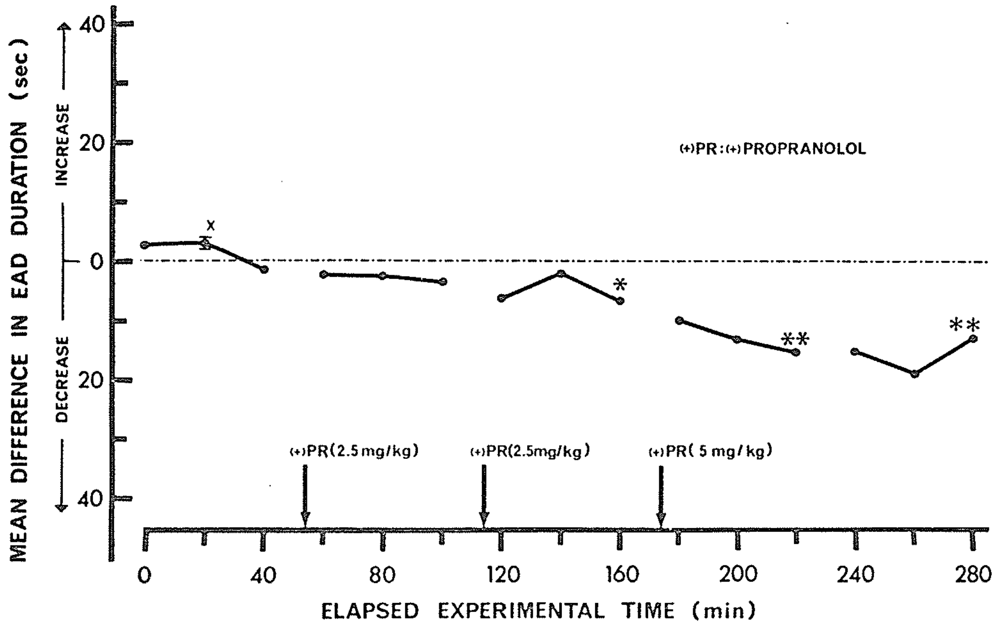


Figure 68.

60 min later, EAD duration returned to control values. A third injection of dl-propranolol, which was 5 mg/kg caused a significant ($P < 0.05$) reduction in EAD duration (10 sec). The final injection in this series was dl-propranolol 10 mg/kg which caused a very significant ($P < 0.01$) decrease in EAD duration (about 20 sec). The response was monitored for 2 hours after the last injection and there was a further decrease in EAD duration of about 30 sec, after which EAD duration appeared to return gradually to control values. There appeared to be cumulation of drug with successive injections. The major peripheral effect seen in all cats, at the higher doses, was hyperventilation. In 3 of the cats a mild calming or sedating effect was observed at these high dose levels.

The β-receptor blockers pronethalol and propranolol have very prominent local anaesthetic or quinidine-like actions (Goodman and Gilman, 1970). To test the degree of this effect the d-isomer of propranolol, which has quinidine-like action, but negligible β-receptor blocking properties, was administered. In 5 cats d-propranolol 2.5 mg/kg produced a slight but not significant decrease in EAD duration (Fig. 68). Another injection of d-propranolol 2.5 mg/kg 60 min later caused a reduction in EAD duration of about 5 sec, which was significantly ($P < 0.05$) different from control. The final injection of d-propranolol 5 mg/kg produced a highly significant ($P < 0.01$) reduction in EAD duration of about 15 sec. It appears that there was cumulation of drug in these experiments. The response remained decreased for almost another 90 min, after which it returned slowly to control levels.

Thus, it seems probable that the decrease in EAD duration

Figure 69. Effect of a β-adrenoceptor antagonist practolol (PT) without local anaesthetic side effects, upon EAD duration. Note initial and brief significant decrease in EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.
$* = P < 0.05$.

Figure 70. Effect of a β-adrenoceptor antagonist MJ-1999, solatol, (MJ) upon EAD duration in the isolated cortex. Note slight, but only significant, decrease in EAD duration after second injection of MJ-1999 at a dose of 5 mg/kg. X refers to the pooled S.E. calculated from the results of experiments on 6 cats.
$* = P < 0.05$.

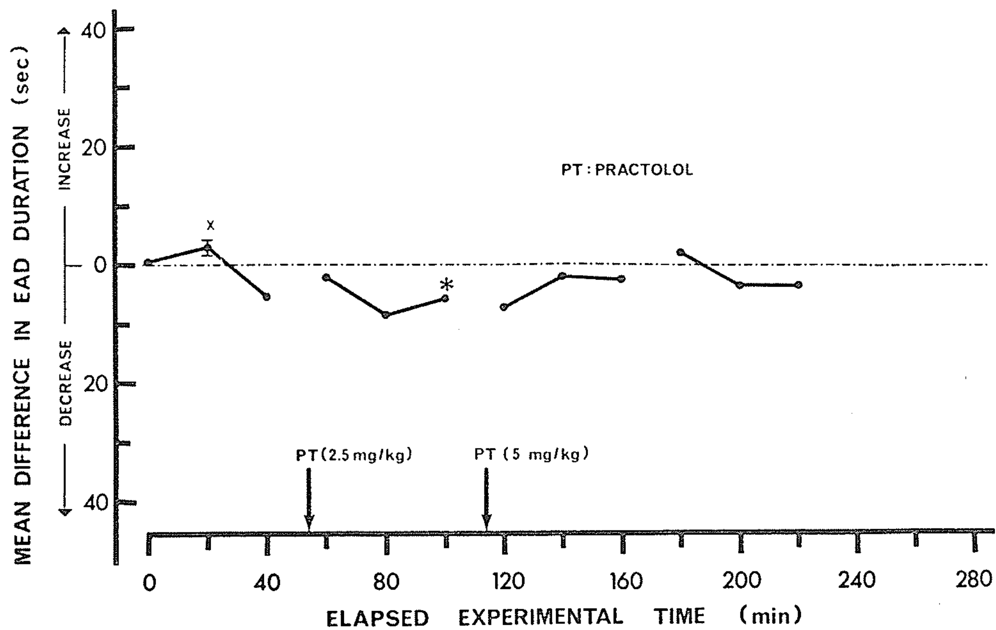


Figure 69.

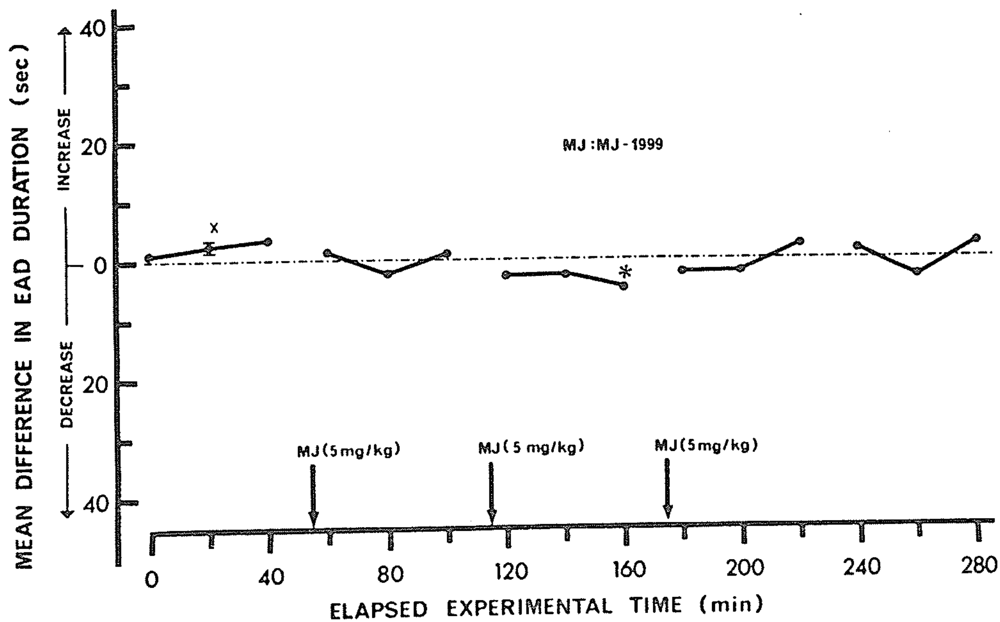


Figure 70.

following the administration of pronethalol and dl-propranolol was most likely due to their non-specific local anaesthetic actions. To test this further, experiments in which practolol or sotalol (MJ-1999), β-receptor antagonists lacking prominent quinidine-like effects (Goodman and Gilman, 1970) were studied.

In 4 cats practolol 2.5 mg/kg (Fig. 69) did cause a small but significant ($P < 0.05$) decrease in EAD duration. A second dose of practolol 5 mg/kg did not result in any change in EAD duration.

Administration of MJ-1999 to 6 cats (Fig. 70) appeared to have little effect on EAD duration. The first injection of MJ-1999 was 5 mg/kg and did not alter EAD duration. The second dose of this agent was also 5 mg/kg and it produced a small (< 5 sec) but significant ($P < 0.05$) decrease in EAD duration. A final dose of MJ-1999, which was also 5 mg/kg, resulted in no change in EAD duration during almost 2 hours after the injection.

Thus, both practolol and MJ-1999 caused brief, temporary decreases in EAD duration which were quite small (< 5 sec). Other than some hyperventilation, little was observed in the way of side effects.

A relatively new β-adrenergic receptor blocking drug tried was an INPEA agent (Murmann _et al._, 1966). The active β-adrenergic blocker is D (-) INPEA, whereas the isomer L (+) INPEA is reported not to antagonize β-adrenergic receptors (Murmann _et al._, 1966). Moreover, these agents apparently lack significant local anaesthetic actions.

In 6 cats D (-) INPEA 2.5 mg/kg (Fig. 71) was injected and produced no change in afterdischarge duration. No change in duration resulted either after injection of 7.5 mg/kg or after injection of

10 mg/kg. The next dose of D (-) INPEA which was 10 mg/kg produced a highly significant ($P < 0.01$) mean increase in EAD duration of about 15 sec above control. It would appear that there was cumulation of the drug during these injections and the final dose probably represented 30 mg/kg of D (-) INPEA. The cats appeared a bit restless at this dose level. More significant, was the occurrence, at the highest dose, of a transient and severe hyperventilation for up to 30 min, which culminated in spontaneous generalized convulsions (in 3 cats).

Another group of 6 cats received the same doses of the inactive isomer L (+) INPEA (no β-receptor antagonism). The first two treatments consisted of injections of 2.5 mg/kg and 7.5 mg/kg (Fig. 72) respectively. At both these doses, no change in EAD duration from control resulted. Following injection of L (+) INPEA at a dose of 10 mg/kg there was a significant ($P < 0.05$) reduction from control in EAD duration of about 10 sec. Another injection of L (+) INPEA 10 mg/kg produced the same reduction in EAD duration of 10 sec, but the response was more stable and this difference was highly significantly ($P < 0.01$) different from control.

c. Effect of β-adrenergic blockers on the response to d-amphetamine

Pretreatment with four different β-receptor blocking drug was done in 4 groups of cats to determine whether these agents might modify the usual decrease in EAD duration produced by d-amphetamine 5 mg/kg.

A dose of dl-propranolol 10 mg/kg (Fig. 73) was injected into 5 cats, producing a transient decrease in EAD duration, which however, returned soon to control levels. This treatment was followed by the

Figure 71. Effect of a β-adrenoceptor antagonist D(-)INPEA, [D(-)I], upon EAD duration in the isolated cortex. Note very significant increase in EAD duration after last injection of D(-)INPEA at a dose of 10 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments in 6 cats.
** = $P < 0.01$.

Figure 72. Example of the effect of a pharmacologically inactive isomer L(+)INPEA, [L(+)I], upon EAD duration in the isolated cortex. Note significant decreases in EAD duration after last two injections of L(+)INPEA at a dose of 10 mg/kg each. X refers to the pooled S.E. of all the mean differences calculated from the results of experiments in 6 cats.
* = $P < 0.05$; ** = $P < 0.01$.

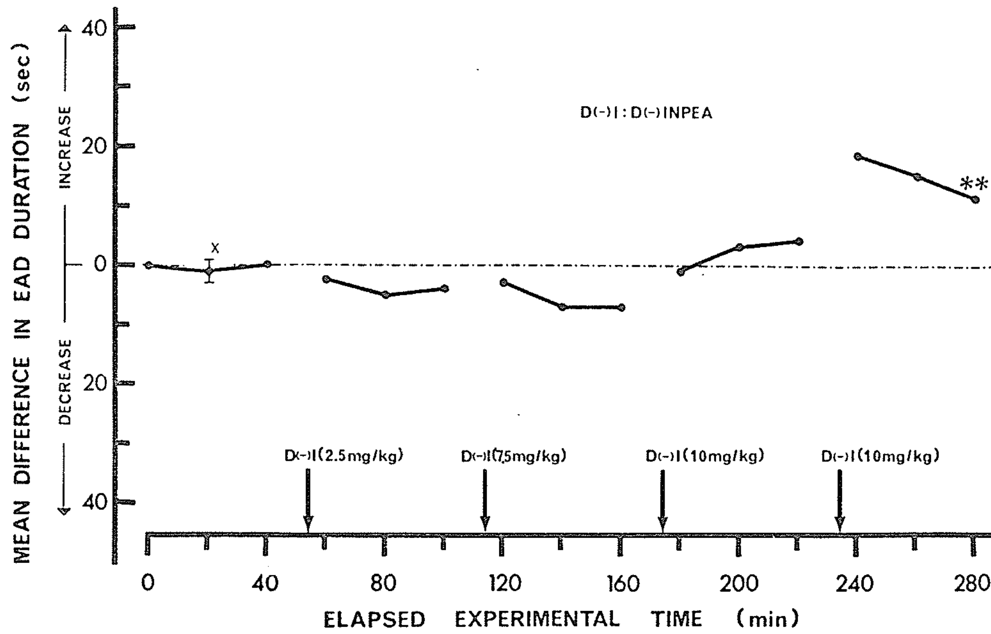


Figure 71.

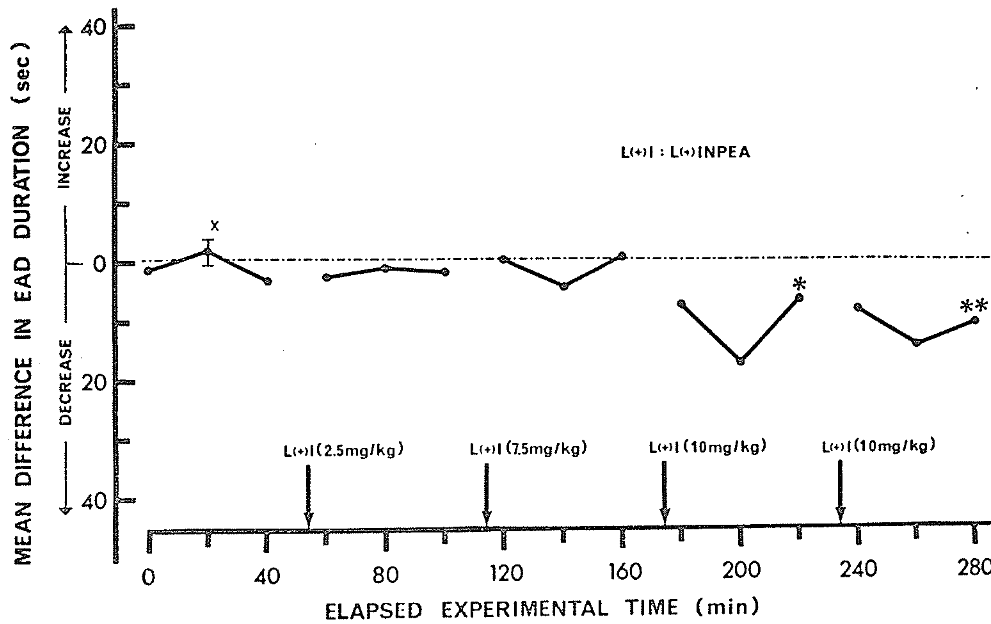


Figure 72.

Figure 73. Lack of effect of a β-adrenoceptor antagonist dl-propranolol, (± PR), 10 mg/kg on the action by d-amphetamine sulfate (d-A) 5 mg/kg upon EAD duration. Note significant decrease in EAD duration caused by d-amphetamine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments in 5 cats.
* = $P < 0.05$.

Figure 74. Lack of effect of a β-adrenoceptor antagonist MJ-1999, (MJ), 10 mg/kg on the action of d-amphetamine sulfate (d-A) 5 mg/kg upon EAD duration. Note very significant decrease in EAD duration caused by d-amphetamine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
** = $P < 0.01$.

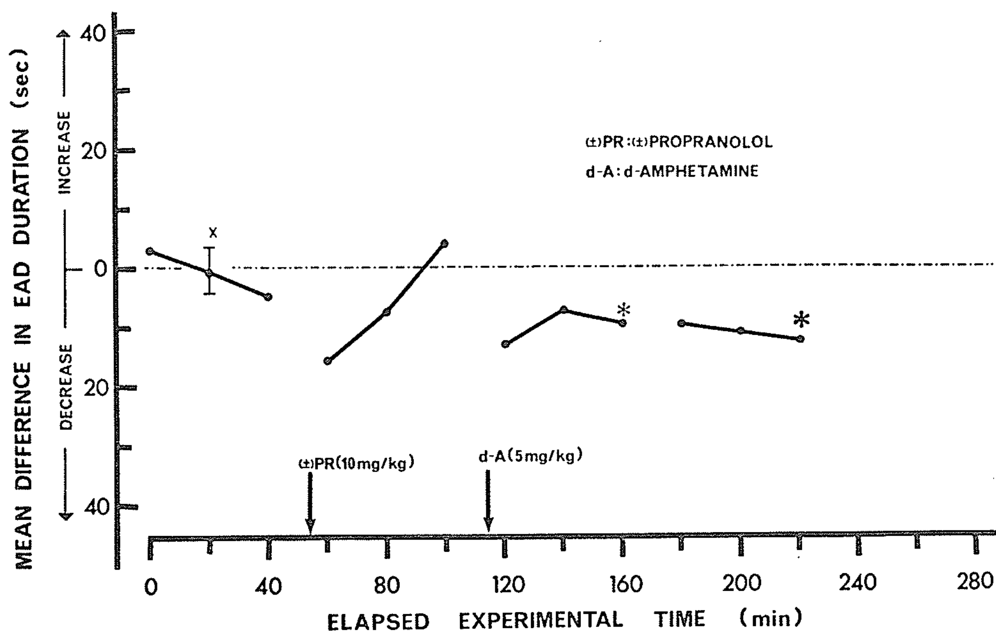


Figure 73.

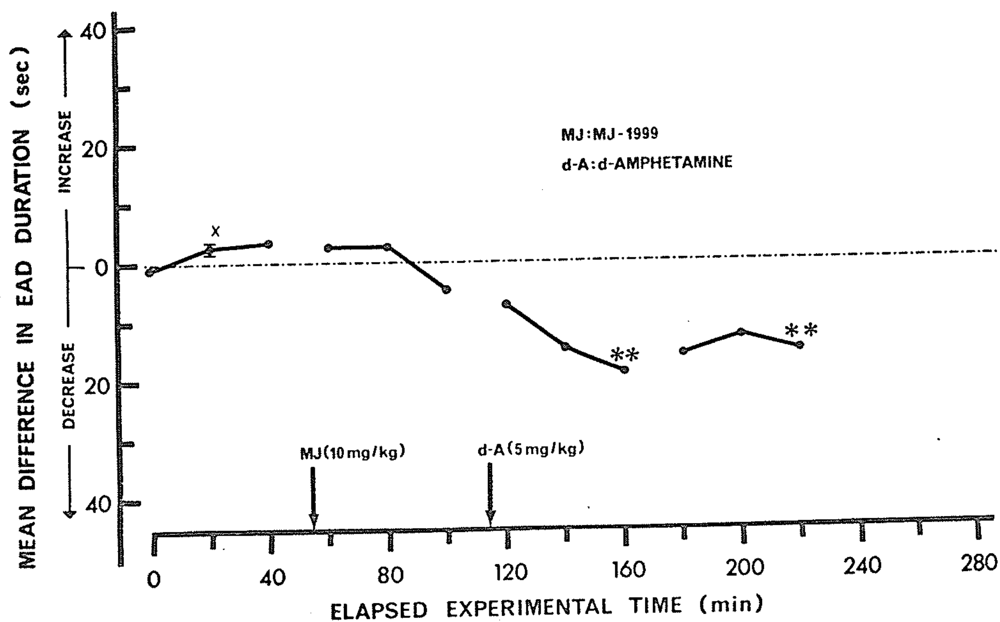


Figure 74.

administration of d-amphetamine 5 mg/kg which produced a significant ($P < 0.05$) decrease in EAD duration of approximately 10 sec.

Another group of 6 cats received MJ-1999 at a dose of 10 mg/kg (Fig. 74) as a pretreatment. This injection was not followed by any change in EAD duration. When d-amphetamine 5 mg/kg (Treatment-2) was injected it produced a highly significant ($P < 0.01$) mean decrease in EAD duration (about 15 sec).

Injection of D(-) INPEA at a dose of 10 mg/kg (Fig. 75) did not change EAD duration in 5 cats. When d-amphetamine 5 mg/kg was injected there was a significant ($P < 0.05$) reduction in EAD duration (about 10 sec).

The inactive isomer L(+) INPEA 10 mg/kg (Fig. 76) was injected in 4 cats and no change in EAD resulted. Administration of d-amphetamine 5 mg/kg produced a significant ($P < 0.05$) reduction in EAD duration (about 10 sec).

Thus, on the basis of the results of 20 experiments, it appears that pretreatment with β-adrenergic receptor blocking drugs does not prevent the action of d-amphetamine upon EAD duration. The greatest decrease in EAD duration in these groups of experiments was in cats pretreated with MJ-1999 (Fig. 74).

d. Effect of β-adrenergic receptor blockers on the response to atropine

Atropine 3 mg/kg (Fig. 77) was injected into 5 cats, and this resulted in the usual highly significant ($P < 0.01$) increase in EAD duration (about 25 sec above control values). Injection of D(-) INPEA at a dose of 20 mg/kg caused a further slight increase in EAD duration. However, this difference was not significantly different

Figure 75. Lack of effect of an active β-adrenoceptor antagonist D(-)INPEA, [D(-)I], 10 mg/kg on the action by d-amphetamine sulfate (d-A) 5 mg/kg upon EAD duration in the isolated cortex. Note that d-amphetamine causes significant decreases in EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. * = $P < 0.05$; ** = $P < 0.01$.

Figure 76. Lack of effect of an inactive isomer L(+)INPEA, [L(+)I], 10 mg/kg on the action by d-amphetamine sulfate (d-A) 5 mg/kg upon EAD duration in the isolated cortex. Note that d-amphetamine decreases EAD duration significantly. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. * = $P < 0.05$; ** = $P < 0.01$.

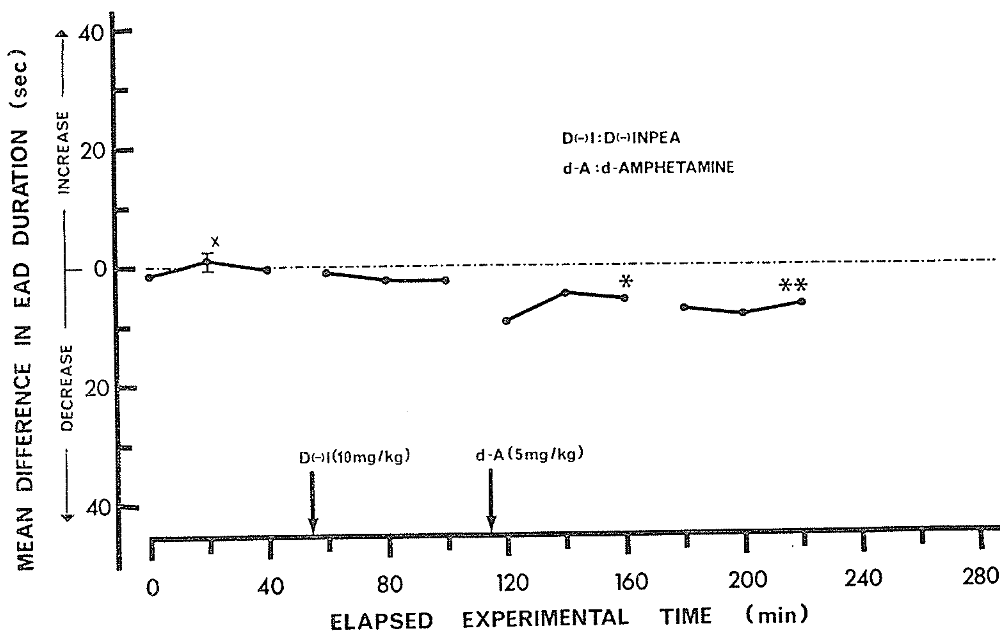


Figure 75.

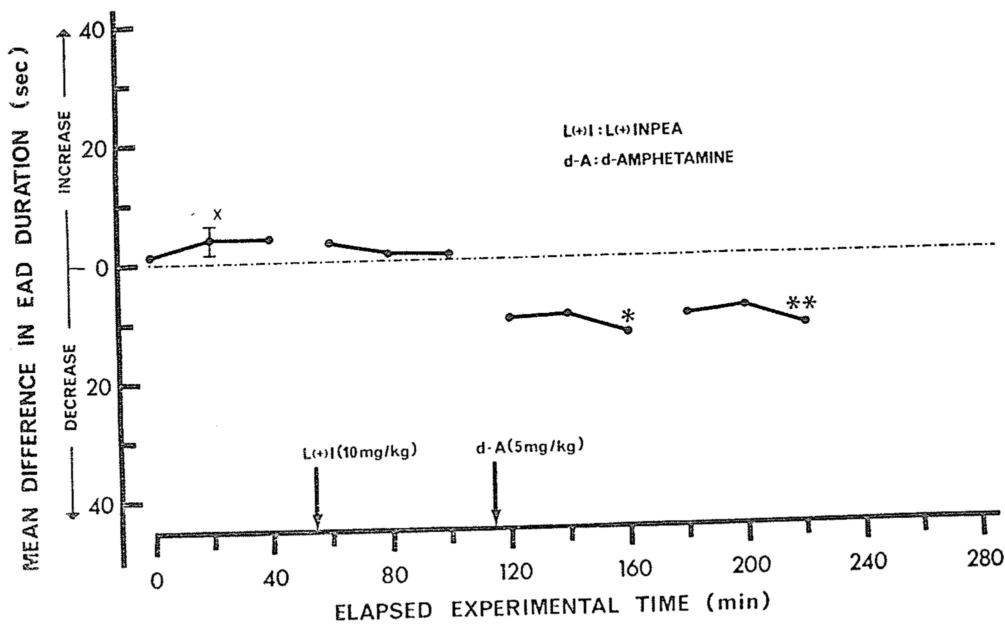


Figure 76.

Figure 77. Lack of modification by D(-)INPEA, [D(-)I], 20 mg/kg of the action by atropine sulfate (At) 3 mg/kg upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. ** = $P < 0.01$.

Figure 78. Lack of effect of a β-adrenoceptor antagonist MJ-1999, (MJ), 10 mg/kg on EAD duration in the isolated cortex already increased by atropine sulfate (At) 3 mg/kg. There is no significant change in EAD duration after injection of MJ-1999. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments in 5 cats. ** = $P < 0.01$.

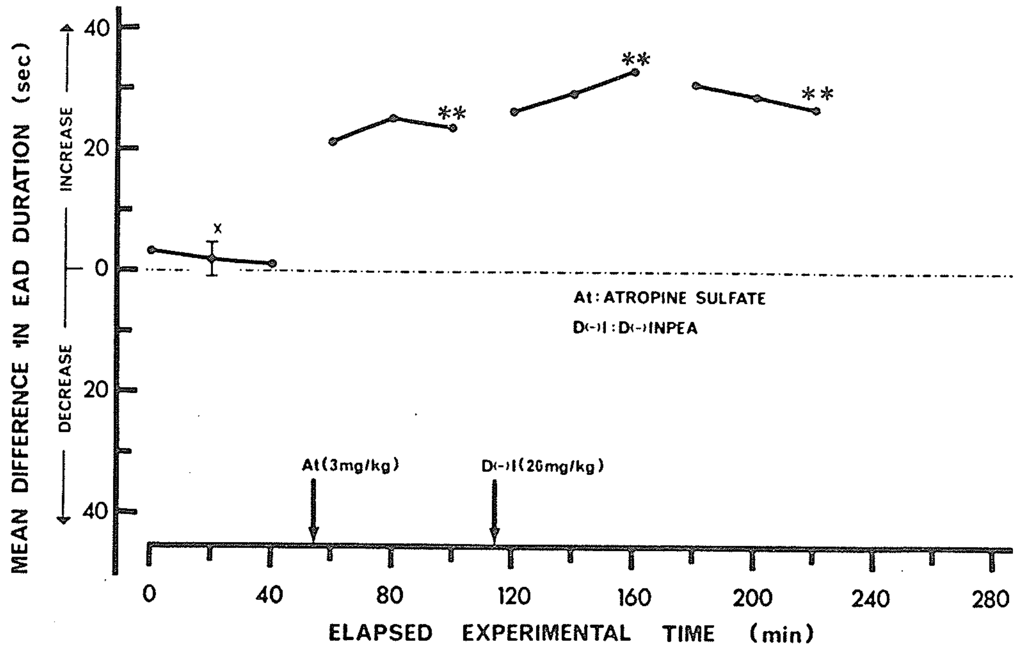


Figure 77.

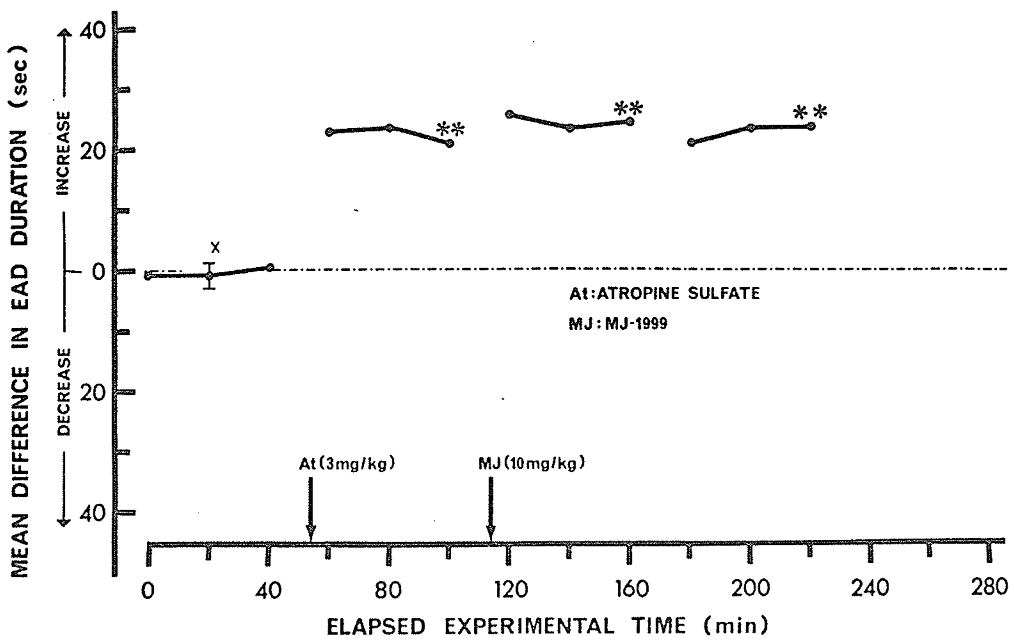


Figure 78.

from the preceding responses. Monitoring the response for a longer period showed that EAD duration remained about 25 sec above control values.

In another group of 5 cats, atropine 3 mg/kg (Fig. 78) produced a similar highly significant ($P < 0.01$) increase in EAD duration, of more than 20 sec. Following this increase, MJ-1999 at a dose of 10 mg/kg was injected and did not produce any further change in EAD duration. Afterdischarge duration remained at the level observed immediately following administration of atropine.

E. Resume

The contradictory and inconsistent effects obtained with the β-adrenergic agonist and antagonist drugs suggest that the resultant changes in EAD duration by some of these agent are due to nonspecific actions of these agents and are not dependent on interaction with the β-adrenergic receptor. On the other hand, the results may reflect, among other things, differences in central distribution of these agents.

The significant decreases in EAD duration caused by pronethalol (Fig. 66) and dl-propranolol (Fig. 67) are most likely due to their local anaesthetic actions (Fig. 68; Fig. 69). A widely used β-receptor antagonist MJ-1999 (Fig. 70) caused little change in afterdischarge duration, while a new agent, D(-) INPEA, reportedly possessing central actions increased duration.

In any event, the β-adrenergic receptor blockers do not antagonize the action of d-amphetamine (Fig. 73; Fig. 74; Fig. 75), and they do not modify the response to atropine (Fig. 77; Fig. 78).

F. Discussion

The results of experiments with adrenergic drugs, particularly amphetamine, show clearly that non-catecholamine sympathomimetic agents with central actions cause significant decreases in EAD duration (Fig. 43-46). Agents such as methoxamine, which do not penetrate into the CNS, do not appear to modify EAD duration (Fig. 48). The adrenergic agents producing these effects are known for their effects on α-adrenergic receptors in the periphery, but there is doubt as to whether they act directly or indirectly to activate central adrenoceptive structures. Increasing cerebral monoamine levels with an inhibitor of monoamine oxidase also decreased EAD duration (Fig. 47). Inhibition of this enzyme would increase the level of dopamine, noradrenaline and serotonin concomittantly. On its own L-DOPA (Table 3) caused significant reductions in EAD duration.

The demonstration of a more direct and specific action on these adrenergic structures was not possible since the preferred compounds, adrenaline or noradrenaline, have to be applied directly to cerebral tissue owing to their inability to cross the blood-brain barrier. Testing two sympathomimetic agents, clonidine and phenylephrine (Table 3), reported to have central effects (Goodman and Gilman, 1970), revealed that only one of them, phenylephrine, was able to reduce EAD duration.

It appears that agents which act upon β-adrenoceptors in the periphery are without effect on afterdischarge duration (Fig. 63-65). Although the results in this thesis suggest that stimulation of adrenoceptors of the α type in the cerebral cortex results in significant decreases in EAD duration, the uncertain mode of action by which these

agents act not only in the CNS but also in the periphery precludes, at present, any definitive statement regarding their role in afterdischarge duration.

Some measure of support for an adrenergic component involved in controlling the duration of afterdischarge has been provided by the report of Killam et al. (1957). These workers demonstrated that after the administration of reserpine the duration of hippocampal afterdischarges induced by direct electrical stimulation is prolonged. However, it is well known that reserpine depletes not only central stores of catecholamines, but also cerebral serotonin. Earlier reports (Chen and Bohner, 1961; Gray et al., 1963; Rudzik and Mennear, 1965, 1966) proposed that the enhancement of seizure activity by reserpine and its antagonism of anticonvulsants involves catecholamines. However, Koslow and Roth (1971) suggest that reserpine antagonism of anticonvulsant agents is not mediated through adrenergic mechanisms. This conclusion is based on their and another observation (Gray and Rauh, 1967) that low doses of reserpine, which do not alter the levels of biogenic amines, antagonize the action of anticonvulsants. Moreover, Koslow and Roth (1971) have reported that reserpine has a dual effect on the MES pattern in the rat, the action consisting of either facilitation or inhibition of the seizure pattern, not really an unexpected finding in view of the many uncertain central actions of reserpine.

As it was found that sympathomimetic drugs shorten the EAD duration in the isolated cortex, it was decided to determine whether blockade with drugs known to antagonize α-adrenergic receptors in the periphery would influence afterdischarge duration. Administration of

α-adrenoceptor blocking drugs did not modify EAD duration (Fig. 49; 51; 53), although the agent Dibozane (Fig. 54) did cause a brief increase in duration. This lack of effect could be due to the absence of an adrenergic tone on the isolated cortex or to a lack of penetration of drug into cortical tissue. However, this latter possibility seems unlikely as various central effects of these agents have been reported (Nickerson and Hollenberg, 1967). Studies with chlorpromazine and haloperidol, two agents with definite central actions and having antagonism against adrenergic drugs in the periphery (Goodman and Gilman, 1970) were also carried out. Both of these substances (Fig. 55; 56) increased EAD duration. However, this does not provide much insight as both of these agents also have many diverse pharmacological antagonistic properties in the periphery in addition to their α-adrenergic receptor blocking activity.

The α-adrenergic receptor blocking drugs appear to be able to antagonize partially the action of amphetamine upon EAD duration (Fig. 50; 52). However, it is also known that both of the agents used, phenoxybenzamine and phentolamine, also antagonize serotonin and histamine, when tested on isolated organs (Nickerson and Hollenberg, 1967). Some measure of the lack of specificity of adrenergic blocking drugs can be seen in the experiments in which phentolamine failed to antagonize the effects of a monoamine oxidase inhibitor (Fig. 62).

In some respects the lack of effect of adrenergic blocking drugs on their own is somewhat puzzling since earlier workers (Munoz and Golstein, 1961) have reported that some of these agents show antagonism to the EEG effects of amphetamine. However, in their study other

adrenergic blocking agents did not show this antagonism. Furthermore, the agents blocking amphetamine, namely phenoxybenzamine, produced depressant effects on their own. Rudzik and Mennear (1966a) have reported that both α and β-adrenergic receptor blocking agents antagonize the anticonvulsant effect of acetazolamide. Only phenoxybenzamine at high doses antagonized the anticonvulsant effects of DPH. Thus, as the role of carbonic anhydrase, through which acetazolamide is presumed to act, is presently not clear, the implication of these findings to the control of epileptiform discharges is at best, preliminary. In any case, Koslow and Roth (1971) have dissociated the action of acetazolamide from involvement with central adrenergic mechanisms.

Some of the β-adrenergic receptor blocking drugs examined in this study decreased EAD duration (Fig. 66; 67; 69). However, this action appears to be related to their non-specific local anaesthetic properties (Fig. 69), since agents such as sotalol (MJ-1999, Fig. 70) and practolol (Fig. 69), that lack this undesirable property, do not cause very significant changes in EAD duration. One β-receptor antagonist, D(-) INPEA (Fig. 71) with reported central actions (Murmann, et al., 1966) increased EAD durations, but only at high doses. The significance of this action is not readily apparent as the pharmacologically inactive isomer, L (+) INPEA (Fig. 72) decreased EAD duration, also at high doses. As both of the INPEA derivatives have central effects not related to their peripheral adrenergic properties, it would appear that there is no intrinsic mechanism having specifically β-adrenergic character in the isolated cortex. In any event, pretreatment with β-blockers did not prevent or modify the action of amphet-

amine (Fig. 73-76).

Thus, although sympathomimetic drugs decrease afterdischarge duration in the isolated cortex, this action does not appear to be exerted entirely by an adrenergic mechanism. Pretreatment with α-receptor antagonists is only partially effective in blocking the decrease in EAD duration produced by adrenergic agents, while prior administration of β-adrenergic receptor antagonists results in no effect on the action of the adrenergic (amphetamine) drugs. Based on these results it appears that the action of these agents seems to be mediated at least in part, via some other system. Selective depletion of cerebral monoamines in laboratory rodents has been successfully applied to solving neuropharmacological problems by many investigators, but the results on cats do not appear to be very promising owing to the profound side-effects.

As it was shown previously that cholinergic drugs were important in the control of EAD durations, the interactions between adrenergic and cholinergic drugs on EAD duration were investigated. Atropine prevented (Fig. 57) and reversed the action of amphetamine (Fig. 58) upon EAD duration. Pretreatment with phentolamine did not block the action of atropine (Fig. 59) nor did it modify the changes in EAD duration produced by prior treatment with atropine (Fig. 60). Moreover, α-adrenoceptor blockers did not prevent the action of a cholinergic drug (arecoline). Thus, it would appear that the amphetamine effect is exerted via some cholinergic inhibitory system in the cortex. The fact that α-adrenergic blocking drugs partially antagonize this action of amphetamine, but not that of cholinergic drugs, suggests that

perhaps other mediator system(s) are involved in this action of amphetamine on EAD duration.

It appears improbable that β-adrenergic receptors are involved in pharmacological modulation of afterdischarge duration from the observations that β-adrenergic blockers do not modify the action of atropine (Fig. 77-78).

It appears therefore, that the action of sympathomimetic drugs upon EAD duration in isolated cortex is not achieved simply via adrenergic structures. Furthermore, our results suggest that if there are adrenergic mechanisms involved in the control of EAD duration they are not exerting any tonic influence in the isolated slab, because adrenergic blocking agents do not alter EAD duration on their own. Since the action of amphetamine is only partially blocked by α-adrenergic receptor antagonists, this suggests that other structures may be mediating the action of amphetamine.

Evidence in favour of amphetamine acting through other mediator systems is suggested by the results of experiments wherein anticholinergic drugs antagonized the effect on EAD duration of amphetamine (Fig. 57; 58), whereas adrenoceptor blockade did not alter the response to cholinergic drugs (Fig. 61). These results suggest further that the cholinergic structures, at this point in the study, are most likely the final link in the network by which adrenergics function.

At this stage it is somewhat difficult to explain clearly the means by which adrenergic drugs act in the isolated cortex to shorten EAD duration. There is evidence for adrenergic terminals in the cerebral cortex (Salmoiraghi, 1966; Dahlstrom, 1969) and it would

appear that these are somehow implicated in this action of amphetamine on EAD duration, since ephedrine and tyramine, which are considered to be relatively specific adrenergic drugs (Trendelenburg et al., 1962) also decrease EAD duration significantly. A critical level of endogenous noradrenaline has been reported to be necessary for the action of amphetamine (Van Rossum et al., 1962; Weissman et al., 1966). However, other studies (Smith, 1963) have shown that prior treatment with reserpine does not abolish the central effects of amphetamine and that it does not act directly on receptor sites (Hanson, 1966; Wolf et al., 1969). Glowinski and Baldessarini (1966) have reviewed the many central effects of amphetamine and it would seem that amphetamine has some direct activity in addition to many other complex indirect effects. The partial blockade by the α-blocking agents suggests that there are several components involved in the response to amphetamine, e.g. dopamine.

Swinyard et al. (1964) have reported that catecholamines alter brain excitability by direct actions on neuronal systems involved in seizure threshold and discharges. Both electroshock and chemoshock thresholds were elevated by small non-toxic doses of sympathomimetics. Other evidence in accord with the findings in this thesis are provided by the studies of Malcom et al. (1967) who showed that cholinomimetics and sympathomimetic substances respectively were powerful inhibitors of evoked cortical responses. However, these authors found that application of very high concentrations of phenoxybenzamine (compared to concentrations of atropine) antagonized the inhibitory action of cholinergics and adrenergics. Atropine did not

alter the response to noradrenaline. Recently, Bernard _et al_. (1969a) have reported that catecholamines applied to motor cortex of rabbits produced an increase in duration of elicited EADs. Previously these authors (Bernard _et al_., 1969) have reported that muscarinic agents shortened while atropinic agents lengthened EAD duration. The apparent discrepancy of their findings with catecholamines might be due to species difference and the use of an acute experimental model as opposed to the chronic model used in my studies. It is possible that in a preparation such as the chronically isolated cortex where hyperexcitability is dominant that catecholamines as well as ACh are mobilized to modulate EAD duration. Moreover, they studied intact cortex.

There is no evidence available concerning the level and distribution of noradrenaline in chronically isolated cortex. Some indirect evidence has been provided by the recent studies of Green _et al_. (1970) who showed that monoamine oxidase activity in chronically isolated slabs increased by over 50 per cent 2 weeks after isolation. These authors speculated that this increased enzyme activity would result in a deficiency of available adrenergic transmitter. The results in this thesis indicate that there might be a reduction of adrenergic tone in isolated cortex. It is not clear that increased enzyme correlates with decreased substrate, for the enzyme could also be increased as a result in response to an increased level of adrenergic substrate.

One possible explanation for the lack of effect of the adrenergic blocking drugs on EAD duration could be their uncertain penetration into the CNS. Although there are no precise quantitative

determinations in this regard, there have been reports that these agents penetrate into the CNS and have relatively specific central effects (Brodie *et al.*, 1954; 1959a; Munoz and Goldstein, 1961). On the other hand, a recent review of the field (Nickerson and Hollenberg, 1967) has cautioned that the observed central actions are usually poorly correlated with α-adrenergic receptor blocking properties. In addition, the available studies have examined these agents for their effects on the whole brain, which would make it more difficult to study their little known direct central actions.

Recent studies with microelectrodes on brain stem neurons by Boakes *et al.* (1971) are in accord with the results presented in this thesis. A similar lack of effect by α and β-adrenoceptor blockers against the depressant but not excitant, action of monoamines on neurons in the pyriform cortex has been reported. These authors could not demonstrate antagonism of the excitatory or inhibitory effects of noradrenaline, either by α-adrenoceptor or β-adrenoceptor blocking drugs. This is in contrast to the findings of Johnson *et al.* (1969a) who reported that cortical neurons were excited by α- and β-adrenergic agonists and were blocked by both α- and β-blocking drugs. Boakes *et al.* (1970) showed that excitatory responses (unit firing) were decreased by minor structural differences of the agonist applied. An interesting observation (Boakes *et al.*, 1971) was that removal of one ring hydroxyl group from the catechol nucleus reduced agonist activity, whereas removal of both ring hydroxyls appeared to restore the excitatory action.

Another explanation for the apparent lack of effect upon EAD duration of the adrenergic blocking agents could be an absence of

adrenergic tone in the chronically isolated slab of cerebral cortex. The adrenergic structure or junction present in neuronally isolated cortex may be of the "potential" (P) linkage type, postulated for central neurons by Sabelli (1964). That is, the receptive structures are present, but the relevant modulator is normally absent. This concept incorporates several classes of linkages at which drugs can achieve various pharmacological effects. In the present situation, chronic deafferentation would damage the presynaptic elements as the cell bodies of central adrenergic neurons are located below the cerebral cortex (Dahlstrom, 1969; section V.B.b.).

Thus, the results of experiments with sympathomimetic agents presented in this thesis support the contention that there are denervated adrenoceptive structures in chronically isolated cortex. More direct proof of the denervated structure is presently lacking. As Salmoiraghi (1966) has postulated an inhibitory function for adrenergic transmitters in the cortex, the apparent deficiency of adrenergic modulation in isolated cortex would conceivably contribute towards a state of increased excitability.

Pepeu and Bartolini (1968) have shown that the administration of amphetamine increased cortical ACh output by 70 percent. This increased ACh release was associated with EEG activation and elevated blood pressure. More recently, these authors (Bartolini and Pepeu, 1970) concluded that the increased ACh output caused by amphetamine was probably due to a central action on β-adrenoceptors, since pretreatment with pronethalol or propranolol completely blocked the increase in central ACh output following amphetamine administration, but

not the pressor response. On the other hand, pretreatment with phenoxybenzamine prevented the hypertensive action of amphetamine, but did not prevent the increase in cortical ACh output. These authors (Bartolini and Pepeu, 1970) concluded that there are β-adrenoceptors in the cortex where catecholamines could act to stimulate more distally situated cholinergic neurons.

The above evidence regarding the role of β-adrenergic structures in cortical phenomena may be misleading. The reduction in ACh output by the cortex could be due to non-specific actions. As I have shown, in this thesis, the most prominent action of β-blockers was a decrease in EAD duration which apparently was related to their marked local-anaesthetic properties. Therefore, it is likely that this generalized inhibitory action would also affect cortical ACh release. Phillis (1968) has shown that anaesthetics decrease the release of ACh from the cortex, and it is known that deepening the degree of anaesthesia depresses the release of ACh (MacIntosh and Oborin, 1953). Goldstein and Munoz (1961) had proposed earlier that central adrenergic responses are mediated through a dual mechanism. These authors postulated that α-effects produced CNS stimulation while β-effects produced CNS depression. Such a dual mechanism does not appear to be operative at the cortical level.

Although Bartolini and Pepeu (1970) found that α-adrenergic blockers were ineffective at the cortical level, other workers have reported contrary findings. Hemsworth and Neal (1968) earlier, have published evidence that the amphetamine evoked increase in cortical ACh ouput from rat cerebral cortex is prevented by α-adrenergic antagonists. Malcolm _et al._ (1967) showed that only α-adrenergic blockers antagonized

inhibition by noradrenaline of evoked responses in the cerebral cortex.

In view of the extensive cardiovascular changes produced by sympathomimetic amines and their antagonists, it would seem that their actions on peripheral structures might be influencing the afterdischarge response. However, Spooner and Winters (1967) have clearly demonstrated using unrestrained conscious chicks that the central and behavioural effects of catecholamines and indoleamines following systemic administration are the result of direct actions on the CNS and are not correlated with any type of cardiovascular change. Rech and Domino (1960) have studied the effect of noradrenaline, adrenaline and serotonin effects on epileptiform activity in the isolated cortex. These authors reported that the central actions of the biogenic amines did not seem to be secondary to their vascular effects. Moreover, the stability of the responses following injections of agents in the experiments presented in this thesis, would also indicate a lack of correlation between central effects and pressor responses. The pressor response to sympathomimetics is usually short-lived and is compensated for by reflex mechanisms. On the other hand, there is no apparent tachyphylaxis or desensitization of the EAD response, as it is apparent for rather prolonged periods following administration of the drug.

Apparently the results of experiments presented in this thesis testing the effects of adrenergic drugs on EAD duration are in contradiction with those published by Minz and Domino (1953). These authors determined on intact cortex that small (1-10 μg/kg) single injections of catecholamines depressed afterdischarge duration. However, after a series of 4-6 such injections, EADs become prolonged. Amphet-

amine, apparently under the same conditions of administration, caused a longer lasting increase in EAD duration. These effects were not related to blood pressure responses. Qualitatively similar results were observed on isolated cortex (Rech and Domino, 1960).

One explanation that can be put forth for this discrepancy is that the schedule of drug administration used by these workers (Minz and Domino, 1953; Rech and Domino, 1960) may have resulted in fatigue or desensitization of central structures. Tachyphylaxis of cortical (Roberts and Straughan, 1967) and other (Phillis and Tebecis, 1967) neurons has been reported and can be a particular problem when interpreting the results of microiontophoretic studies of neurons wherein drugs are applied in a series. Thus, if a normally inhibitory network was inhibited (i.e. disinhibition), the resultant loss of inhibition would result in increased excitability.

In another study on isolated cortex (Maiti and Domino, 1961) it was reported that systemically administered amphetamine and adrenaline, apparently single injections, did not produce consistent changes in EAD duration, in spite of marked cardiovascular changes. These workers (Maiti and Domino, 1961) conducted their experiments on acutely isolated cortex.

It appears therefore, that these authors (Maiti and Domino, 1961) were unable to repeat the previous finding that sympathomimetics increase afterdischarge duration (Minz and Domino, 1953; Rech and Domino, 1960).

It is apparent that catecholamines do not appear to alter the excitability of the isolated cortex in a direct manner. The evidence

presented suggests that there may be present a mixed mechanism and that biogenic amines function in association with central cholinergic mechanism. The fact that adrenergic blocking drugs were only partially effective in antagonizing the action of amphetamine indicates that it acts, at least in part, through other structures. Longo (1962) showed that scopolamine antagonized EEG activation by eserine or amphetamine. Whereas eserine surmounted blockade of its action by scopolamine, such was not the case with amphetamine, whose action was blocked. None of the adrenolytic agents used by Longo (1962) in his study were effective central antagonists of amphetamine. White and Daigneault (1959) showed that electrocortical patterns produced by the administration of adrenergic or cholinergic drugs were prevented by atropine. These studies were carried out on intact brain, and my results indicate that similar effects may be produced at the cortical level. It appears that at this level amphetamine has an action, direct or otherwise, which is somehow channeled into a higher cholinergic inhibitory component, as has been revealed and discussed by Dren and Domino (1968; 1968a).

It has been considered by some investigators that the central effects of sympathomimetics might be related to the function of serotonin in the brain. Costa _et al_. (1960), and Bueno _et al_. (1968) suggested that the balance between serotonin and catecholamines may be crucial for the EEG effects of adrenergic drugs. Vane (1960) described actions of amphetamine on tryptamine receptors of peripheral organs. In this context, the next aspect of this study was to examine the effects on EAD duration of agents known to influence 5-HT receptors in peripheral organs.

XII. 5-HYDROXYTRYPTAMINE (SEROTONIN)

A. Effects of Serotonin Agonists and Antagonists on Afterdischarge Duration

A major difficulty in studying any central effects of serotonin (5-HT) is that drugs affecting cerebral 5-HT levels inevitably affect cerebral catecholamine levels in the same direction (see V.C.). Moreover, since serotonin does not cross the blood-brain barrier, it becomes necessary to use its precursor, 5-hydroxytryptophane (5-HTP) which is capable of penetrating into the brain. It is presumed that 5-HTP is then decarboxylated within the CNS to 5-HT and that the central effects observed are due to 5-HT (Udenfriend *et al.*, 1957). On top of these problems is the lack of specificity of serotonin antagonist compounds (Goodman and Gilman, 1970). Since these agents are known to block several types of receptors in the periphery they probably affect other receptor types in the CNS whenever they are administered. Functionally, the role of 5-HT as a neurotransmitter or modulator in the CNS is not clear.

a. Effect of serotonin on EAD duration

The compounds 5-hydroxytryptamine creatinine sulfate and 5-hydroxytryptamine hydrogen maleate were administered to 2 cats each. The results of these experiments are shown in Table 4.

Table 4. Effect of 5-Hydroxytryptamine on EAD Duration

SALINE CONTROL			TREATMENT - 1
Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	Mean Difference in EAD Duration (sec) ± S.E. (N)
-1.3 ± 2.1 (2)	5-HT Creatinine	0.2 mg/kg	-0.8 ± 1.8 (2)
3.0 ± 1.6 (2)	5-HT Maleate	0.2 mg/kg	1.4 ± 2.0 (2)

N = number of cats.

Thus, systemically administered serotonin did not appear to influence afterdischarge duration. Monitoring the response for a longer period (60 min) did not show any further change. A second injection 120 min after the first dose caused no significant changes.

Administration of 5-HTP to 5 cats (Fig. 79) did not result in any significant change in EAD duration. Injections were 5 mg/kg, 5 mg/kg and 15 mg/kg at intervals of 60 min. Higher doses (50 mg/kg) were tried in a few cats, but these invariably caused changes in respiratory rate and diarrhea.

A report was noticed (de la Torre and Mullan, 1970) in which a peripheral decarboxylase inhibitor Ro 4-4602 which is N(DL-seryl)N^1-(2,3,4-trihydroxybenzyl) hydrazine, was used to minimize the degree of extracerebral metabolic conversion of 5-HTP to 5-HT. This treatment allowed, according to these authors, an effective proportion of the administered dose of 5-HTP to enter the brain, where it is decarboxylated to 5-HT. This cerebral accumulation of 5-HTP is rapid and evident 4 hours after injection (Carlsson and Lindqvist, 1970). In 3 cats, administration of 15 mg/kg of Ro 4-4602 did not produce any significant

change in EAD duration during a period of 6 hours in which EAD duration was monitored.

In 6 experiments administration of the decarboxylase inhibitor Ro 4-4602 at a dose of 15 mg/kg (Fig. 80) did not result in any change in EAD duration. This treatment was followed by an injection of 5-HTP of 2.5 mg/kg which caused a small but significant ($P < 0.05$) decrease in EAD duration. A second injection of 5-HTP at a dose of 2.5 mg/kg, resulted in a very significant ($P < 0.01$) decrease in EAD duration (almost 20 sec). This decrease was not prolonged, and within 4 hours EAD duration had returned to control values.

Thus, the injection of 5-HTP when an extracerebral decarboxylase inhibitor is present, results in a significant decrease in EAD duration. It is assumed that the 5-HTP enters the brain and is converted to 5-HT, and that it is this latter substance that acts pharmacologically lowering EAD duration.

B. Results After the Administration of Serotonin Antagonists

Lysergic acid diethylamide (LSD) derivatives, including LSD itself, are very potent antagonists of 5-HT when tested on peripheral structures (Burger, 1970). Other derivatives used are 2-bromo-LSD, dihydroergotamine and 1-methyl-d-lysergic acid butanolamide (methysergide). Of these methysergide is the most readily available and reasonably specific agent presently available. The major difficulty with most antagonists of 5-HT is that they display a rather wide spectrum of pharmacological actions when tested on peripheral structures (Goodman and Gilman, 1970).

The serotonin blocking drug methysergide was tested in 5 cats

Figure 79. Example of lack of effect of 5-hydroxy-dl-tryptophan (HTP) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.

Figure 80. Effect of 5-hydroxy-dl-tryptophan (HTP) upon EAD duration in the isolated cortex, after pretreatment with an extracerebral decarboxylase inhibitor Ro4-4602 (Ro4). Note significant decrease in EAD duration after injection of 5-HTP at a dose of 2.5 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
$* = P < 0.05$; $** = P < 0.01$.

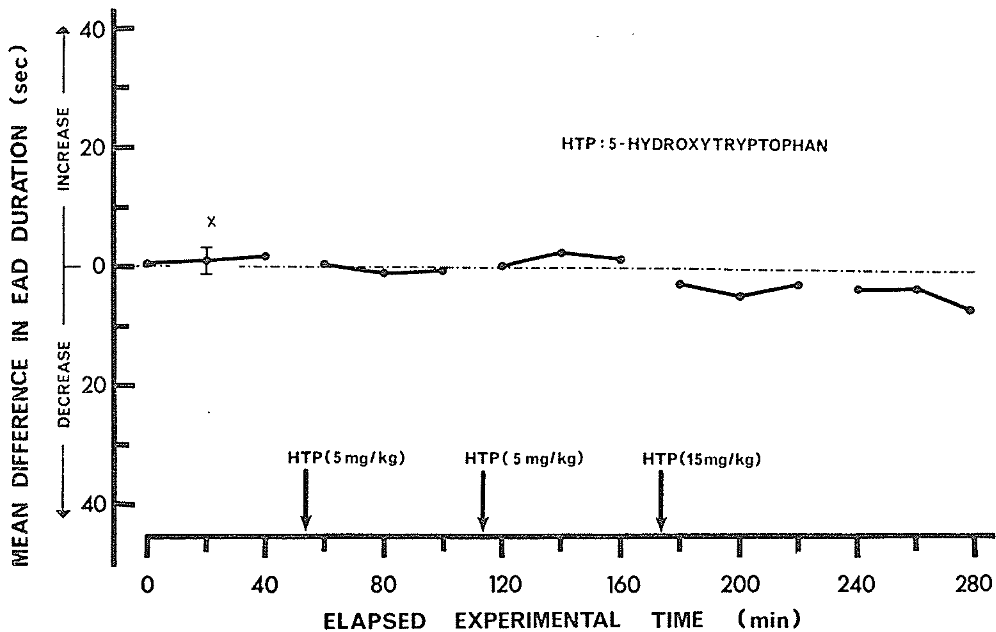


Figure 79.

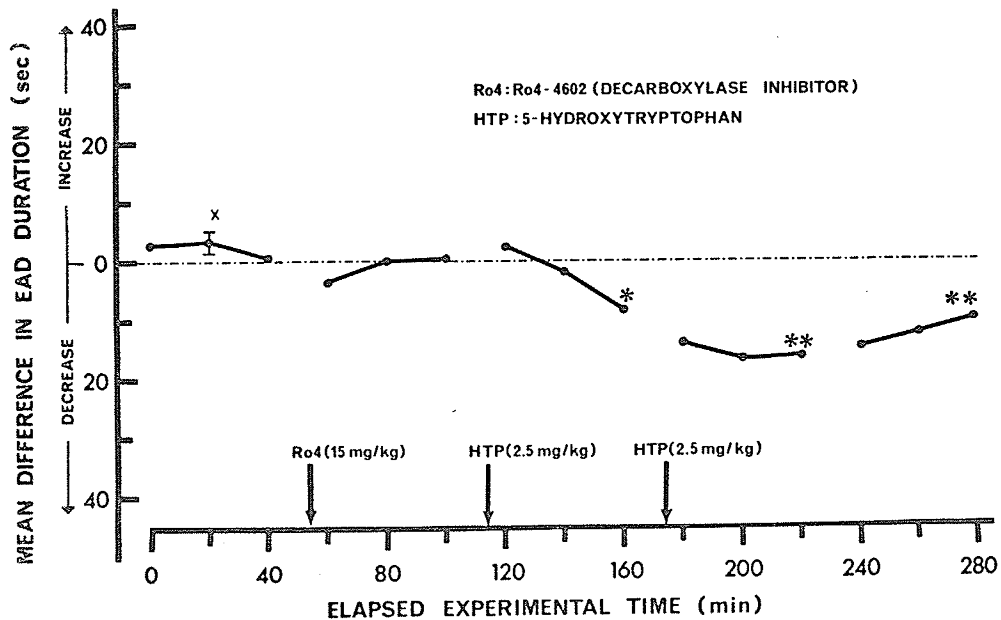


Figure 80.

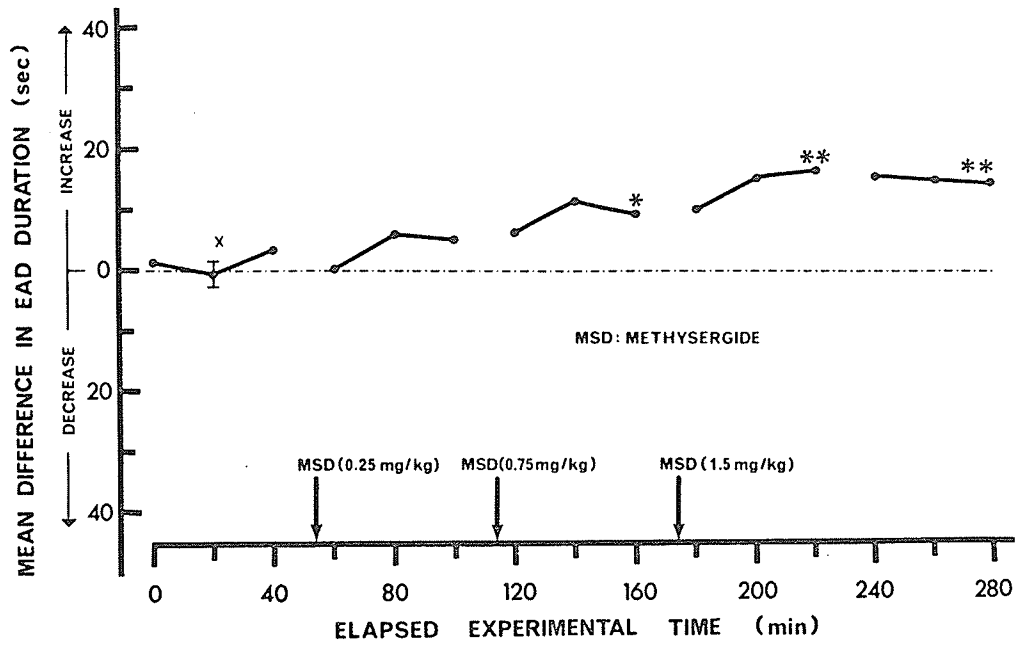
(Fig. 81). The first injection of methysergide which was 0.25 mg/kg, did not result in any significant change in EAD duration. Increasing the dose of methysergide to 0.75 mg/kg produced a significant ($P < 0.05$) increase in EAD duration (approximately 10 sec). A higher dose of methysergide 1.5 mg/kg was tried next and it resulted in a highly significant ($P < 0.01$) increase in EAD duration of almost 20 sec. This increased EAD duration lasted for almost two hours, after which EAD duration decreased rapidly and returned towards control levels. It would appear that there was some cumulation of drug.

Cyproheptadine was another serotonin antagonist that was studied in 6 cats (Fig. 82). Following administration, of cyproheptadine 1 mg/kg there was a significant ($P < 0.05$) increase in EAD duration (almost 5 sec). The second injection of cyproheptadine was 1.5 mg/kg which resulted in a slightly greater, but highly significant ($P < 0.01$) increase in EAD duration (about 10 sec above control). The next injection of cyproheptadine which was 2.5 mg/kg, caused a slight further increase in EAD duration. The maximum increase in afterdischarge duration with this agent was approximately 15 sec. This increased EAD duration was not maintained for long, returning to control values within 2 hours after the last injection. There might have been cumulation of drug with this series of injections, but this is not clear.

Several other compounds reputed to have serotonin blocking actions in the periphery were also tested. Even less information is available regarding the possible central penetration and pharmacological activity of these agents, although, one of these, methergoline, has been reported to be centrally active (Mawson and Whittington, 1970). The re-

Figure 81. Effect of a serotonin antagonist methysergide (MSD) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. * = $P < 0.05$; ** = $P < 0.01$.

Figure 82. Effect of a serotonin antagonist cyproheptadine (CHN) upon EAD duration in the isolated cortex. Note significant increases in EAD duration at all doses of cyproheptadine tested. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. * = $P < 0.05$; ** = $P < 0.01$.



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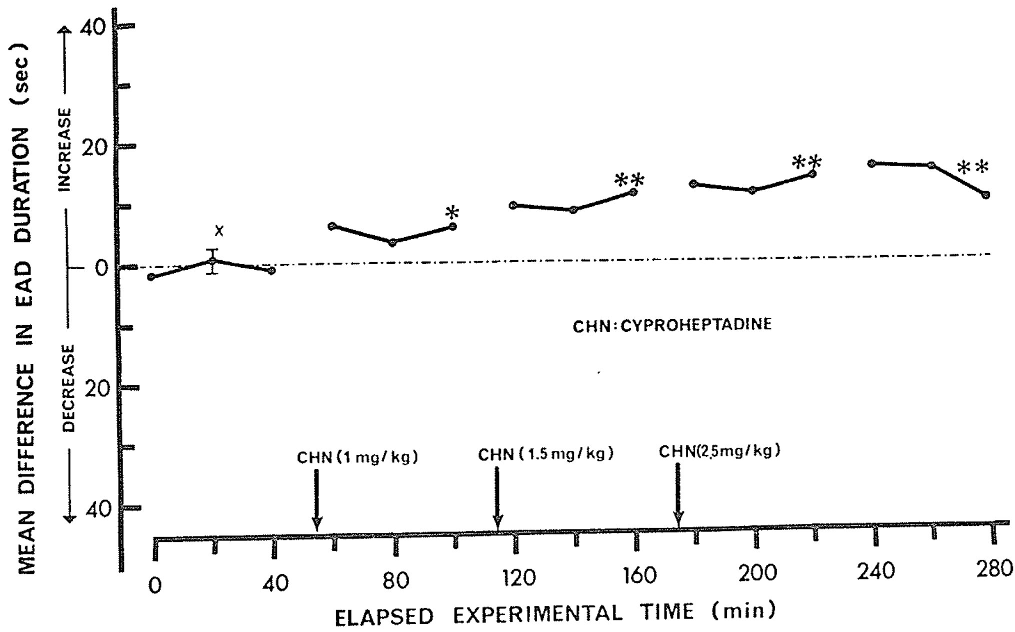


Figure 82.

sults of these studies are summarized in Table 5. The agents tested were 2-bromo-LSD or (BOL) 1 mg/kg in 1 cat, benzo-(4,5) cyclohepta (1,2-b) thiophene or BC-105 at a dose of 5 mg/kg in 3 cats, and methergoline 1 mg/kg in 3 cats.

Table 5. Effect of Agents with Serotonin Blocking Actions on EAD Duration

SALINE CONTROL Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	TREATMENT - 1 Mean Difference in EAD Duration (sec) ± S.E. (N)
-1 ± 3.6 (1)	BOL	1 mg/kg	4.6 ± 2.7 (1)
0.5 ± 2.4 (3)	BC-105	5 mg/kg	8.5 ± 2.9 (3)**
1.9 ± 2.1 (3)	Methergoline	1 mg/kg	7.7 ± 1.7 (3)*

N = number of animals
* = $P < 0.05$
** = $P < 0.01$

Thus, two other agents, BC-105 and methergoline, caused significant increases in EAD duration. Unfortunately, little more can be said about these effects as very little is available concerning possible other actions of these agents.

a. Effect of serotonin antagonists on the response to 5-HTP

After pretreating a group of 4 cats with Ro 4-4602 at a dose of 15 mg/kg (which did not alter EAD duration), the administration of methysergide 1 mg/kg (Fig. 83) 60 min later produced a very significant ($P < 0.01$) increase in EAD duration (about 10 sec). After this pretreatment, the administration of 5-HTP at a dose of 5 mg/kg did not produce an immediate change in EAD duration. Monitoring the response for a longer period showed that there was a gradual decrease in after-

discharge duration towards control levels.

Another group of 4 cats were pretreated with the same dose of the peripheral decarboxylase inhibitor and 60 min later received cyproheptadine 5 mg/kg (Fig. 84). This treatment resulted in an increase in EAD duration of 10 sec, which was highly significantly ($P < 0.01$) greater than control. The next treatment was an injection of 5-HTP at a dose of 5 mg/kg which caused a small, but significant ($P < 0.05$), decrease in afterdischarge duration to control values. The response remained slightly below control values during almost 2 hours of testing.

On the basis of results in 4 experiments, methysergide appears to have blocked the action of 5-HTP upon EAD duration. In the case of cyproheptadine, it is not clear whether this agent is an effective antagonist of this action of 5-HTP, or if it was administered at insufficient dose levels.

b. Effect of serotonin antagonists on the response to d-amphetamine

Treatment of 4 cats with methysergide 1 mg/kg (Fig. 85) resulted in a highly significant ($P < 0.01$) increase in EAD duration (almost 12 sec). This treatment was followed by an injection of d-amphetamine 5 mg/kg, which did not result in any further change in EAD duration.

A similar study was conducted with cyproheptadine in 5 cats (Fig. 86). Injection of cyproheptadine 5 mg/kg increased EAD duration very significantly ($P < 0.01$) by about 10 sec. This treatment was followed by d-amphetamine 5 mg/kg which resulted in no further change in EAD duration.

Thus, on the basis of 9 experiments, serotonin antagonists

Figure 83. Antagonism by methysergide (MSD) 1 mg/kg of the action on EAD duration by 5-hydroxy-dl-tryptophan (HTP) 5 mg/kg. The peripheral decarboxylase enzyme was previously inhibited by Ro4-4602, (Ro4), 15 mg/kg. Note lack of any immediate effect of 5-HTP upon EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments in 4 cats.
$* = P < 0.05$; $** = P < 0.01$.

Figure 84. Example of the partial antagonism by cyproheptadine (CHN) 5 mg/kg of the action on EAD duration by 5-hydroxy-dl-tryptophan (HTP) 5 mg/kg. The decarboxylase enzyme present peripherally was previously inhibited by Ro4-5602 (Ro4) 15 mg/kg. Note partial reduction in EAD duration immediately after injection of 5-HTP at a dose of 5 mg/kg. X refers to the pooled S.E. of all the mean differences calculated from the results of experiments on 4 cats.
$** = P < 0.01$.

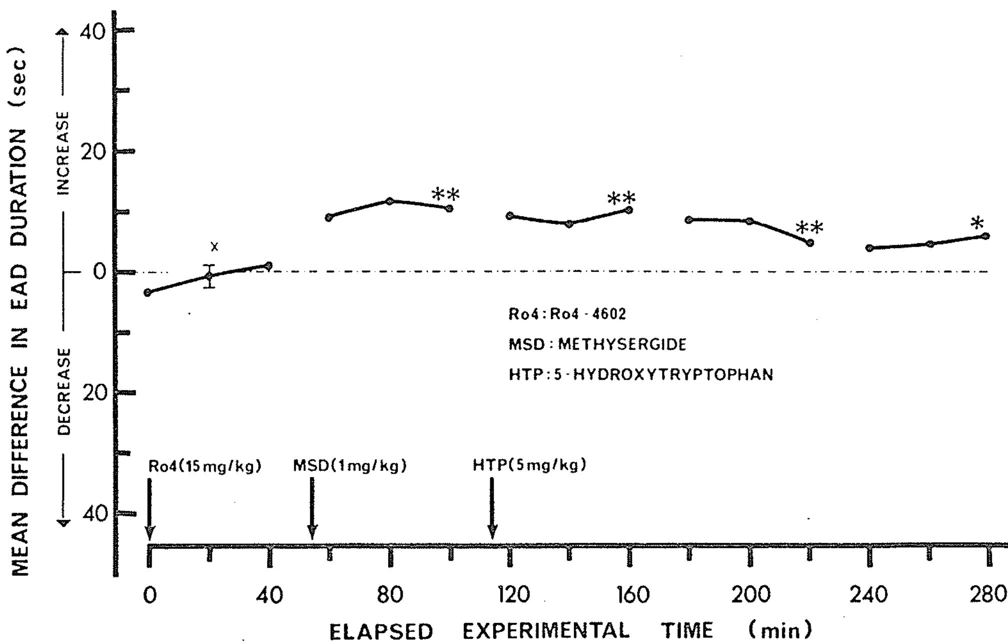


Figure 83.

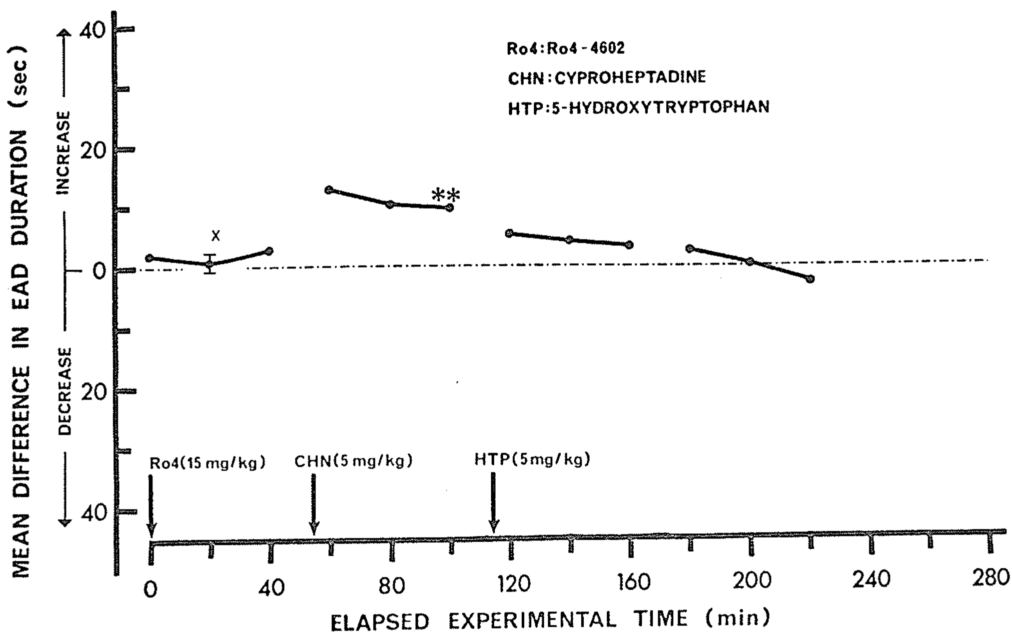


Figure 34.

Figure 85. Antagonism by methysergide (MSD) 1 mg/kg of the action upon EAD duration by d-amphetamine sulfate (d-A) 5 mg/kg. Note lack of any significant change in EAD duration after injection of d-amphetamine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. ** = $P < 0.01$.

Figure 86. Antagonism by cyproheptadine (CHN) 5 mg/kg of the action on EAD duration by d-amphetamine sulfate (d-A) 5 mg/kg. Note lack of any significant change in EAD duration after injection of d-amphetamine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats. ** = $P < 0.01$.

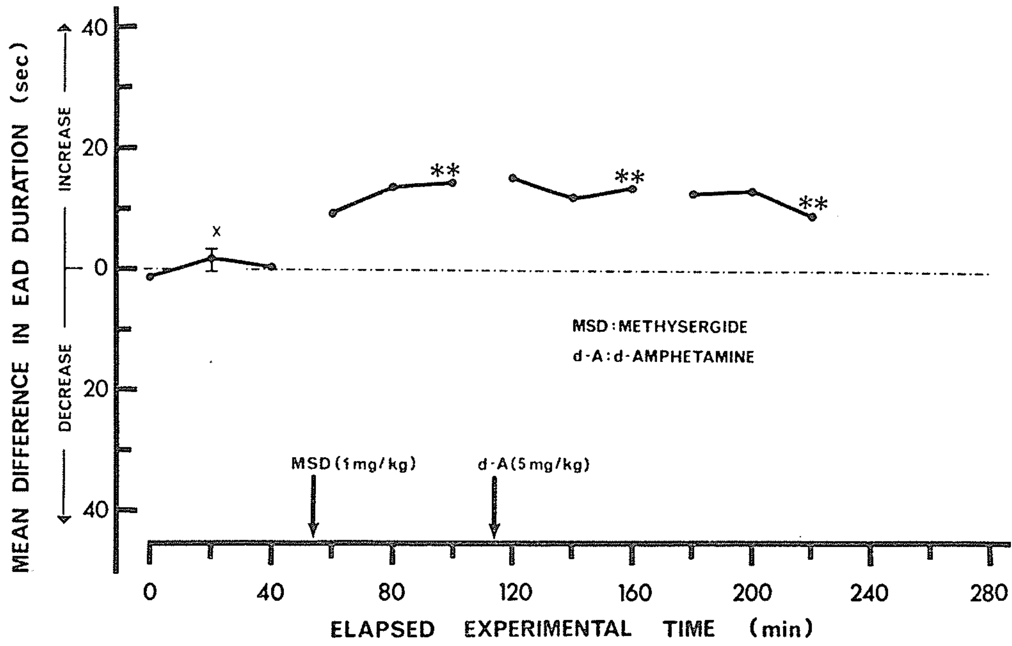


Figure 85

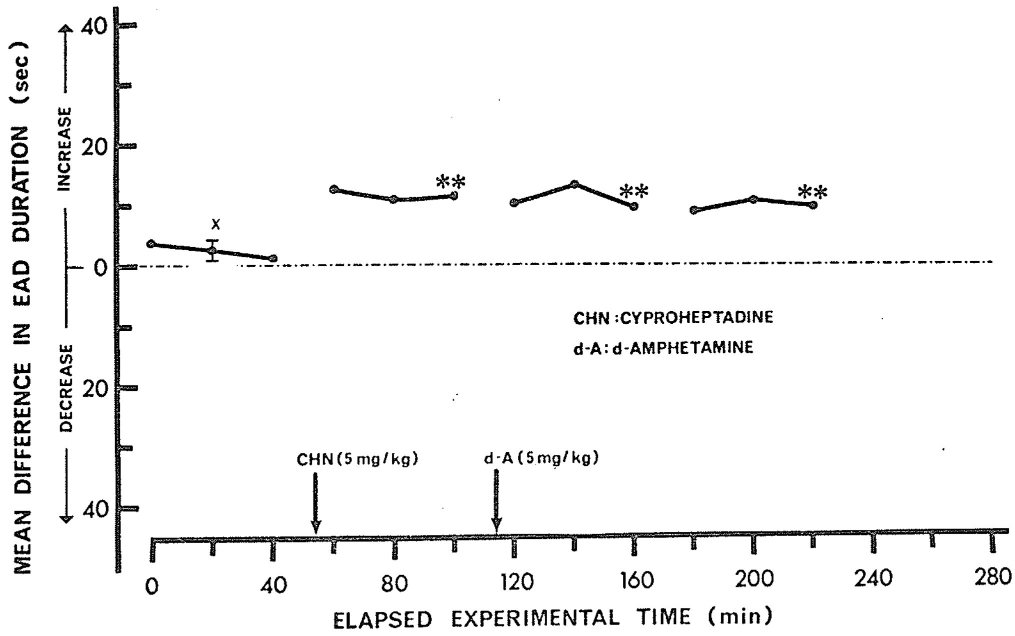


Figure 86.

appear to block the action of d-amphetamine on afterdischarge duration. The action of cyproheptadine is puzzling in that this agent was not able to block more effectively the effect on EAD duration of 5-HTP (Fig. 84).

C. Effect of Phentolamine on the Action of 5-HTP

Five cats were pretreated with the decarboxylase inhibitor Ro 4-4602 60 min before an injection of phentolamine 8 mg/kg (Fig. 87). Neither treatment altered EAD duration. The next injection 60 min later was 5-HTP at a dose of 5 mg/kg, which decreased EAD duration by more than 10 sec. This difference was very significantly ($P < 0.01$) different from control. Afterdischarge durations following the 5-HTP treatment returned gradually to control values, having returned completely 2.5 hours later.

D. Effect of Atropine on the Response to 5-HTP

A group of 5 cats was pretreated with the decarboxylase inhibitor 60 min before injection of atropine 3 mg/kg (Fig. 88). The atropine treatment caused a very significant ($P < 0.01$) increase in EAD duration (more than 20 sec). The injection 60 min later of 5-HTP at a dose of 5 mg/kg did not change the EAD responses increased by atropine. Thus, atropine appears to have antagonized the effect of 5-HTP upon afterdischarge duration.

E. Effect of Methysergide on the EAD Response to Cholinergic or Anticholinergic Drugs

In 4 cats the injection of methysergide 1 mg/kg (Fig. 89) resulted in a very significant ($P < 0.01$) increase in EAD duration (about 12 sec). Fifty min later these animals were injected with methylatropine

Figure 87. Lack of effect by an α-adrenoceptor antagonist, phentolamine hydrochloride (PA) 8 mg/kg, against the action on EAD duration of 5-hydroxy-dl-tryptophan (HTP) 5 mg/kg. The peripheral decarboxylase enzyme was inhibited previously by Ro4-4602 (Ro4) 15 mg/kg. Note lack of effect of phentolamine but the significant decrease in EAD duration after injection of 5-HTP. X refers to the pooled S.E. for all the mean differences calculated from the results on 5 cats.
** = $P < 0.01$.

Figure 88. Antagonism by the antimuscarinic agent atropine sulfate (At) 3 mg/kg of the action upon EAD duration by 5-hydroxy-dl-tryptophan (HTP) 5 mg/kg. Pre-treatment with Ro4-4602 (Ro4) inhibited the peripheral decarboxylase enzyme. Note lack of any significant change in EAD duration after injection of 5-HTP. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

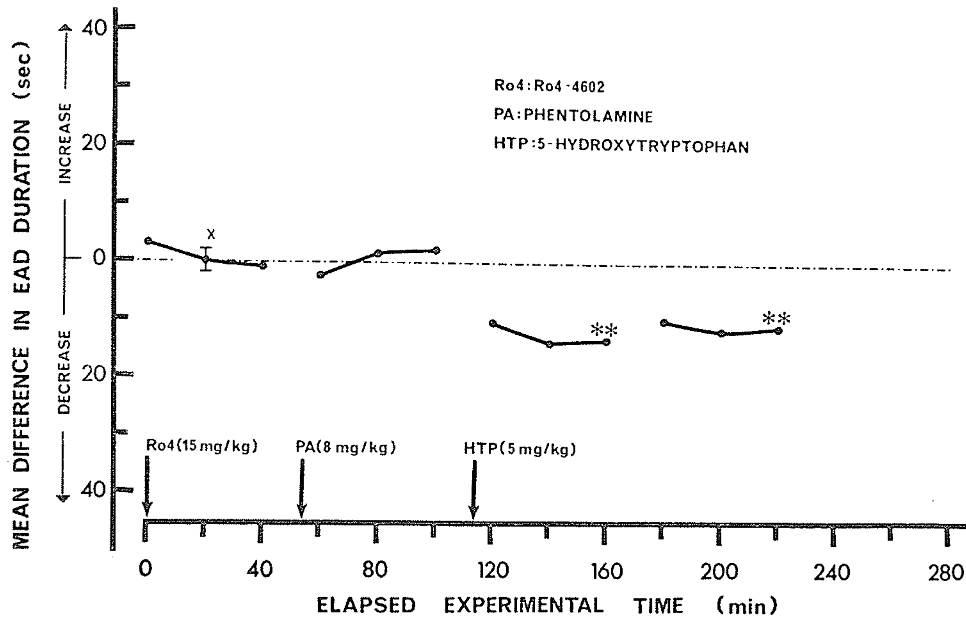


Figure 87.

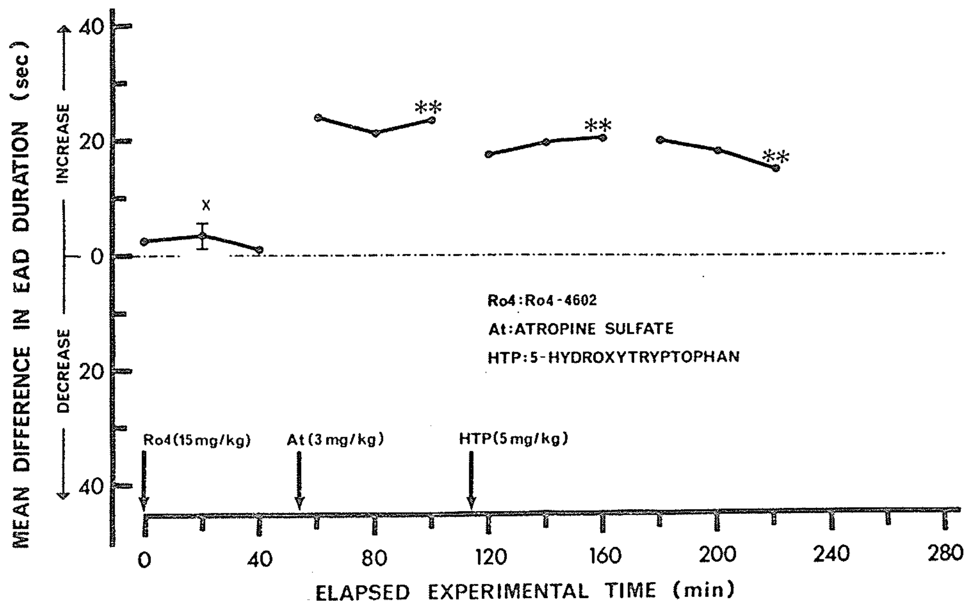


Figure 88.

4 mg/kg to provide protection from the peripheral effects of arecoline 1 mg/kg which was injected 10 min later. This dose of arecoline significantly ($P < 0.05$) reduced EAD duration towards control levels, however it still remained significantly ($P < 0.05$) different from control.

Another group of 4 cats responded to methysergide 1 mg/kg (Fig. 90) with a highly significant ($P < 0.01$) increase in EAD duration (about 10 sec). The injection of atropine 3 mg/kg 60 min later increased afterdischarge duration very significantly ($P < 0.01$) to approximately 25 sec above control levels. The response due to atropine was significantly ($P < 0.05$) different from the response due to methysergide.

F. Effects on EAD Duration of Selective Serotonin Depletion

The use of substances which specifically inhibit cerebral tryptophan hydroxylase causing marked depletion of central serotonin levels is another approach to the study of central serotonergic mechanisms. Two compounds capable of depleting serotonin from storage sites are p-chloroamphetamine (Fuller et al., 1965) and p-chlorophenylalanine (Koe and Weissman, 1967). Cross tolerance is observed in animals treated with p-chloroamphetamine and p-chlorophenylalanine (Knoll and Vizi, 1970).

The results of studies with these agents in a limited number of cats are shown in Table 6. The agents used were p-bromomethamphetamine 7 mg/kg administered to 3 cats, p-chloroamphetamine 5 mg/kg administered to 2 cats, and p-chlorophenylalanine 2 injections of 100 mg/kg 12 hours apart administered to 3 cats. The responses in the first two groups of cats treated with the p-halogenated amphetamine derivatives were measured 6 hours after the injection. The responses obtained from

Figure 89. Lack of antagonism by the serotonin antagonist methysergide (MSD) 1 mg/kg of the action on EAD duration by arecoline hydrochloride (Ar) 1 mg/kg. Methyl atropine nitrate (MAt) 4 mg/kg was administered previous to the injection of arecoline. Note that the responses after the injection of arecoline are significantly different from control and from the increased responses immediately after injection of methysergide. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.
* = $P < 0.05$; ** = $P < 0.01$.

Figure 90. Partial antagonism by the serotonin antagonist methysergide (MSD) 1 mg/kg of the action upon EAD duration in the isolated cortex produced by atropine sulfate (At) 3 mg/kg. Note that there is still a further significant increase in EAD duration after the injection of atropine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats.
** = $P < 0.01$.

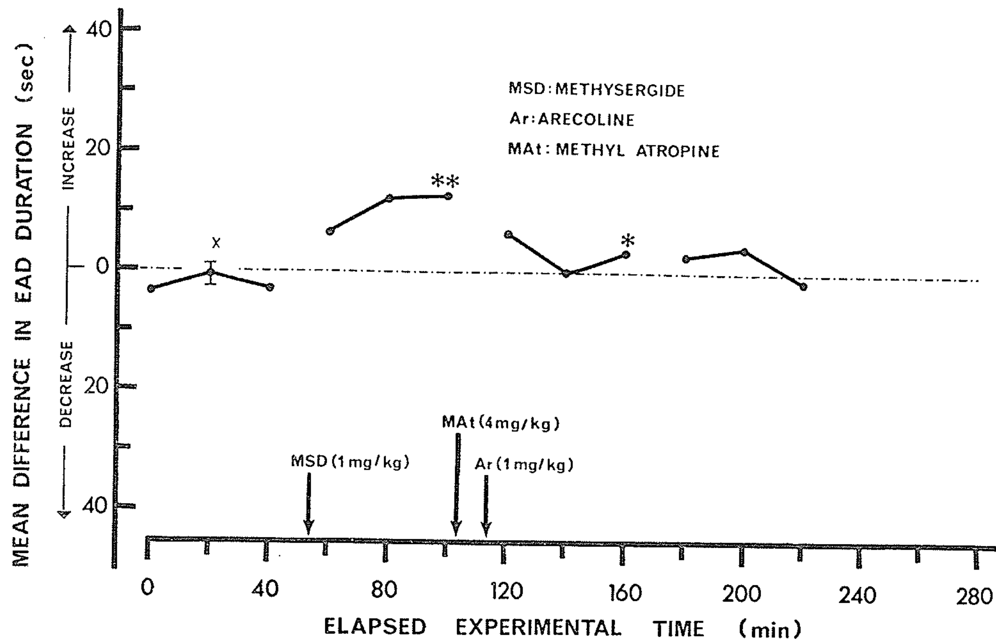


Figure 89.

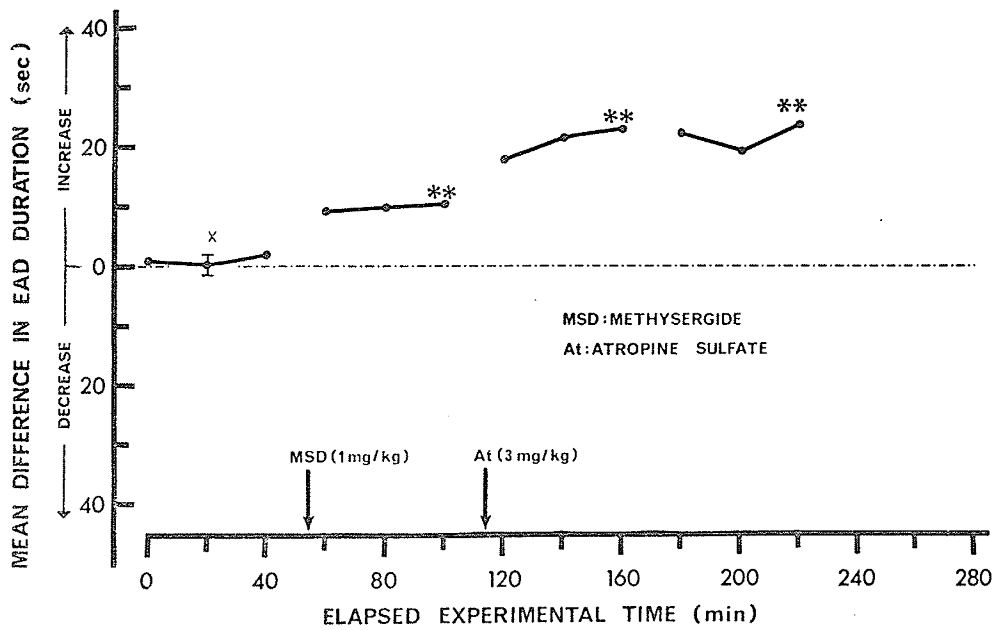


Figure 90.

the p-chlorophenylalanine treated cats were measured 30 hours after the first injection and 18 hours after the second injection.

Table 6. Effects of Treatment with Serotonin Depleting Agents on EAD Duration

SALINE CONTROL			TREATMENT - 1
Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	Mean Difference in EAD Duration (sec) ± S.E. (N)
-0.3 ± 2.7 (3)	p-Bromo-MA	7 mg/kg	9.6 ± 3.1 (3)**
-0.9 ± 4.3 (2)	p-Chloro-A	5 mg/kg	1.1 ± 3.3 (2)
1.5 ± 3.5 (3)	p-Chloro-PA	200 mg/kg	16.6 ± 5.8 (3)**

N = number of animals
** = $P < 0.01$
A = amphetamine, MA = methamphetamine, PA = phenylalanine

Thus, all treatments except p-chloroamphetamine produced highly significant ($P < 0.01$) increases in EAD duration. The side effects in the first two groups of cats treated with the halogenated amphetamines were similar and consisted for the most part in a restless pacing about the cage and apparently seeking attention. Treatment with p-chlorophenylalanine resulted in similar behaviour a few hours after the initial injection, but at the time of testing the cats appeared very restless. Immediately prior, and just after testing sessions all the cats appeared to be quite lethargic.

Another agent shown to deplete central 5-HT levels was also tried. This was α-propyldopacetamide (Corrodi *et al.*, 1967). A dose of 200 mg/kg was injected intraperitoneally and the animal returned to its cage. Approximately 6 hours later it was observed that this animal

was quite lethargic and subject to periodic tremors. This condition deteriorated and 2 hours later this cat was killed. Another cat was injected with α-propyldopacetamide 50 mg/kg, but testing 6, 12 and 24 hours after injection revealed no change in EAD duration. Further experiments with this agent were not planned.

Each of the cats treated with p-chlorophenylalanine was further tested after the above data had been obtained. On cat received Ro 4-4602 (15 mg/kg) and was followed 60 min later by 5-HTP (5 mg/kg), which decreased EAD duration by 12 sec. Another cat received atropine 3 mg/kg which caused a further increase of 20 sec in EAD duration. The third cat was treated with d-amphetamine 5 mg/kg which did not alter EAD duration during 2 hours of testing. A second dose of amphetamine 5 mg/kg, two hours after the first, caused no significant change in EAD duration.

G. Resume

The results presented in this section strongly suggest that 5-HT causes significant reductions in EAD duration (Fig. 80). Serotonin antagonists on their own cause significant increases in EAD duration (Fig. 81; 82). The action of 5-HT on EAD duration is blocked effectively by methysergide (Fig. 83) but not by cyproheptadine (Fig. 84). Both of these antagonists are able to block the action of d-amphetamine upon EAD duration (Fig. 85; 86). Pretreatment with an adrenergic blocking agent did not antagonize the effect of serotonin (Fig. 87). Serotonin action on EAD duration was blocked by atropine (Fig. 88), while atropine potentiated methysergide (Fig. 90). Cholinergic drugs were not antagonized by methysergide (Fig. 89).

H. Discussion

Effects of 5-Hydroxytryptamine on EAD Duration Elicited in the Isolated Cortex

The results of studies with drugs which stimulate or antagonize 5-hydroxytryptamine (5-HT, serotonin) receptors suggest that this system of pharmacologically reactive structures has a significant role in the modulation of EAD duration. Since serotonin itself does not enter into the brain from the systemic circulation, it was necessary to use the metabolic precursor 5-hydroxytryptophane or 5-HTP, as this form distributes into the CNS and is decarboxylated to 5-HT (Udenfriend *et al*., 1957). The utilization of an extracerebral inhibitor of DOPA-decarboxylase appears to be a valid procedure for enhancing the entry of 5-HTP into the brain. It has been reported that 5-HTP accumulates rapidly (< 7.5 min) in the brain after injection of the inhibitor Ro 4-4602, and that the increased 5-HT levels persists up to 4 hours (Carlsson and Lindqvist, 1970). This treatment does not appear to modify EAD duration on its own and permits the use of a considerably lower dose of 5-HTP.

Administration of 5-HTP with presumably subsequent cerebral conversion to 5-HT resulted in significant reduction of EAD duration in my experiments (Fig. 80). On their own, 5-HTP (Fig. 79) and salts of serotonin (Table 4) did not significantly alter EAD duration. The decrease in EAD duration produced by increasing cerebral levels of serotonin is blocked by the 5-hydroxytryptamine antagonists methysergide (Fig. 83) and cyproheptadine (Fig. 84), whereas the adrenergic blocking agent phentolamine (Fig. 87) is incapable of antagonizing this action of 5-HTP on EAD duration. Even if these results are far

from conclusive they do suggest that there are serotonin type receptors present in the isolated cortical slab capable of decreasing the spread of epileptiform potentials. This observed activity of 5-HT in my experiments agrees with findings reported by Prockop et al.(1959) for electroshock studies in which increasing brain levels of 5-HT by treatment with monoamine oxidase inhibitors were correlated with protection from convulsions. Lessin and Parkes (1959) found that 5-HTP in the presence of a monoamine oxidase inhibitor protected mice against seizures better than did L-DOPA under the similar conditions. Schlesinger (1968) using a special strain of mice responding to audio stimulation showed that the peak increase in cerebral 5-HT levels due to administration of 5-HTP corresponded to the lowest susceptibility to audiogenic seizures.

It is unlikely that cardiovascular effects of 5-HT are involved in the action upon EAD duration of 5-HT. Longo (1962) has reported a slight bradycardia with no decrease in blood pressure at a time when maximal changes in electrocortical potentials were produced by 5-HTP at a dose of 60 mg/kg. Rech and Domino (1960) found the hypertensive effect of serotonin was the least when compared to catecholamines. These authors observed changes in the electrical activity of the isolated cortex at this dose of serotonin (20-60 μg/kg).

Gangloff and Monnier (1957a) have shown in unanaesthetized rabbits that serotonin decreased cortical excitability by increasing the threshold for eliciting cortical afterdischarges by direct stimulation. Monnier and Tissot (1958) compared low (15 mg/kg) and high (100 mg/kg) doses of 5-HTP and found that the low doses decreased elec-

troencephalographic activity whereas the large doses were excitatory. Rech and Domino (1960) studied the effects of serotonin on the electrical potentials of isolated cortex. These authors reported serotonin depressed strychnine or d-tubocurarine spiking evoked in the cortical slab. Thus, there appears to be some basis for serotonin being able to decrease epileptiform potentials in the cerebral cortex.

As methysergide (Fig. 83) and cyproheptadine (Fig. 83) are capable of altering EAD duration on the isolated slab it becomes necessary to assume that there is normally present a certain degree of tone mediated by serotonergic structures. If this were not so, these blockers, and others (Table 5) should be devoid of any activity when administered alone.

As the adrenergic blocking agents used (phenoxybenzamine and phentolamine) were not completely effective in blocking the actions of amphetamine (Fig. 50; 52), whereas methysergide (Fig. 85) and cyproheptadine (Fig. 85) blocked d-amphetamine completely, it is tempting to suggest that the observed effects of the adrenergic drugs to decrease EAD duration might be exerted through serotonin type receptors.

Vane (1960) has discussed the possibility that amphetamine might be acting on serotonin receptors in the brain, a situation analogous to its actions in the periphery. This conclusion was based on observations that reflexes elicited by 5-HT were also elicited by amphetamine and that there was no relationship to noradrenaline-releasing actions. In addition, it was found that all sympathomimetic agents with central nervous system stimulant properties were all also able to stimulate serotonin receptors with a similar order of potency. Such a

mechanism might explain the central effects of amphetamine in animals and patients treated with reserpine and having depletion of brain catecholamines (Goodman and Gilman, 1970). Vane (1960) speculated that the psychotomimetic effects of large doses of amphetamine might conceivably be due to its acting on 5-hydroxytryptamine receptors.

Further investigation revealed that atropine completely antagonized the action of 5-HT (Fig. 87). Furthermore, methysergide did not block the action of a muscarinic drug, arecoline (Fig. 89), or antagonize the action of atropine (Fig. 90). Since blockade of the cholinoceptive system influences the result of stimulation of the serotonergic system, whereas blockade of the serotonergic structures is without significant effect upon stimulation of the cholinergic system, it seems reasonable to assume that the serotonergic system is subservient to the cholinergic one. Thus, it would appear that in the pharmacological control of EAD duration a serotonergic mechanism is "driving" a cholinergic neuron, which appears to be in direct connection with the effector site for generating the EAD in the cerebral cortex.

The most likely arrangement of the serotonin-type synapses as suggested by the results in this thesis is in a series system of tryptaminergic and cholinergic synapses respectively. Blockade of the serotonergic or cholinergic system therefore increases EAD duration, whereas selective stimulation of these mechanisms results in inhibitory effects against afterdischarges. The placement of the adrenoceptive structure poses some problems, but the evidence presented in this thesis appears to favour an adrenergic terminal (probably post-synaptic) which impinges on the serotonergic structure.

There are many problems encountered when attempting to elucidate the functional role of several mediators for a given population of neurons as there is much conflicting evidence in the literature. The experiments with selective depleting agents of monoamines (see XI. A.d; Table 6) show that increases in EAD duration result following treatment. However, as these results are preliminary and most of these agents are relatively recent additions as investigative tools it is perhaps premature to speculate further on the significance of these results. Moreover, without exception these agents cause rather profound central and peripheral side-effects at the time the testing was carried out. It is strange that of the agonist types studied, i.e. cholinergic, adrenergic and serotonergic, that there were no reciprocal actions of at least one type upon EAD duration.

Some workers have reported specific localization and uptake of 5-HT by central neurons (Aghajanian and Bloom, 1967). However, there is also evidence suggesting that 5-HT may also be accumulated by catecholaminergic neurons (Fuxe and Ungerstedt, 1967; Sashkan and Snyder, 1970). This interplay is understandable in view of the close structural similarities and metabolic handling of catecholamines and 5-hydroxytryptamine.

There is some agreement that amphetamine produces many of its pharmacological effects in the brain by releasing catecholamines from central stores (Hanson, 1966; Weissman _et al._, 1966). The depletion of brain levels of 5-HT by the p-chloro-analogues of amphetamine (Pletscher _et al._, 1968) prompted investigation of the effects of d-amphetamine on 5-HT metabolism. Earlier work (Laverty and Sharman, 1965) failed to demonstrate any depletion of cerebral 5-HT due to amphetamine, however,

more recent work has revealed that although tissue levels of this biogenic amine remain unchanged, amphetamine increases the metabolic turnover rate of brain 5-HT (Reid, 1970).

Beani *et al.* (1968) have shown that amphetamine can cause the release of ACh in the brain. This would help to explain in part why anticholinergics antagonized the action of amphetamine on EAD duration. Therefore, it is likely that amphetamine can act not only upon adrenergic neurons in the cortex, but also upon neurons having 5-HT as their transmitter. Beleslin and Myers (1970) have studied the release of 5-HT and ACh from the colliculi of unanaesthetized monkeys and found that more superficial sites released ACh, whereas deeper levels released predominantly 5-HT. A similar arrangement might exist in the cortex but this assumption can only be based on pure speculation at present.

Monoamine oxidase inhibitors have been shown to have anticonvulsant effects (Prockop *et al.*, 1959) in addition to their capability of counteracting the increased tendency towards seizures after reserpine treatment (Chen and Bohner, 1956; Lessin and Parkes, 1959; Lewis, 1963). However, elevation of brain 5-HT by direct or indirect means did not offer protection against metrazol seizures in one study (Bonnycastle *et al.*, 1957), but was effective in another study (Lessin and Parkes, 1959). Some workers (Schmidt and Mathies, 1962) have prevented the convulsant effects of reserpine treatment by intracerebral injection of serotonin or noradrenaline, whereas others (Prockop *et al.*, 1959) did not observe any effects after such treatment.

The increase in EAD duration produced by the serotonin antagonists in my experiments suggests that interruption of a serotonergic net-

work in the cortex results in removal of one of the mechanisms controlling EAD duration. However, it is necessary to be cautious in interpreting these results in a pharmacological context, because it is known (Gyermek, 1966; Burger, 1970; Goodman and Gilman, 1970) that the serotonin receptor blocking drugs lack specificity of action, and effect practically all other presently known receptor systems, e.g. acetylcholine, noradrenaline, and histamine. Reid (1970) and Schubert *et al.*, (1970) have reported that many different types of drugs influence serotonin metabolism in 5-HT neurons. This may be due in part to the vague concept most authors have of serotonin receptors and the suggestion that there might be more than one type of 5-HT receptor (Gyermek, 1966). The observation that LSD mimics rather than antagonizes 5-HT when tested on some tissues (Gyermek, 1966) further complicates the issue. Moreover some drugs, e.g. chlorpromazine, known more for their tranquillizing properties are also known to antagonize peripheral actions of 5-HT (Goodman and Gilman, 1970).

Additional controversy with respect to the central role of 5-HT has arisen from the results of studies with agents known to antagonize the effects of 5-HT on peripheral organs. This was based on the assumption that LSD, a potent peripheral serotonin antagonist, might be acting in the CNS in a similar manner to produce its marked psychotomimetic effects (Gaddum, 1957). This relationship has not been conclusively demonstrated, although LSD has been shown to antagonize a specific excitant action of 5-HT on brain stem neurons (Boakes *et al.*, 1970). Unfortunately, a number of derivatives of LSD, e.g. 2-bromo-LSD or BOL which are also peripheral and central (Boakes *et al.*, 1970) anta-

gonists of 5-HT, lack psychotomimetic effects (Isbell _et al_., 1959).

Questions regarding the role of brain 5-HT, in experimental seizures have been discussed in a recent monograph on serotonin (Garattini and Valzelli, 1965). Unfortunately, it has not yet entirely been resolved what is the exact relation between changes in serotonin levels in specific areas of the brain and epileptiform activity. Moreover, it is not clear whether the changes result from the experimental procedures or are causally related. The results presented in this thesis support an anticonvulsant role at the cortical level for 5-HT, in association with cholinergic structures. Serotonin has been administered to epileptic patients and shown to antagonize abnormal paroxysmal discharges (Canali and Grisoni, 1957). Electroshock appears to raise brain levels of serotonin, but this is probably due to resultant stress, as it also occurs in the presence of anticonvulsant drugs (Garattini and Valzelli, 1965). Rather specific increases in cerebral 5-HT levels have been found to result from the administration of anticonvulsant drugs (Bonnycastle _et al_., 1957; 1962), and reserpine treatment is known to antagonize the effects of anticonvulsants (Lewis, 1963). However, others (Prockop _et al_., 1959) have not been able to duplicate these findings.

Recent studies by de la Torre _et al_. (1970) using chemoshock seizures indicate that serotonin exerts a protective role against experimental seizures induced by metrazol. The anticonvulsant effects achieved by increasing cerebral serotonin were greater than that seen after treatment with phenobarbital 6 mg/kg. Selective cerebral depletion of serotonin produced by p-chlorophenylalanine was shown to exacer-

bate metrazol provoked seizures (Alexander and Kopeloff, 1970) and decreased thresholds for tonic extensor seizures (Koe and Weissman, 1966). In another study selective depletion of cerebral noradrenaline (de la Torre and Mullan, 1970) achieved with α-methyltyrosine showed that decreased cerebral catecholamines and apparently decreased adrenergic function, did not significantly alter seizure thresholds, as did alteration of serotonin levels in the brain. This is in contrast to the findings of Schlesinger (1968) that decreased cerebral levels of noradrenaline with α-methyltyrosine resulted in increased susceptibility to audiogenic seizures in special strains of mice.

Administration of 5-HTP alone does not have much of a protective effect against chemoshock and extensor seizures (Kobinger, 1958; Chen _et al._, 1968) except if the 5-HTP is combined with a monoamine oxidase inhibitor, in which case there is a marked elevation of thresholds. This enhancement of the protection offered by 5-HT does not permit a clear conclusion since the inhibitor is also affecting cerebral catecholamine levels and under the same conditions (monoamine oxidase inhibition) L-DOPA will also protect against seizures (Kobinger, 1958). Other investigators (Pfeifer and Galambos, 1967; 1967a) have studied brain monoamine levels and convulsions and propose that noradrenaline has a more important role in the change of susceptibility to seizures than do L-DOPA or 5-HT. Maynert (1969) has reviewed the relationship of brain serotonin brain levels and seizure thresholds and it is apparent that there are still some gaps in our knowledge of the neurohumoural factors involved in seizure activity. Thus, it appears that serotonin has some specificity of action and plays an intermediate (compared to adrenergic and cholinergic activity) role in

cortical EAD modulation. The blockade of action of amphetamine by methysergide, although on first inspection an apparent non-specific effect, can be considered as a specific effect in other respects. This observation suggests, not convincingly, that the adrenoceptive structure might be collateral to and in synaptic contact with tryptaminergic neurons. Thus, in this arrangement amphetamine would in fact be acting along an alternating network of synapses and through serotonergic structures. Since both atropine and methysergide block the action of amphetamine, the adrenoceptive structure is apparently further removed from the effector site than are the succeeding serotonergic and cholinergic neurohumoural linkages in this proposed network. Therefore, modulation by catecholamines of afterdischarge duration is through serotonin receptors, and, in turn, modulation by serotonin is through cholinergic receptors. In this way, both catecholamines and 5-hydroxytryptamine apparently modulate the action of acetylcholine in the maintenance and spread of the EAD.

The possibility for the existence of mixed adrenergic and serotonergic mechanisms to explain the actions of drug acting in the CNS has been outlined in the proceedings of a recent symposium by Jacob (1969). Takagi _et al._ (1968) used tetrabenazine pretreatment to deplete central stores of monoamines before studying the EEG response to L-DOPA and 5-HTP. These authors interpreted their findings as suggesting that dopamine and noradrenaline ("a catecholaminergic system") have a facilitatory role while serotonin has an inhibitory role in the EEG activating system. Steiner and Himwich (1962) reported earlier that cholinergic mechanisms are also involved in the EEG alerting system. Thus, it is not difficult to perceive that many problems will

arise concerning the explanation of possible interactions between postulated neurohumours, even when limiting the discussion to dopaminergic, noradrenergic, serotonergic, and cholinergic systems. Jacob (1969) has concluded that the complexity of the mechanisms of drug-induced central actions just on the basis of considering two mediators, e.g. serotonin and catecholamines, provides for many pitfalls during interpretation of the results. The present author has not escaped this tradition.

The L-amino acid decarboxylase inhibitor Ro 4-4602, which was used to facilitate the entry of 5-HTP into the brain has been shown to increase the level of dopamine in neurons (Constantinidis _et al._, 1968; Butcher and Engel, 1969). Apparently when L-DOPA is administered to animals treated with Ro 4-4602 it accumulates as a result of displacement of dopamine in 5-HT neurons (Butcher _et al._, 1970). There was no loading with L-DOPA in the experiments in this thesis in which Ro 4-4602 treatment preceded administration of 5-HTP. This is not to say that some endogeneous L-DOPA never accumulated in other neurons in the isolated slab. Since there are fewer neurons and an apparent increase in sensitivity to other pharmacological agents there might have been significant accumulation of dopamine to produce effects on EAD duration. On its own L-DOPA decreased EAD duration significantly (Table 3). Studies on brain slices (Ng _et al._, 1970) have demonstrated that L-DOPA markedly increased the efflux of tritiated serotonin, even after selective destruction of catecholamine containing nerve terminals with 6-hydroxydopamine.

The reason for consideration of the above possibility of

interference by one transmitter agent with the metabolism of another amine is that Faiman _et al_. (1971), have made a careful study of the role of monoamines in convulsions induced by hyperbaric oxygen. These authors discovered that the absolute levels of noradrenaline or serotonin were not the important factors in altering seizure susceptibility. Their investigation showed that there were greater increases in brain dopamine after treatment with monoamine oxidase inhibitors or inhibitors of dopamine β-hydroxylase and that this change in dopamine corresponded with the period of greatest protection against seizures. In accord with these results, Bennet (1970) has demonstrated in the frog that L-DOPA inhibited convulsive movements due to strychnine. Administration of L-DOPA was less effective against convulsions induced by metrazol.

A convulsant action for serotonin has also been reported (Tedeschi _et al_., 1959). These authors have used tryptamine to stimulate central 5-HT neurons. Tryptamine is a substance closely related to 5-HT and known to activate serotonin receptors in the periphery. Since these investigators found that LSD antagonized tryptamine induced convulsions, apparently due to actions on serotonergic neurons, they reasoned that tryptamine was a useful substance for studying the role of 5-HT in the CNS. It has been reported that high doses of 5-HTP cause CNS excitation (Costa and Rinaldi, 1958; Monnier and Tissot, 1958). However, the significance of these findings regarding epileptiform phenomena does not appear to be tenuous since Tedeschi and his co-workers (1959) also found that classical anticonvulsants did not antagonize the convulsant action of tryptamine. Furthermore, the fact that monoamine oxidase inhibitors potentiated these effects of tryptamine does not

substantiate or clarify a specifically convulsant role for serotonin.

Therefore, on the basis of the results in this thesis it appears that serotonin has prominent anticonvulsant activity at the cortical level, which is apparently mediated through cholinergic synapses. If adrenergic structures have a role at this level, it cannot be clearly outlined. On the basis of the experiments in this thesis catecholamines could be modulating in some fashion the activity of the serotonin neurons. These complex relationships of neurotransmitters at the cortical level cannot, unhappily, be considered in other than the most speculative terms at the present moment.

XIII. ANTI-EPILEPTIC AGENTS

A. Action of Anticonvulsant Drugs on EAD Duration

It has been pointed out (Toman and Goodman, 1948) that the majority of drugs effective in treating convulsive disorders bear some resemblance in their structure to that of phenobarbital. This suggests that there might be a common mechanism or receptive structure at which these agents exert their well-known anticonvulsant effects. As the previous results have shown, the EAD elicited in the chronically neuronally isolated slab of cat's cerebral cortex shows a comparitively preferred susceptibility in terms of changes in its duration, when treated with relatively specific types of pharmacological agents, e.g. muscarinic drugs decrease EAD duration, antimuscarinic drugs increase EAD duration, and nicotinic drugs have no significant effect.

Therefore, it was decided to study some types of anticonvulsant drugs to determine whether there was any preferred susceptibility of EAD duration to particular classes of these agents, e.g. anti-*grand mal* or anti-*petit mal* drugs. A preference for one type of agent could prove useful in helping to investigate possible pharmacological mechanisms involved in cortical epileptiform activity and whether there is any resemblance or relationship to the effects of the pharmacological agents studied in the previous sections (see X, XI, XII). As discussed in the preceding sections there is strong evidence for pharmacological mechanisms in the spread and maintenance of seizure discharges.

a. Effect of some anticonvulsant drugs on cortical EAD duration

Since diphenylhydantoin sodium is a relatively insoluble substance it was necessary to solubilize it in a special vehicle (see VIII.O.).

The effects of increasing amounts of this vehicle on EAD duration were tested (Fig. 16). Injecting volumes of this vehicle of 2.5 cc/kg was observed to be deleterious as this amount caused significant reductions in EAD duration. Injection volumes of this DPH vehicle containing the required dose of DPH usually administered were of the order of 0.5 cc/kg. Not only did this amount of vehicle have negligible effects on EAD duration, but it also allowed a wide margin for any cumulation of the components of the vehicle due to subsequent doses.

Increasing doses of diphenylhydantoin (DPH) were tested in 8 cats (Fig. 91). The first injection in this study consisted of DPH vehicle 0.5 cc/kg which produced no change in EAD duration. The next injection 60 min later was DPH 5 mg/kg which caused a highly significant ($P < 0.01$) decrease in EAD duration (approximately 10 sec). The second injection of DPH 5 mg/kg 60 min after the first injection produced a further decrease in EAD duration. The total decrease from control was now more than 20 sec and was highly significant ($P < 0.01$). The final injection of DPH 5 mg/kg resulted in a further decrease in EAD duration, the total decrease being 35 sec and highly significant ($P < 0.01$). Thus, the duration of the afterdischarge was effectively decreased by DPH. It would seem that there is cumulation of drug following each injection and that the decrease in EAD duration is proportional to the cumulated dose, i.e. 10 mg/kg and 15 mg/kg. These decreases in duration persisted for at least 3 hours of testing after the drug injection. In 2 cats EAD duration had not returned to control values 18 hours later, although it was gradually returning at this time. Apart from this effect on duration, DPH did not appreciably alter the afterdischarge pattern or threshold.

Figure 91. Effect of cumulated doses of diphenylhydantoin sodium (DPH) 5 mg/kg upon EAD duration in the isolated cortex. Note that drug-free vehicle (VEH), less than 0.5 cc/kg, does not change EAD duration. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 8 cats.
** = $P < 0.01$.

Figure 92. Effect of cumulated doses of phenobarbital sodium (PHB) upon EAD duration in the isolated cortex. Note the immediate decrease in EAD duration following the first injection. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

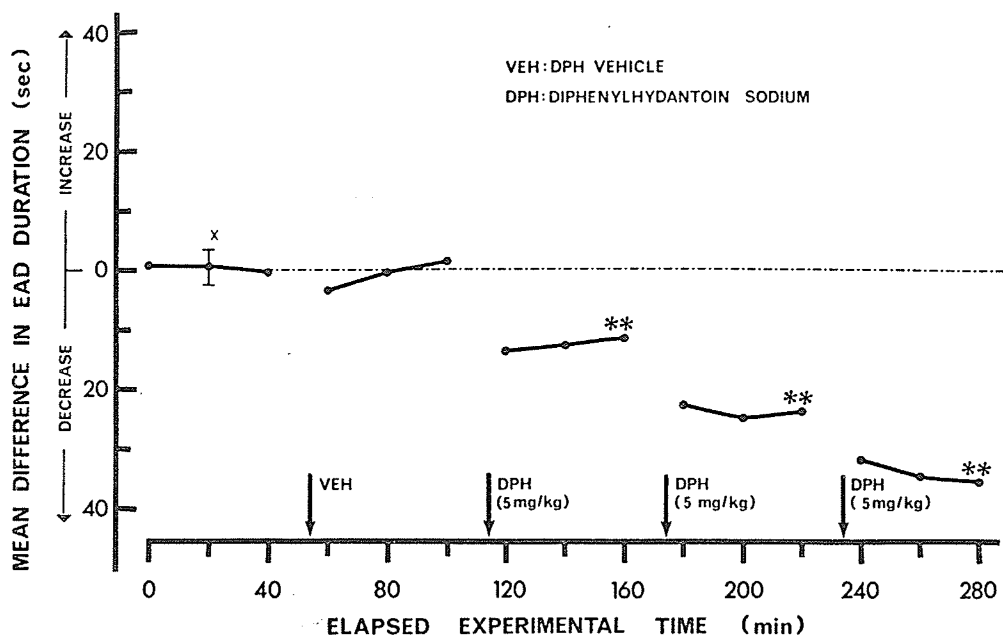


Figure 91.

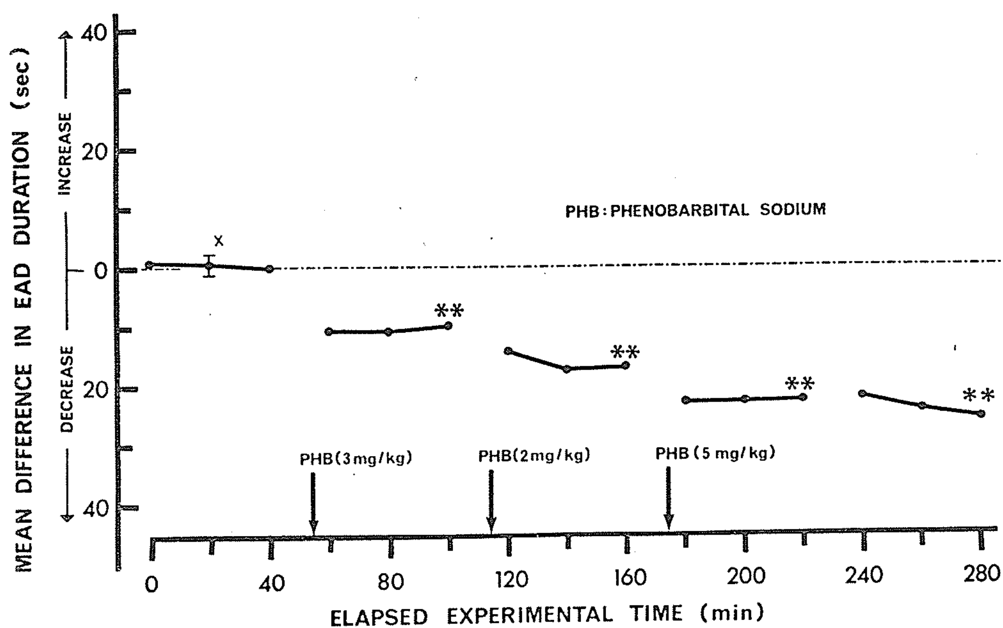


Figure 92.

No effects were evident on the ECoG recorded over intact cortex of DPH-treated animals at any of the dose levels tested. With doses of DPH 15 mg/kg noticeable behavioural changes were evident a short time after injection. The animals were lethargic and depressed, staring with a fixed gaze. They remained awake and reasonably alert. The smaller doses had no such effects.

Phenobarbital sodium was studied in a group of 5 cats (Fig. 92). Injection of phenobarbital 3 mg/kg produced an immediate decrease in EAD duration which was highly significant ($P < 0.01$). Injection of another dose of phenobarbital 2 mg/kg, 60 min later produced a further decrease in EAD duration, the total mean change from control being a highly significant ($P < 0.01$) decrease in EAD duration of almost 20 sec. The final injection in this series was phenobarbital 5 mg/kg which reduced EAD duration very significantly ($P < 0.01$) to a total of about 25 sec below control values. It appears that there was cumulation of drug following this series of injections, and that the final dose was probably near 10 mg/kg. The animals appeared mildly sedated, but were able to walk around the cage at all dose levels tested.

Pentobarbital sodium was administered to a group of 6 cats (Fig. 93). Two separate injections of pentobarbital 5 mg/kg 60 min apart did not cause any significant change in EAD duration. The third injection of pentobarbital which was 20 mg/kg produced a brief and transient decrease in EAD duration of about 8 sec, which was significantly ($P < 0.05$) different from control. Afterdischarge duration returned to control values soon after the injection. The most striking changes produced by pentobarbital were on the consciousness and loco-

Figure 93. Lack of effect of pentobarbital sodium (PTB) upon EAD duration in the isolated cortex. Note slight but significant decrease after injection of pentobarbital 20 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats. * = $P < 0.05$.

Figure 94. Effect of sodium bromide (NaBr) upon EAD duration in the isolated cortex. Note progressive decrease in EAD duration after injection of bromide. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. * = $P < 0.05$; ** = $P < 0.01$.

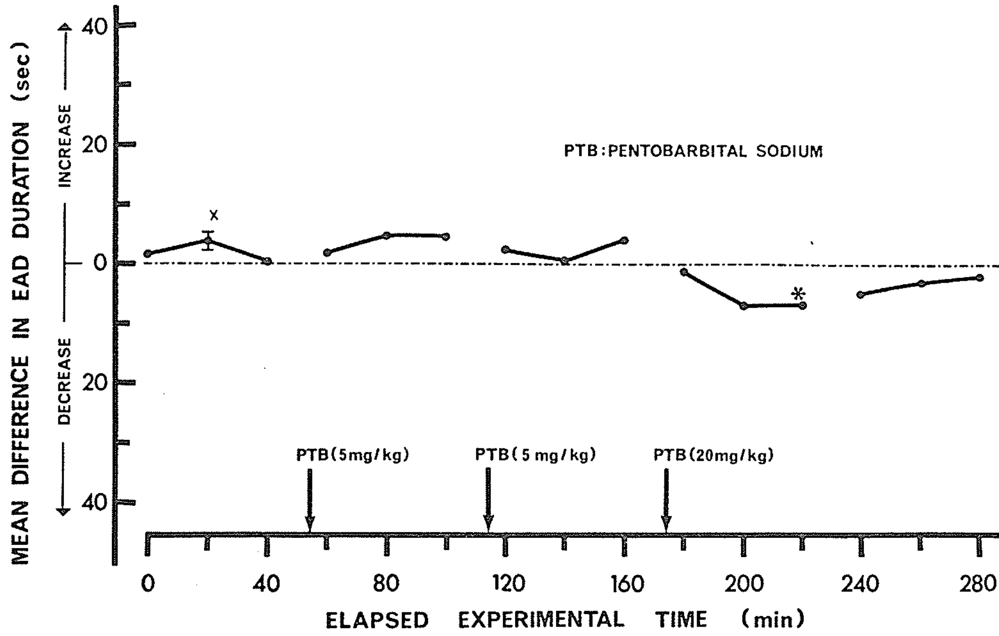


Figure 93.

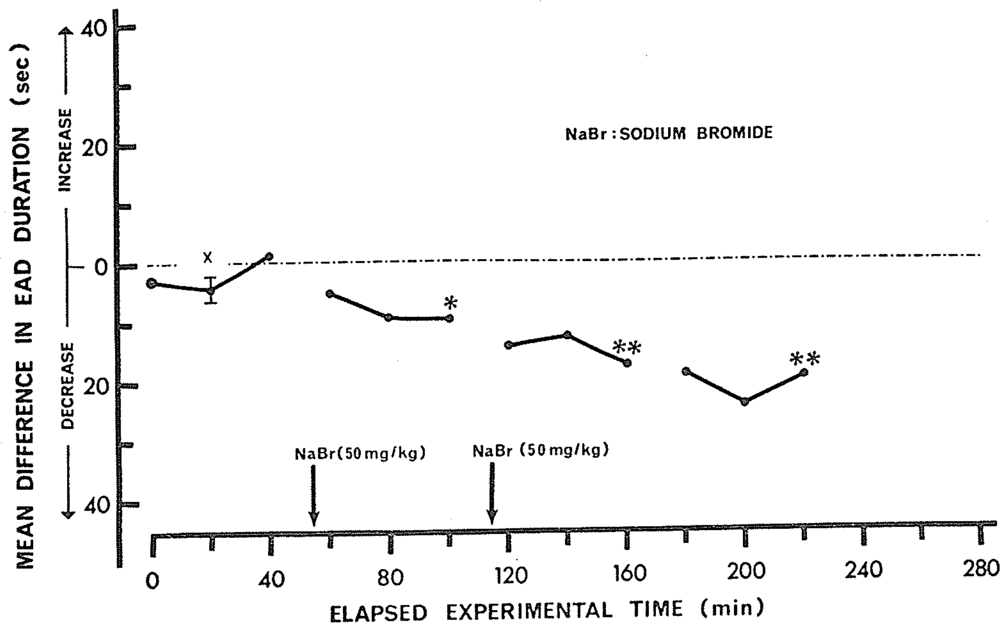


Figure 94.

motor ability of the animals. The first injection of 5 mg/kg produced a pronounced ataxia and drowsiness. After the next injection of pentobarbital 5 mg/kg the animals were barely awake. Following the dose of 20 mg/kg, the cats were virtually anaesthetized.

Sodium bromide was administered to a group of 4 cats (Fig. 94). The first dose of bromide 50 mg/kg produced a small (5 sec), but significant ($P < 0.05$) decrease in EAD duration. The second injection of bromide 60 min later was also 50 mg/kg and it reduced EAD further to about 12 sec below control, which was a highly significant ($P < 0.01$) difference. EAD duration remained approximately at this level for almost 2 hours, after which it began to return slowly to control values. The cats appeared to be drowsy and remained in a sitting position. No significant changes were observed in the spontaneous ECoG.

Two agents known to be effective against petit mal seizures were investigated in two groups of 6 cats each.

Treatment with trimethadione injected at a dose of 50 mg/kg (Fig.95) resulted in no change in EAD duration. The second injection of trimethadione was also 50 mg/kg and this also did not alter EAD duration. The last dose of trimethadione was 100 mg/kg, which did not cause any change in afterdischarge duration. Although there was no change observed for almost 3 hours following the last injection, there was probably cumulation of the drug following each injection. After the second injection (cumulated dose probably 100 mg/kg) the cats appeared slightly sedated. After the last injection all the cats were moderately sedated.

In another set of 6 experiments ethosuximide at a dose of 50 mg/kg (Fig. 96) was administered at 60 min intervals on 3 occasions. This treatment did not alter afterdischarge duration after each injection

Figure 95. Lack of effect of an anti _petit mal_ agent trimethadione (TMD) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.

Figure 96. Lack of effect of an anti _petit mal_ agent ethosuximide (ES) upon EAD duration in the isolated cortex. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.

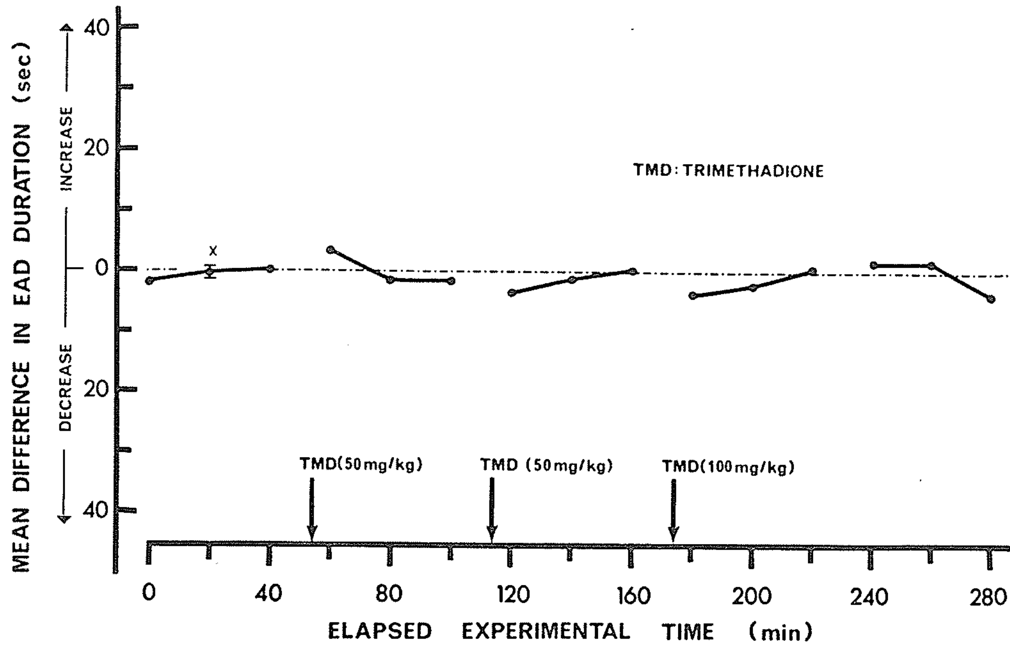


Figure 95.

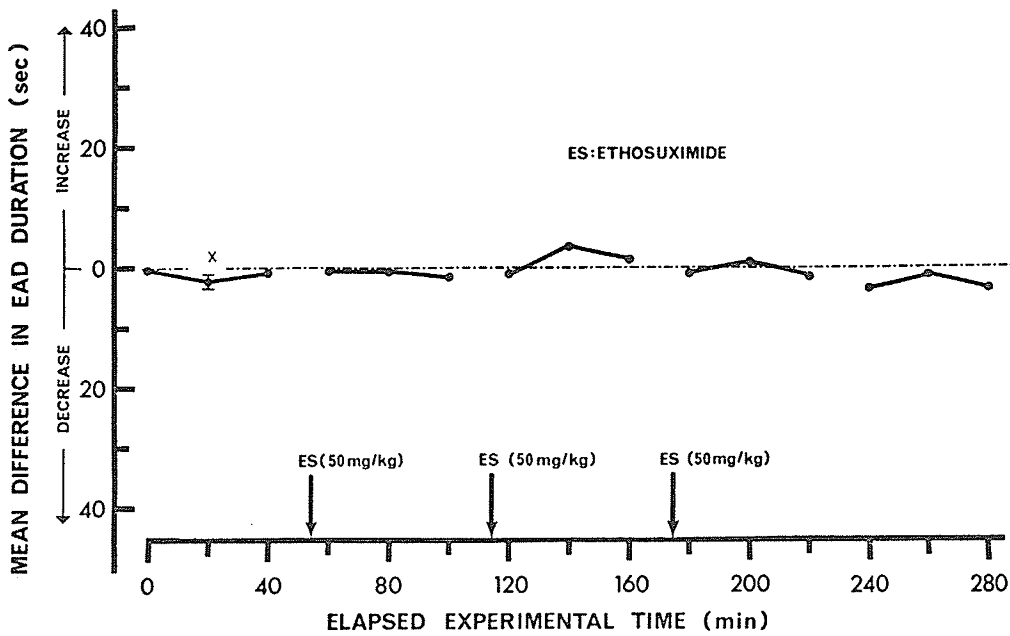


Figure 96.

or for 3 hours after the last injection of drug. There was probably significant cumulation of drug following this series of injections. The cats became increasingly drowsy after the second injection of ethosuximide (cumulated dose probably near 150 mg/kg).

Thus, it is quite apparent that the duration of afterdischarges elicited in chronically neuronally isolated slabs of cat suprasylvian cerebral cortex is preferentially sensitive to the effects of anti-*grand mal* but not anti-*petit mal* agents.

b. *Effects of anticonvulsant drugs upon the response of the isolated slab to antimuscarinic agents*

Four cats were injected with trimethadione 100 mg/kg (Fig. 97). This treatment did not alter EAD duration. The next injection was scopolamine 1 mg/kg which produced a highly significant ($P < 0.01$) increase in EAD duration of 20 sec above control. A second injection of trimethadione 100 mg/kg caused no further change in the response which remained about 20 sec above control for almost 3 hours after the last injection.

Another group of 4 cats received ethosuximide 100 mg/kg (Fig. 98) which did not change EAD duration. Administration of scopolamine 1 mg/kg resulted in a highly significant ($P < 0.01$) increase in EAD duration (over 20 sec). This increase in EAD duration was still evident 3 hours following the injection of scopolamine.

Thus, on the basis of 8 experiments, trimethadione and ethosuximide are not capable of preventing the changes in afterdischarge duration caused by scopolamine.

The effect of scopolamine 1 mg/kg on the response to diphenylhydantoin (DPH) was studied in 6 cats (Fig. 99). Scopolamine 1 mg/kg

Figure 97. Lack of antagonism by trimethadione (TMD) 100 mg/kg of the action upon EAD duration by scopolamine hydrochloride (Sc) 1 mg/kg. Note that there is no further change in the EAD duration already increased by scopolamine when trimethadione 100 mg/kg is injected. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. ** = $P < 0.01$.

Figure 98. Lack of antagonism by ethosuximide (ES) 100 mg/kg of the action upon EAD duration by scopolamine hydrochloride (Sc) 1 mg/kg. Note lack of change immediately after injection of ethosuximide. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 4 cats. ** = $P < 0.01$.

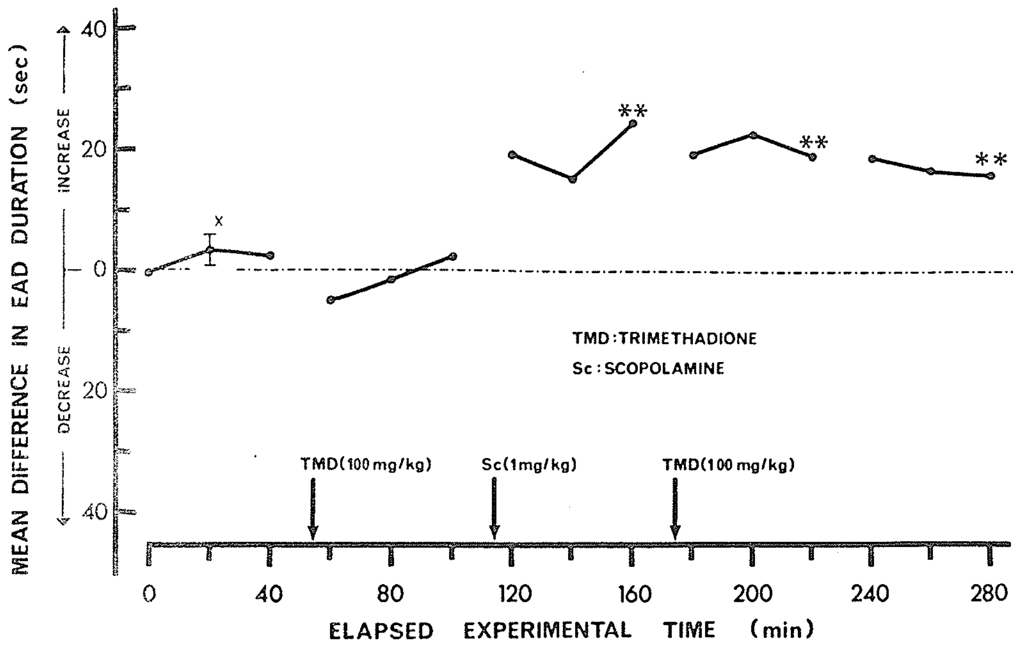


Figure 97.

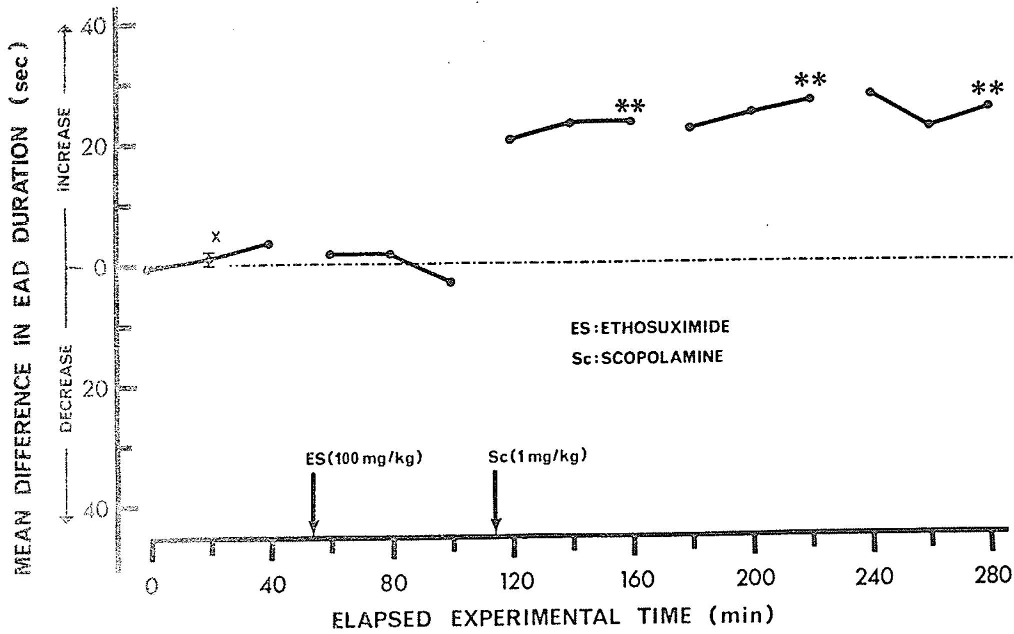


Figure 98.

caused a highly significant ($P < 0.01$) increase in EAD duration (more than 20 sec). Administration of DPH 10 mg/kg did not result in any immediate significant change in EAD duration. However, two hours after the administration of DPH there was a significant ($P < 0.05$) decrease in EAD duration.

Another group of 6 cats received DPH 10 mg/kg (Fig. 100) which reduced EAD duration almost by 20 sec, a highly significant ($P < 0.01$) change. The next injection was scopolamine 1 mg/kg. There was no immediate change in EAD duration but after an hour a gradual increase in EAD duration began, and three hours after the injection of scopolamine EAD duration had returned to control levels.

In 6 cats atropine 3 mg/kg (Fig. 101) increased EAD duration by almost 20 sec which was a highly significant ($P < 0.01$) change in afterdischarge duration. The next injection was DPH at a dose of 10 mg/kg did not cause any further change in EAD duration. Another dose of DPH which was 5 mg/kg produced only a slight decrease in this level of EAD duration, the total difference still being very significantly ($P < 0.01$) different from control. However, within 3 hours after the last dose of DPH, afterdischarge duration had returned to control values, and an hour later had decreased 10 sec below control values, this last change being a significant ($P < 0.05$) difference from control.

c. Effects of diphenylhydantoin and cholinergic agents on the EAD in isolated cortex

Atropine 3 mg/kg was administered to 5 cats (Fig. 102) producing a highly significant ($P < 0.01$) increase in EAD duration (over 30 sec). The second injection in this series was DPH at a dose of

Figure 99. Antagonism by scopolamine hydrochloride (Sc) 1 mg/kg of the action upon EAD duration by diphenylhydantoin sodium (DPH) 10 mg/kg. Note lack of change in EAD duration after injection of diphenylhydantoin. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
** = $P < 0.01$.

Figure 100. Example of antagonism by diphenylhydantoin sodium (DPH) 10 mg/kg of the action upon EAD duration by scopolamine hydrochloride (Sc) 1 mg/kg. Note lack of immediate change in EAD duration after the injection of scopolamine 1 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
* = $P < 0.05$; ** = $P < 0.01$.

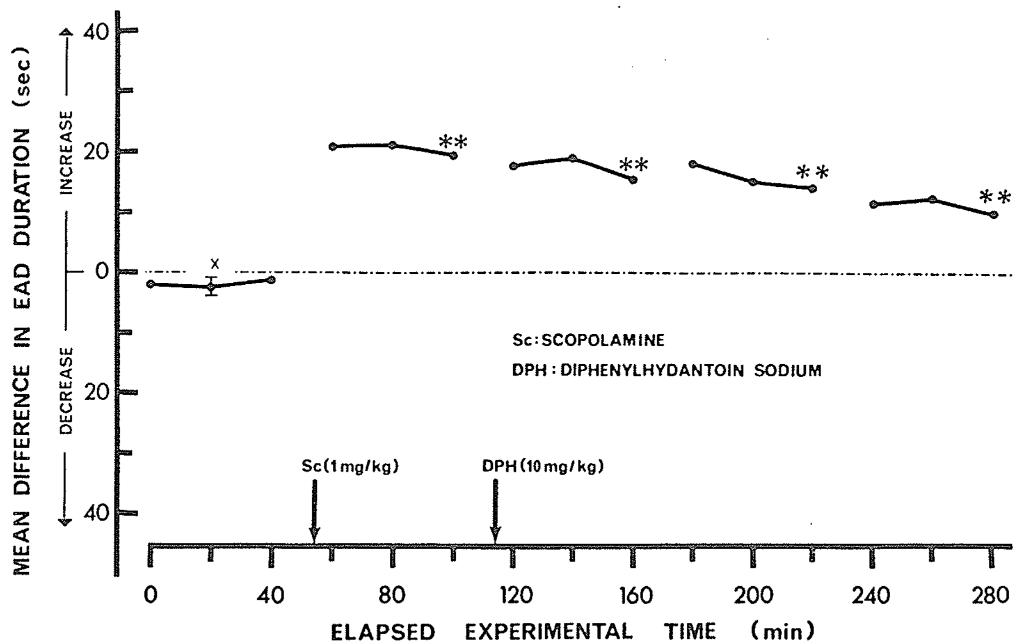


Figure 99.

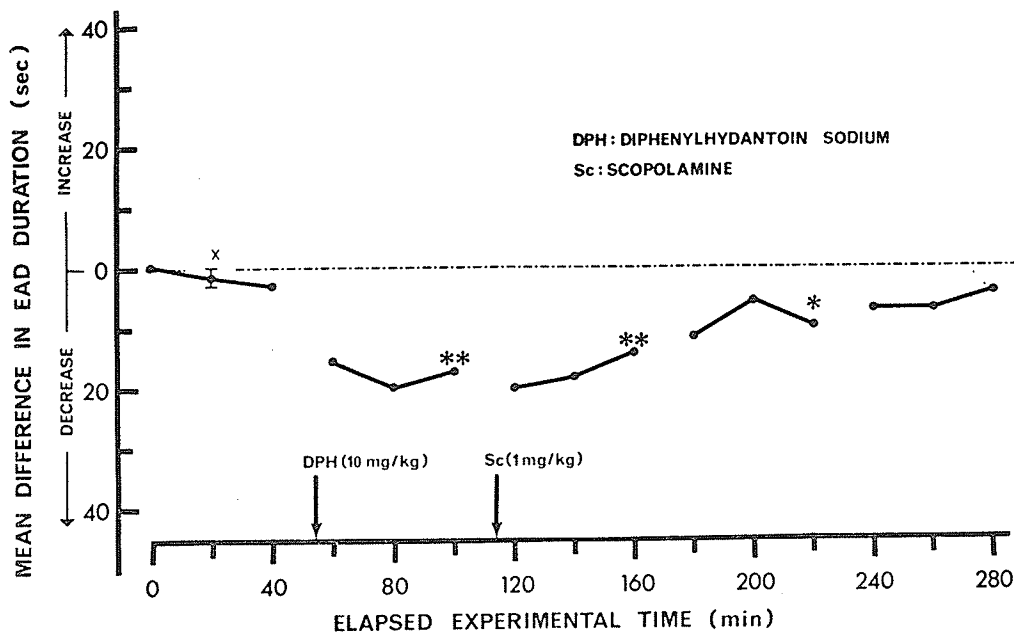


Figure 100.

10 mg/kg which slightly reduced the EAD response (about 10 sec) but EAD duration still remained significantly ($P < 0.05$) different from control values. The next injection was arecoline 2 mg/kg which caused only a slight decrease to a value of 20 sec above control responses. This set of responses (Treatment-3) was just significantly ($P < 0.05$) different from the responses following atropine (Treatment-1). Afterdischarge duration kept decreasing gradually after the last injection and returned to control levels almost 4 hours later.

Administration of scopolamine 1 mg/kg in 8 cats (Fig. 103) increased EAD duration very significantly ($P < 0.01$) by 25 sec above control. The next injection, which was DPH at a dose of 10 mg/kg did cause a slight, but just insignificant decrease in EAD duration of about 7 sec. Another injection of scopolamine 1 mg/kg (Treatment-3) did not result in any further change in EAD duration. The final injection in these experiments consisted of pilocarpine 4 mg/kg which reduced EAD duration by a few more sec to a level about 15 sec above control levels. This level of EAD duration was significantly ($P < 0.05$) different from the first responses to scopolamine (Treatment-1). Afterdischarge duration decreased gradually from this point onward and 3 hours later was significantly below control levels by 10 sec.

In 5 experiments DPH at a dose of 10 mg/kg was injected 10 min after a pretreatment dose of methylatropine 4 mg/kg (Fig. 104). DPH produced a highly significant ($P < 0.01$) decrease in EAD duration of about 20 sec. The next treatment was arecoline 2 mg/kg which caused a further small reduction in EAD duration of about 7 sec, which however, was not significantly different from the previous (Treatment-1) responses.

Figure 101. Antagonism by atropine sulfate (At) 3 mg/kg of the action upon EAD duration by diphenylhydantoin sodium (DPH). Note that there are no immediate significant changes in EAD duration after the injections of DPH. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 6 cats.
** = $P < 0.01$.

Figure 102. Interactions between an antimuscarinic agent (atropine sulfate, At) diphenylhydantoin sodium (DPH) and a muscarinic agent arecoline hydrochloride (Ar). Note lack of significant change in EAD duration after the injection of DPH and arecoline. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

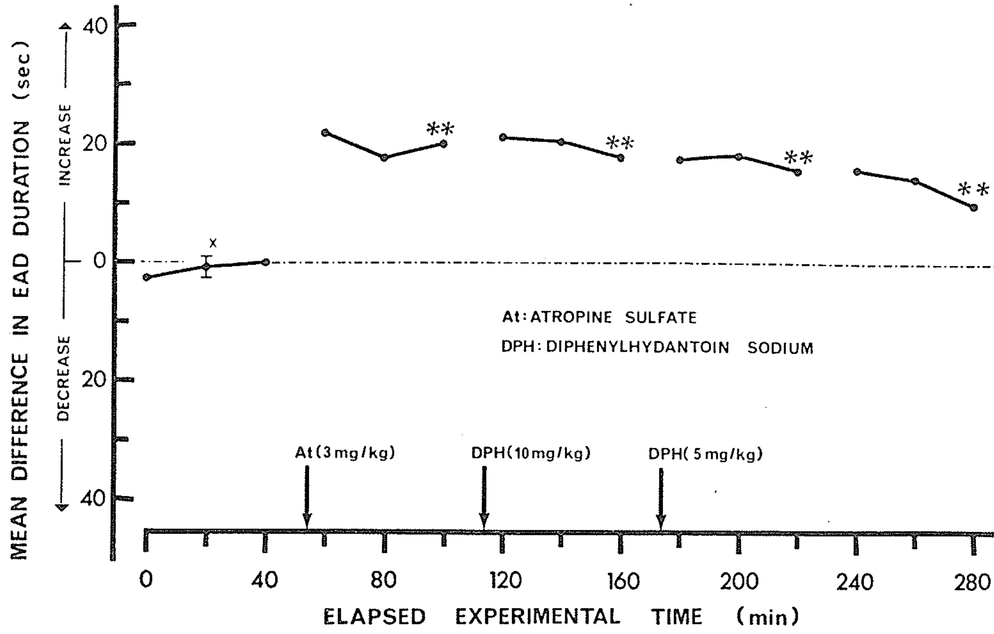


Figure 101.

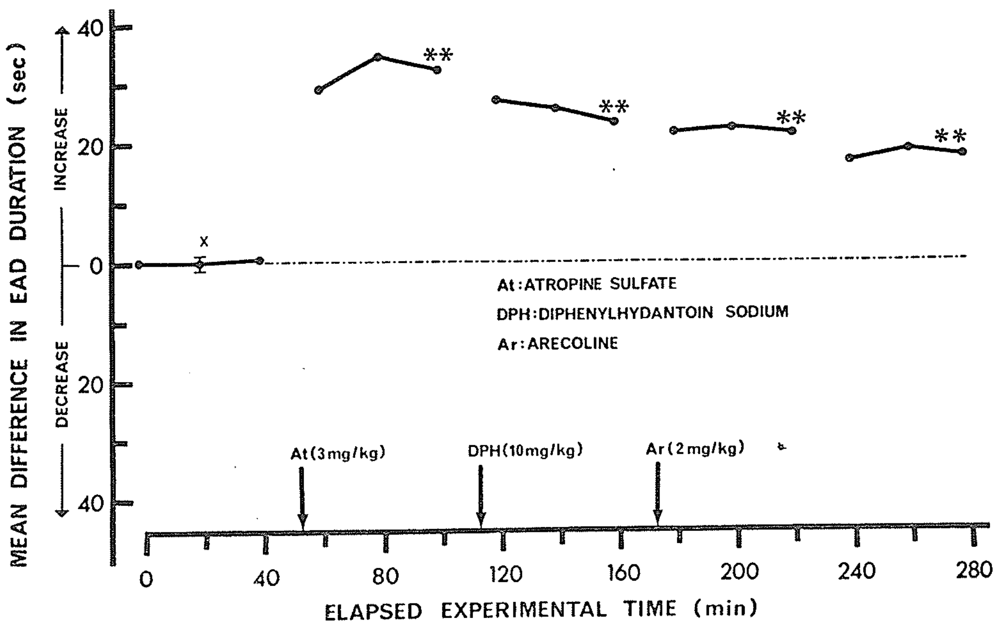


Figure 102.

Figure 103. Interactions between an antimuscarinic agent scopolamine hydrochloride (Sc), diphenylhydantoin sodium (DPH), and a cholinergic agent pilocarpine hydrochloride (Pc). Note lack of immediate significant changes in EAD duration after the injection of DPH and pilocarpine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 8 cats.
** = $P < 0.01$.

Figure 104. Antagonism by diphenylhydantoin sodium (DPH) of the action upon EAD duration by arecoline hydrochloride (Ar). Methyl atropine nitrate (MAt) was given as a pretreatment. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

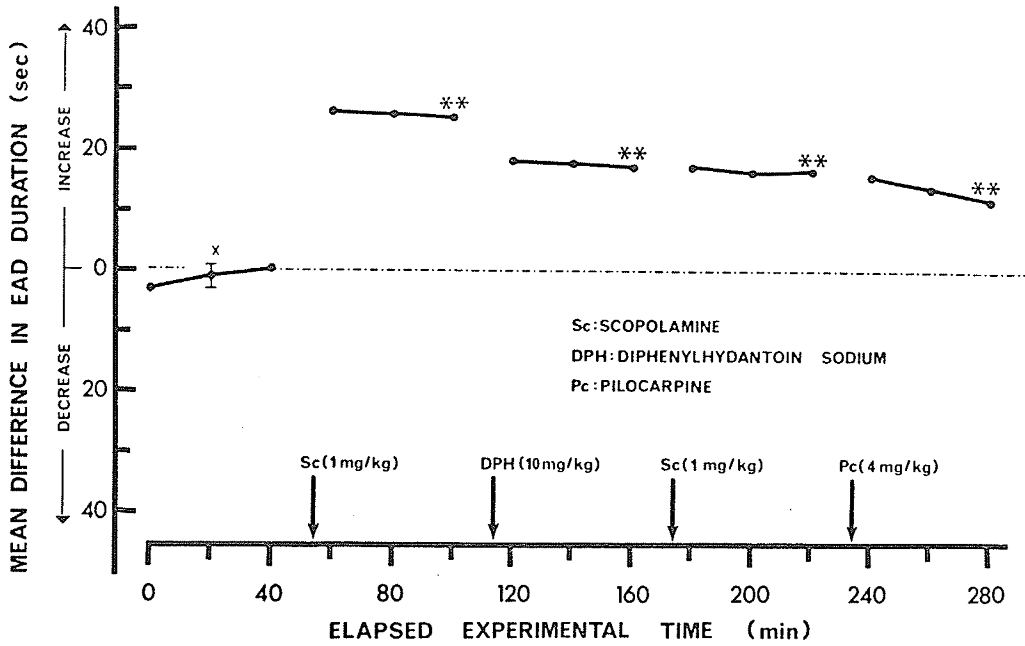


Figure 103.

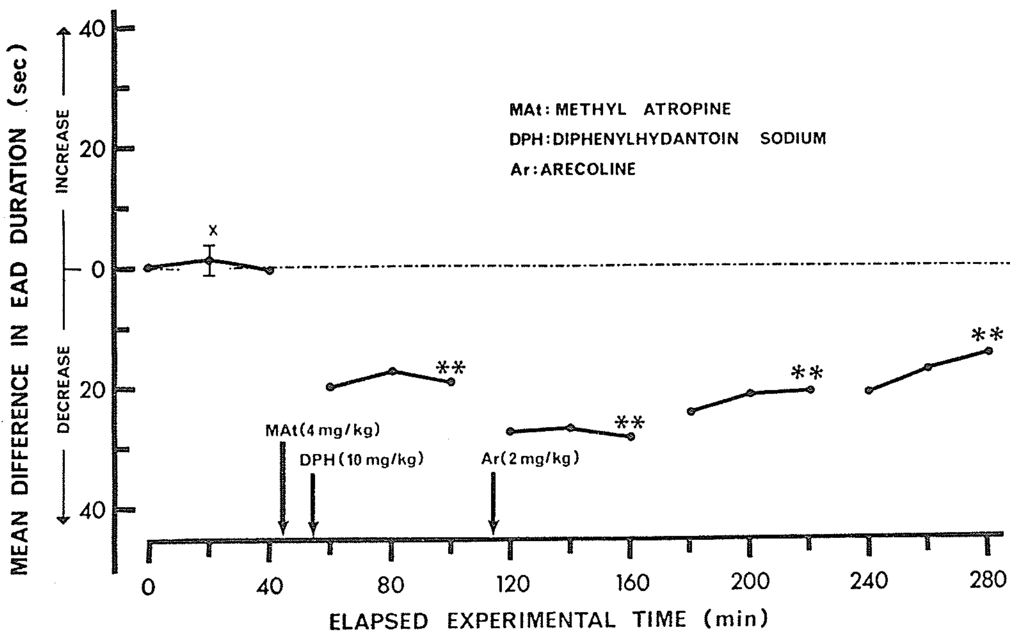


Figure 104.

Figure 105. Interaction between atropine sulfate (At) 3 mg/kg and phenobarbital sodium (PHB) 5 mg/kg upon EAD duration in the isolated cortex. Note lack of significant change in EAD duration immediately after the injection of phenobarbital. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
* = $P < 0.05$; ** = $P < 0.01$.

Figure 106. Interaction between phenobarbital sodium (PHB) 5 mg/kg and atropine sulfate (At) 3 mg/kg upon EAD duration in the isolated cortex. Note lack of immediate change in EAD duration immediately after the injection of atropine. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

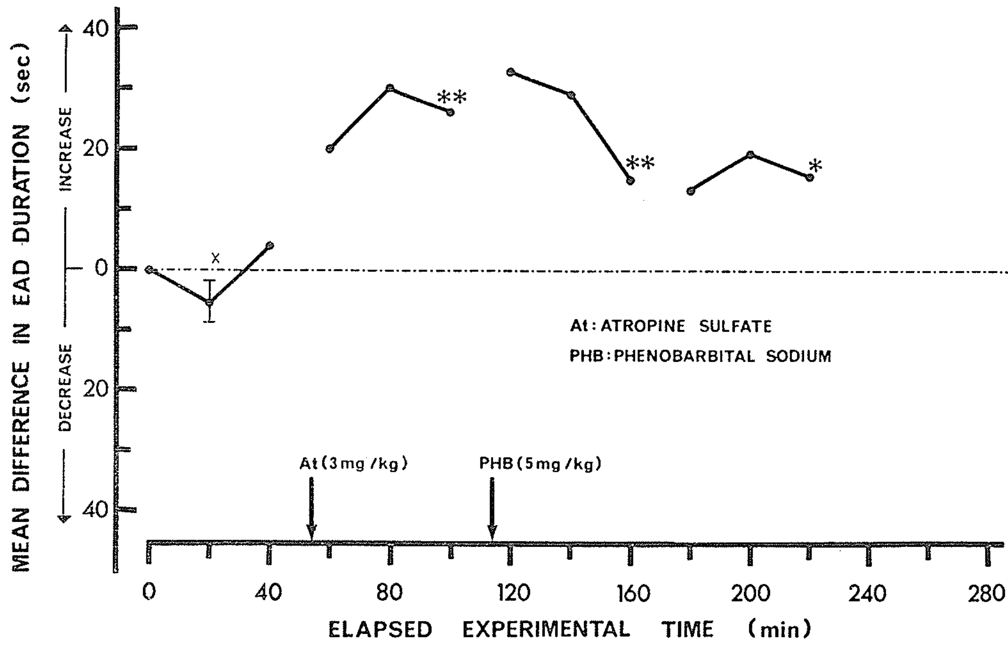


Figure 105.

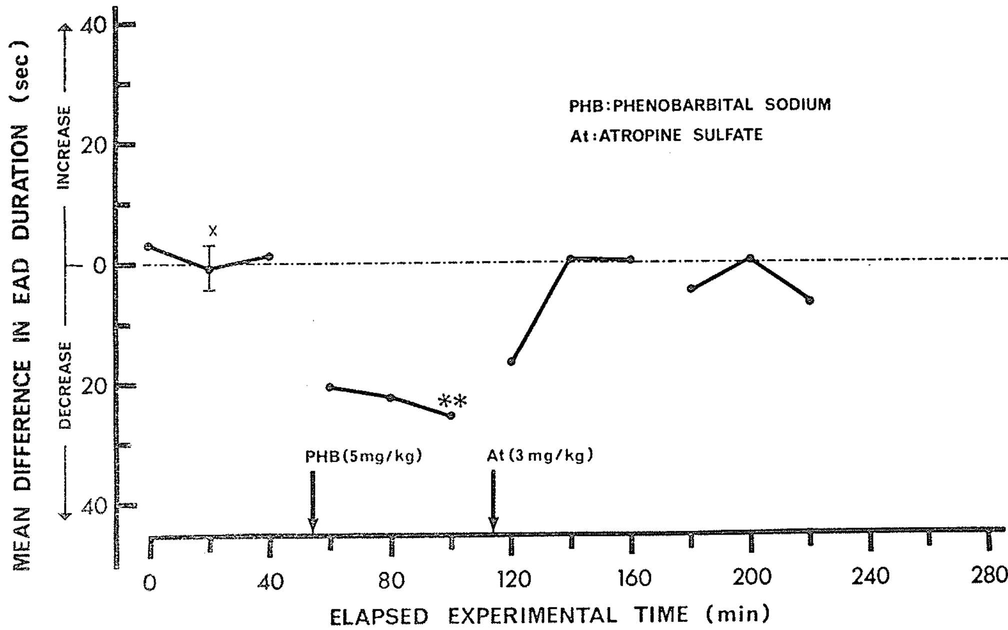


Figure 106.

Afterdischarge duration gradually increased after this and remained at 12-15 sec below control during a further 3 hours of testing.

d. Effect of phenobarbital upon the EAD response to atropine

Five cats were injected with atropine 3 mg/kg (Fig. 105) which produced a highly significant ($P < 0.01$) increase of 25 sec in EAD duration. This treatment usually maintained EAD duration elevated at this level for up to 3 hours. Following this response, an injection of phenobarbital 5 mg/kg did not produce any immediate change. Forty-five min after the administration of phenobarbital, EAD duration had decreased, but still was significantly different from control ($P < 0.05$). This difference was also significantly ($P < 0.05$) different from the response caused by atropine.

Another group of 5 cats received phenobarbital 5 mg/kg (Fig. 106). This treatment resulted in a highly significant ($P < 0.01$) decrease in EAD duration of over 20 sec. The second treatment consisted of atropine 3 mg/kg which caused EAD duration to return to control values almost immediately, where it remained for several hours.

B. Resume

Afterdischarge duration was significantly reduced by the anti-grand mal agents diphenylhydantoin sodium (Fig. 91) and phenobarbital (Fig. 92), but not by the anti-petit mal agents trimethadione (Fig. 95) or ethosuximide (Fig. 96). Pentobarbital sodium, a barbiturate with observed marked sedative properties, did not alter EAD duration (Fig. 93).

Trimethadione (Fig. 97) and ethosuximide (Fig. 98) did not modify the changes in EAD duration produced by scopolamine. On the other hand, scopolamine (Fig. 99) and atropine (Fig. 101) appeared to

antagonize the immediate effect on EAD duration of diphenylhydantoin for some time. In turn, diphenylhydantoin (Fig. 100) antagonized, for a while, the action by which scopolamine increases EAD duration. Atropine antagonized the action of phenobarbital (Fig. 105), whereas phenobarbital did not seem to be as effective an antagonist for the effects of atropine (Fig. 106) upon EAD duration.

The effect on EAD duration of the cholinergic drugs arecoline (Fig. 102) and pilocarpine (Fig. 103) were antagonized by pretreatment with atropinic agents and diphenylhydantoin. When arecoline (Fig. 104) was administered only in the presence of DPH, there was a slight decrease in EAD duration, which was not significant.

C. Discussion

As the results presented in this thesis indicate the EAD elicited in the chronically neuronally isolated cortical slab responds to the same anticonvulsant agents as does *grand mal* epilepsy. This conclusion is based on the observations that diphenylhydantoin sodium (Fig. 91) and phenobarbital sodium (Fig. 92) each significantly reduced EAD duration, whereas two anti-*petit mal* agents trimethadione (Fig. 95) and ethosuximide (Fig. 96) had no effect on this parameter. Sodium bromide (Fig. 94) which was used at one time to manage major seizures and it also reduced EAD duration significantly. This preferred susceptibility of isolated cortex for anti-*grand mal* agents has been reported previously (Halpern, 1961; Sanders and Gravlin, 1968).

Whether the isolated cortical slab is a suitable experimental model for the study of agents effective against *grand mal* epilepsy remains an open question. It is useful from the point that it provides a

reliable chronic model having a lesion consisting of a given population of neurons. It is necessary to bear in mind that as with all experimental models there are limitations in application to all facets of the problem. As discussed earlier (I.A.; I.B.; IV) epilepsy is a symptom-complex and presents a vast array of potential mechanisms, all somehow interrelated. Drugs capable of controlling and managing seizure activity do so by acting on different structures, e.g. upon pathologically altered neurons, upon normal neurons, upon non-neural structures, and other factors involved in the maintenance of excitability of nervous tissue. The isolated cortex allows at best an opportunity to study the part played in epileptogenesis by altered neurons and non-neural elements. In spite of removal of extracortical influences there still remains a relatively complex meshwork, but it is important to have an adequate population of neurons to investigate since sampling only one or a few neurons probably has little relationship to epileptiform EEG phenomena (sections IV. A.c.; IV. B.b.; IV. B.d.).

The onset of action of diphenylhydantoin is quite rapid and this is an interesting effect since it is generally assumed that a long period is required before therapeutic concentrations in cerebral tissues are achieved (Goodman and Gilman, 1970). However Kutt _et al_. (1968) have observed that DPH accumulated rapidly in the brain after systemic administration and quickly abolished "epileptic activity" (Louis _et al_., 1968). Furthermore, Riehl and McIntyre (1970) have reported acute EEG effects due to DPH within 10-15 min after injection in epileptic patients.

The action of DPH was antagonized by scopolamine (Fig. 99)

and atropine (Fig. 101; 103) and in turn DPH antagonized the action of scopolamine (Fig. 100). On the other hand, the anti-*petit mal* agents trimethadione (Fig. 97) and ethosuximide (Fig. 98) did not alter the EAD response to scopolamine. These results with DPH and atropinic drugs were qualitatively similar to those observed between muscarinic and antimuscarinic drugs. These results suggest that there might be a common basis for the maintenance and spread of epileptiform potentials in the isolated cortical slab.

Pentobarbital (Fig. 93) had no significant effect on EAD duration except for a small and brief reduction at higher doses. This barbiturate has little usefulness for the treatment of epilepsy since it increases cortical thresholds only at doses that produce marked overt depression (Keller and Fulton, 1931; Domino, 1962). In the present context this apparent lack of effect of pentobarbital on EAD duration, especially when compared to the considerable action of phenobarbital, suggests that the effects of DPH and phenobarbital against EAD duration were relatively specific and not achieved by general CNS depression.

A qualitatively similar pattern of interactions was also observed to occur when testing phenobarbital and atropine. Atropine appeared to antagonize the action of phenobarbital (Fig. 105) and phenobarbital temporarily antagonized the action of atropine (Fig. 106). These preliminary results suggest that DPH and phenobarbital might be acting on a common pathway and that part of this action might occur on the cholinergic structures that seem to be capable of limiting after-discharge duration. Other experiments revealed that DPH antagonized

the action on EAD duration of arecoline (Fig. 102) and pilocarpine (Fig. 103). In these experiments atropinic agents were present. In experiments in which only DPH was the pretreatment (Fig. 104), a dose of arecoline produced a further small decrease in EAD duration, but these were not significantly different from the previous set of responses decreased by DPH. Thus, on the basis of these very preliminary experiments it is possible that there might be a common basis by which anti-*grand mal* drugs and cholinergic agents act to limit the duration of epileptiform activity in the cerebral cortex.

There are many contradictory studies of the effects of anti-epileptic drugs on epileptiform afterdischarges at various cerebral foci. Part of these discrepancies might be due to difference in animal species used, technique and dosage of drug administered. It would seem that a careful study of these agents on the isolated cerebral cortex is advantageous and necessary since it permits the investigation of these drugs in a localized discharging focus in a particular part of the brain free from the influence of other active regions.

Gangloff and Monnier (1957) appear to have been the first to study the effects of anticonvulsant drugs on self-sustained electrical activity at supraspinal levels. These authors administered high doses of DPH to rabbits and reported that it did not change EAD duration or threshold in the cortex, whereas trimethadione produced an increase in cortical EAD thresholds and a decrease in EAD duration. Their results with phenobarbital are even more puzzling, as this agent decreased in their experiments EAD threshold in the cortex with an apparent increase in excitability, although EAD duration was not increased. Strobos and

Spudis (1960) studied these agents for their effect on afterdischarges elicited on the cat's cerebral cortex and found that DPH increased threshold and decreased EAD duration in some cortical areas, but did not alter the spread of the EAD, while phenobarbital had little effect on threshold, but limited duration and spread of the afterdischarge. Trimethadione produced only slight reductions in duration and spread of the EAD. Vastola and Rosen (1960) also studied anticonvulsants in cats at relatively physiological doses and reported that intravenous doses of DPH, phenobarbital and to a lesser degree pentobarbital, depressed EAD amplitude and duration. Trimethadione produced no significant effects. Therefore, it is apparent that the results reported earlier by Gangloff and Monnier (1957) are at variance with later studies (Strobos and Spudis, 1960; Vastola and Rosen, 1960; Halpern, 1961; Sanders and Gravlin, 1968) and the results presented in this thesis. Schallek and Kuehn (1963) have reported that DPH raised thresholds for the afterdischarges only in the thalamus, while DPH decreased afterdischarge duration in the cortex and hippocampus.

It is curious that a depressant barbiturate like pentobarbital did not reduce EAD duration as did phenobarbital. The major structural difference between these agents is at the C-5 position of the barbiturate nucleus (Burger, 1970). Pentobarbital has alkyl substituents whereas phenobarbital has a phenyl group attached at this position. This may not be the explanation for this difference in activity but it is perhaps pertinent to also consider along these lines the other anticonvulsants. It has been stated (Burger, 1970) that 5-phenylsubstitution on the barbiturate nucleus confers anti-grand mal activity to compounds. Diphenylhydantoin has 2 phenyl substituents at this position,

whereas trimethadione and ethosuximide have relatively low alkyl substitution at the C-5 position of a similar parent nucleus. Therefore, on the basis of very preliminary structural considerations it seems that aryl substitution confers to a common chemical nucleus the ability to manage grand mal epilepsy and apparently to shorten EAD duration in isolated cortex.

The lack of anticonvulsant effect of pentobarbital has been discussed previously (Aston and Domino, 1961). Keller and Fulton (1931) and Merritt and Putnam (1938) found that phenobarbital abolished motor cortical responses at low doses in monkeys and increased the convulsive threshold in cats respectively, whereas pentobarbital had little effect, except at high doses that produced signs of anaesthesia. Aston and Domino (1961) reported that although pentobarbital was more potent in elevating motor cortical thresholds, it had little effect on seizure duration, in contrast to their findings with phenobarbital or DPH which significantly shortened seizure duration.

Berry (1965) has injected high doses of pentobarbital to rabbits and reported negligible effects on afterdischarge duration in the cortex. It is possible that phenobarbital and pentobarbital act to limit epileptiform potentials at different sites in the brain for Aston and Domino (1961) have demonstrated that pentobarbital increased reticular and motor cortical thresholds whereas phenobarbital increased primarily motor cortical thresholds. The fact that these authors worked with whole brain preparations suggested that the effect of pentobarbital on the cortex was probably acting from subcortical centres. Domino (1962) has discussed further the problems of explaining the apparent

differences in central activity between phenobarbital and pentobarbital. Pentobarbital appears to be unique in that it produces suppression-burst activity in the cortex (Domino, 1957), a form of increased electrical potentials. Goldring *et al*. (1961) showed that pentobarbital increased some components of DCRs. Preston (1955) reported depression.

Straw and Mitchell (1967) have compared quantitatively phenobarbital and pentobarbital using the threshold for motor cortical seizures in cats prepared with indwelling electrodes for chronic experiments. These authors reported that phenobarbital was more selective than pentobarbital in increasing motor cortical thresholds. These results are in accord with those reported by Aston and Domino (1961) on monkey cortex. Sharpless and Jaffe (1966) studied chronic barbiturate intoxication in cats with isolated cortical slabs in an attempt to determine whether the barbiturate withdrawal syndrome (convulsions) was related to neuronal deafferentation. The compound used was pentobarbital and it was administered daily in anaesthetic doses. These authors used EAD duration as a parameter but could not show that barbiturate intoxication and sudden withdrawal resulted in EAD durations that were augmented. Chronically isolated and untreated cortex produced EAD durations considerably longer than the above two groups. It might prove interesting to see whether the expected result (increased EAD duration) would result after treatment with other barbiturates, e.g. phenobarbital.

Cortical or subcortical lesions were employed by Dilts and Berry (1968) to study the patterns of excitability of cat suprasylvian cortex. This region of the cortex was more sensitive to depression by

pentobarbital after midbrain transection. After undercutting, circumscription, or lesions of the ventralis anterior of the thalamus, pentobarbital produced no depression or increase in excitability. These results suggested to Dilts and Berry that pentobarbital affects cortical excitability acting at subcortical sites, most likely thalamic. Therefore, this appears to explain the lack of action of pentobarbital on EAD duration in the experiments in this thesis. On the other hand, phenobarbital would act directly on the cortex to shorten EAD duration significantly.

Straw and Mitchell (1966b) reported that phenobarbital in low doses prolongs cortical afterdischarge duration in cats with chronically implanted electrodes. This investigation showed that there were two groups of cats on the basis of the severity of the overt component of the seizure since these experiments were performed on cats with intact cortex. The cats exhibiting, the most severe generalized seizures, which were depressed by phenobarbital, were also the ones that showed prolongation of EAD duration. The other group of cats showed less severe overt seizure components and EAD durations were depressed by the same dose of phenobarbital. No explanation is available to better understand this result, except that it appears in a select group of animals and may be due to genetic or pathological factors, or both.

Sharpless (1970) has discussed in a recent monograph the effects of barbiturates on chemical transmission across synaptic junctions and it appears that neuronal and neuroeffector junctions might be more susceptible to action by these agents than are comparitively non-

specific sites along fibres. Whether or not these agents influence the release of mediators is not clear but decreases in ACh release and increases in noradrenaline release were reported. Sharpless (1970) suggests that the depressant action of barbiturates is exerted selectively on polysynaptic pathways. Thus, if EADs are dependent on repetitive activity in multineuronal networks, this might be another explanation for the ability of phenobarbital to limit EAD duration.

If the capacity of CNS structures to respond with repetitive or epileptiform discharges is determined by disruption of physiological tonic control mechanisms as effected by a balance between excitatory and inhibitory inputs on that structure, then other possibilities for modulation or restoration of control by means of drugs become available, e.g. reduction of post-tetanic potentiation (PTP), increased pre- or postsynaptic inhibition, excitation, and others (Esplin and Zablocka, 1969). Esplin (1957; 1963) has shown that DPH selectively depresses PTP in spinal reflexes, whereas pentobarbital was not effective. Low doses of phenobarbital were shown to increase polysynaptic discharges, while DPH only slightly depressed repetitive responses. This latter effect was distinct from the effects by these agents on PTP.

Post-tetanic potentiation might be one of the mechanisms responsible for the spread and sustainment of epileptiform seizures, but PTP is not implicated in the initial discharge of the seizure focus (Toman and Taylor, 1952; Toman, 1959). DPH is known to stimulate the membrane Na+ - K+ pump ATPase (Woodbury, 1955) and it has been postulated that one possibility for the anti-epileptic action of DPH is the stabilization (Woodbury and Esplin, 1959) of the cell membrane and

prevention of the hyperpolarization of the cell membrane associated PTP (Morrell, 1959). However, Gage and Hubbard (1966) have ruled out hyperpolarization and speculated that increased amounts of transmitter released following a train of repetitive pulses might be the causal factor of PTP. In synaptic networks with a high degree of dendritic branching Esplin (1957) has proposed that PTP may represent release from normally existing inhibition. Since DPH blocks PTP (Rand _et al_, 1966) and antagonizes spinal cord convulsions (Esplin and Freston, 1960), it seems reasonable to assume that DPH may be acting in a similar manner at the cortical level. In any event, it seems likely that conditions for PTP probably exist at a seizure focus and the result of such potentiation would be expansion of the discharge zone. Perhaps the basis for anticonvulsant action might be a selective action against repetitive high frequency discharges. Morrell _et al_. (1959) have shown quite clearly that anti-epileptic agents limit propagation of epileptiform activity rather than acting directly on epileptiform epileptogenesis.

Toman (1952) has shown that DPH protects peripheral nerve from the hyperexcitability induced by lowering external Ca++ concentration. The level of excitability achieved by repetitive stimulation, a phenomenon similar to PTP, reaches the same level as that achieved by removing some extracellular Ca++, and supramaximal repetitive stimulation will not produce a further increase in excitability (Toman, 1969). Morrell, _et al_. (1958) reported that DPH reversed the increased excitability of oxalate-treated nerve fibres _in situ_.

An intriguing possibility is that DPH through its apparent "anti low calcium" action may augment the efficacy of the Ca++ available

at cholinergic nerve terminals, and in this manner increases the amount of ACh liberated at cortical cholinergic inhibitory neurons. It is known that calcium ions are essential for the liberation of ACh (Hunter and Kostial, 1954; Randic and Pajden, 1967). Therefore, whether DPH enhances ACh release directly or not is unclear, but through an action involving Ca++, DPH might be promoting and improving cholinergic activity in conditions, e.g. denervation, where cholinergic function is compromised.

It appears to be difficult to fit the data presented in this thesis into a scheme in which DPH limits EAD duration by acting, at least in part, through central cholinergic synaptic structures. McLennan and Elliott (1951) have demonstrated in vitro that low concentrations of DPH and phenobarbital stimulate the production of free ACh by brain slices. At high concentrations this production was depressed, which is in agreement with findings reported by Bose *et al.* (1958) that anti-epileptics decrease brain level of ACh. Tower (1955) has reported that epileptogenic cortex is characterized by a failure in the production of bound ACh, and that phenobarbital and DPH, but not trimethadione, counteract this defect, increasing the levels of bound ACh in such tissues. However, Pappius and Elliott (1958) and Wolfe and Elliott (1962) have raised important questions regarding the validity of the earlier findings. Nevertheless, circumstantial evidence suggests that alterations in ACh levels play an important role in epileptiform activity. The relation of ACh to the action of anti-convulsants, though not confirmed, deserves some attention.

Disordered neuromuscular transmission in patients receiving DPH on a chronic basis has been described on the basis of preliminary

observations (Norris et al. 1964). These authors also carried out experimental studies showing that at the neuromuscular junction large (> 10 mg/kg) doses of DPH appeared to block end-plates as a result of either end-plate depolarization or anticholinesterase activity. At lower doses of DPH the effects observed by these investigators were interpreted in favour of DPH acting as a competitive inhibitor of ACh.

Woodbury (1969) has demonstrated at the hypothalamic level that low doses of DPH stimulate the release of TSH, whereas high doses of DPH inhibited this effect. In other studies (Woodbury and Kemp, 1970) on the isolated ileum of the rat it was shown that low doses of DPH stimulate the release of ACh from various cholinergic structures, parasympathetic nerve endings, and intramural ganglia of the ileum. Higher doses of DPH were found to block this release. Low doses of DPH stimulated contraction of the ileum, an effect which was blocked by atropine. The higher doses of DPH which inhibited contraction of the gut were shown to act through two different mechanisms. A competitive component which was associated with ACh release because when DPH was present it enhanced the antagonistic effect of atropine on ACh induced contractions presumably by direct competition with ACh. At these higher concentrations of DPH the response of the gut to ACh was inhibited. This latter entirely inhibitory effect of DPH was designated the second component by Woodbury and Kemp (1970) and considered to be an action of DPH on excitation-contraction coupling. Contractions of the ileum induced by barium ions were blocked non-competitively by the same dose range of DPH.

In his extensive review on the mechanisms of action of anti-convulsants (Woodbury (1969a) has provided other examples of DPH having

a cholinergic activity. This agent has been shown to produce vagal stimulation which results in A-V block, an effect of DPH which is blocked by atropine. High doses of DPH abolished spontaneous contractions and tone of various smooth muscle preparations by an apparent inhibition of ACh release of ACh. Physostigmine enhanced the effect of DPH on smooth muscle.

Diphenylhydantoin does not appear to have affected electrocortical function at the doses and administered in these experiments. Some high frequency repetitive discharges are still present in the ECoG and there was no apparent modification of the ECoG recorded from intact cortex. This finding is in accord with results observed by Sanders and Gravlin (1968) and by Halpern and Ward (1969), who showed that DPH does not affect the DCR. This suggests further that DPH does not depress cortical neurons but that it probably prevents the spread of seizure discharges along cortical networks.

DPH has been known to exacerbate seizure activity, especially of the _petit mal_ type, (Goodman and Gilman, 1970). Rumke (1967) has demonstrated that prolonged treatment with high doses of some anticonvulsant drugs can decrease seizure thresholds in mice. This toxic effect of DPH might therefore be due to less specific effect of this agent.

It has been shown by Neal (1967) that several convulsant agents (strychnine, brucine, picrotoxin, bemegride, metrazol and nikethamide), depress the twitch response in the coaxially-stimulated guinea pig ileum. This investigator showed that an approximately linear correlation exists between the impairment of this muscarinic response by the convulsant compounds he tested and their known ability to produce

convulsions in mice. His results revealed further, that this inhibitory effect of the convulsants was associated with a decrease in ACh output. This result was incompatible with Neal's hypothesis that convulsants should increase the twitch response as they are known to increase the electrical activity of the brain and the amount of ACh released from the surface of the cerebral cortex (Mitchell, 1963). However, these latter effects of the convulsant drugs were studied on whole brain structures, and it is not clear what actually influenced the increased EEG potentials and ACh output. On the other hand, the model studied by Neal (1967) may have much in common with the significance of the results presented in this dissertation, for others (McKinstry and Koelle, 1967) have concluded that strychnine reduces the amount of ACh released from nerve terminals.

That is, if convulsant agents do indeed decrease ACh release specificially at cholinergic synapses in the cortex as they do cholinergic structures in the ileum this may impair the function of a cholinergic mechanism in the cortex. A reduction in neuronal ACh release would compromise the effectiveness of the inhibitory action upon EAD duration of muscarinic neurons which the results of this thesis suggest as being operative in the cerebral cortex. Such a loss of inhibitory tone would, therefore, enhance the spread and maintenance of epileptiform potentials in isolated cortex. In view of the evidence presented for a muscarinic inhibitory system in the cerebral cortex and the inter-action of DPH with this system, the possibility of DPH acting partially through a mechanism involving ACh cannot be ruled out in light of the present evidence.

XIV. OTHER PHARMACOLOGICAL AGENTS

A. Effect of Systemic *gamma*-Aminobutyric Acid (GABA) on EAD Duration

There has been a report (Krnjevic *et al.*, 1970a) that intravenous administration of GABA resulted in the arrest of seizure discharges in chronically isolated cortex. In addition, Godfraind *et al.*, (1970) reported that bicuculline, reputed to be a specific antagonist of GABA at various sites in the CNS (Curtis *et al.*, 1970), either applied topically or injected systemically in low doses evoked local paroxysmal activity.

GABA was administered to 5 cats (Fig. 107), there were 4 consecutive injections of GABA administered at 60 min intervals. The first two injections of GABA at a dose of 50 mg/kg each did not produce any significant changes in EAD duration. Following the two injections of this agent 100 mg/kg there appears to be a gradual decrease in EAD duration ($<$ 5 sec), but these changes were not significantly different from control. No effects of GABA on behaviour or activity of the animals were noted.

In one cat in this group, there was a brief ($<$ 30 min) increase in EAD duration following the last injection of GABA (cumulated dose near 300 mg/kg), but did not occur in the other 4 cats.

B. Discussion

Numerous reports are available indicating that GABA may interfere with seizure discharges and act as an inhibitory transmitter (Killam, 1957; Killam and Bain, 1957; Eidelberg *et al.* 1959; Bhattacharya *et al.*, 1964). There are many uncertainties in these studies with GABA, because GABA does not penetrate into the brain in significant amounts

Figure 107. Lack of effect of gamma-aminobutyric acid (GABA) administered intraperitoneally upon EAD duration in the isolated cortex. Note lack of significant changes in EAD duration after each injection of GABA at a dose of 50 mg/kg or 100 mg/kg. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.

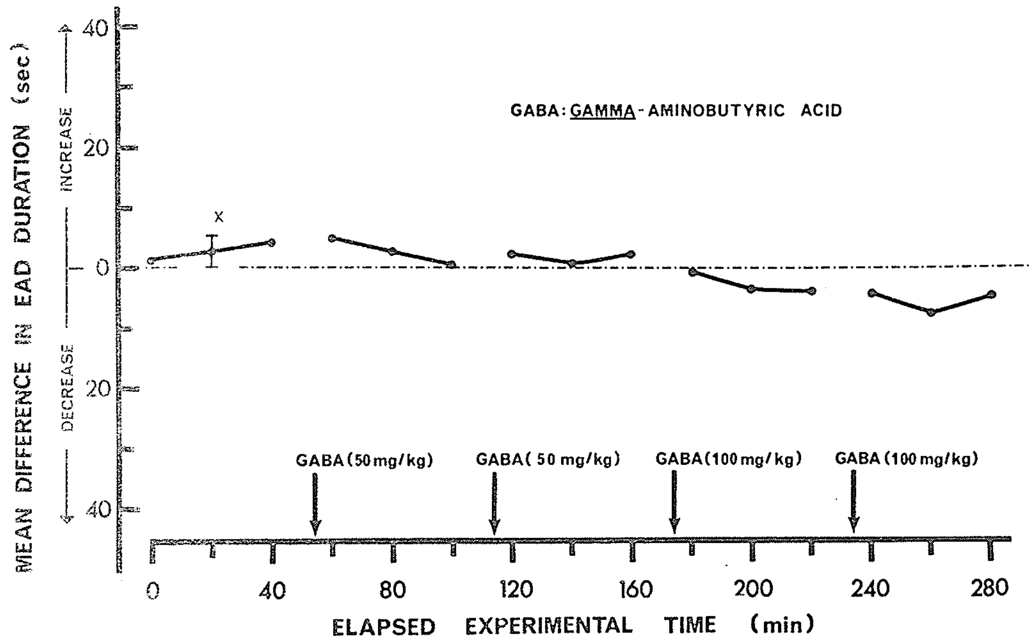


Figure 107.

(Purpura *et al.*, 1958) and antagonism of its effects or modification of its release still cannot be achieved experimentally. As discussed previously (section V.D. Curtis, 1963; 1969) has reviewed extensively the possible function of GABA as a central inhibitory transmitter. The vast majority of his evidence is based on the results of microiontophoretic experiments on single neurons. Recently, evidence that GABA is a transmitter at certain inhibitory synapses in the mammalian cerebral cortex has been provided by Iversen *et al.* (1970) who showed a Ca++ dependent release of endogenous GABA from the cortex during synaptic inhibition. Krnjevic *et al.* (1970a) reported that intravenous injections of GABA stopped seizure discharges in isolated cortex.

Another approach has been to alter central levels of GABA and observe the pattern of electroshock and chemoshock seizures (section V.D.c). On the whole, it appears that whenever brain GABA stores are depleted there is an increased frequency of tonic seizures in mice (Maynert, 1969). However, although amino-oxyacetic acid, an agent that increases GABA levels in the CNS, has some anticonvulsant activity in mice (Kuriyama *et al.*, 1966), recent evidence (Meldrum *et al.*, 1970) suggests that the primary abnormality is not corrected, and that elevated GABA levels only modify the expression of the seizure discharge, but do not suppress spontaneous epileptiform spikes, and spikes and waves.

Rech and Domino (1960a) have shown that GABA topically applied to the isolated cortex of the dog reverses or depresses chemically and electrically evoked activity. However, their results cannot be explained simply by assuming that GABA is an inhibitory transmitter,

because GABA treatment also facilitated the ability of strychnine to produce epileptiform in their preparation. Maynert and Kaji (1962) have questioned a role for GABA in the prevention of seizures. A GABA-related compound has been found to be a useful anticonvulsant experimentally, but was not successful clinically (Moyersoons _et al._, 1969). In cortical lesions induced by freezing intravenous GABA suppressed focal epileptiform activity, but this effect could not be correlated with an increase in the level of GABA in the tissue immediately concerned (Berl _et al._, 1961)

Eidelberg _et al._, (1959) studied the effects of hydroxylamine, another agent which increases significantly GABA levels in the brain, on the duration of afterdischarges elicited on intact cortex in cats. Marked reductions in duration and spread of EADs were observed at a time when cortical GABA levels showed increases of 100 percent.

In the experiments reported in this thesis in which GABA was administered intraperitoneally, there is no clear action of this agent on EAD duration. Although Gottesfeld _et al._, (1971) have considered the possibility that GABA penetrates into the CNS, they interpreted their studies on intact and chronically isolated cortex as showing that there was no general increase in permeability to GABA in isolated slabs. As a criteria these authors used unit firing and the appearance of labelled GABA in the cortex. That the blood-brain-brain barrier may be altered in chronically isolated cortex might be inferred from my results of experiments with DPH. It is generally held that the onset of action of DPH is slow, due to its slow penetration into the CNS (Goodman and Gilman, 1970). However, in my experiments the action of DPH on EAD duration was evident quite soon (about 5 min) after

injection. This could be due to an increased permeability to drugs in the region of the isolated slab. More precise studies are required in order to clarify and elaborate this point.

As discussed previously (section V.D.c) thiosemicarbizide has been shown to lower cerebral GABA levels and make animal susceptible to seizures (Preston, 1955). It is possible that the decrease in GABA levels and the onset of seizures are unrelated and products of a more general metabolic derangement. Mennear (1969) has shown that the convulsant effect of thiosemicarbizide may be attributed to a lowering of brain amines. In a more definitive study it has recently been reported (Sze *et al.*, 1971) that the convulsant action of thiosemicarbizide may be due to an action on non-neural elements or due to a direct neural action of a metabolic derivative of the hydrazide. Definitive metabolic studies by Tapia and Awapara (1967) have suggested that the rate of GABA formation, independent of its total concentration, is probably a factor in some forms of epileptogenesis. Moreover, it appeared to these authors that more critical studies with respect to the site(s) of formation of GABA are necessary. Sze *et al.*, (1971) have studied various possible sites of GABA formation and their results suggested to them a non-neural site of action in the brain for GABA, i.e. as a modulator of neuronal excitability.

Krnjevic and his associates (Godfraind *et al.*, 1970) have shown that systemic administration or local application of the substance bicuculline on the cortex provokes local paroxysmal activity. The demonstration by others (Curtis *et al.*, 1970; McLennan 1970a) that bicuculline is apparently an antagonist of the actions of GABA has renewed interest for GABA as a possible central inhibitory transmitter.

However, other workers (Godfraind et al., 1970) have questioned the pharmacological properties of this relatively unknown agent.

A publication by Worum and Parazasz (1968) attempts to correlate GABA metabolism to the effect of cholinergic drugs on electroshock convulsions. Physostigmine increased in their experiments the intensity of electroshock seizures and at the same time GABA levels were reduced considerably. More relevant appears to be the action of pilocarpine which significantly reduced seizure activity, this effect was associated with a considerable increase in cerebral GABA levels. This anticonvulsant effect of pilocarpine is consistent with the findings of Zablocka (1963), and the effect on EAD duration of pilocarpine described in this thesis (section X.).

It is possible but not probable that the actions against epileptiform discharges of cholinergic drugs may result from an interference with GABA metabolism rather than from a cholinergic effect. Roberts et al., (1958) have speculated that GABA might be antagonistic to ACh in regulating activity in the CNS. This speculation is based on Tower's (1960) hypothesis that ACh has a stimulant role in epileptogenesis. Relying solely on a balance between two opposing systems in the CNS is perhaps not a realistic evaluation of endogeneous neurochemical influences. Hance et al., (1963) have compared gamma-aminobutyrylcholine, GABA, eserine and atropine on neocortical sites. No significant interactions were observed between these agents suggesting that these substances probably do not interact.

C. Effects of Benzodiazepines on Afterdischarge Duration

There are reports that the benzodiazepine class of compounds exhibit marked anticonvulsant activity in experimental animals (Schallek

and Kuehn, 1963; Straw, 1968) and in man (Sawyer et al., 1968; Toman, 1970). Preliminary experiments were conducted in which increasing doses of these agents were tested on EAD duration in the isolated cortex.

Diazepam was administered to 5 cats (Fig. 108). After the first dose of 0.25 mg/kg EAD duration decreased significantly ($P < 0.05$) by over 10 sec. The next injection of diazepam was 0.75 mg/kg (Treatment-2) which brought down EAD duration to approximately 25 sec below control, a very significant ($P < 0.01$) decrease. The final injection of diazepam tested was 2.5 mg/kg which caused EAD duration to increase very significantly ($P < 0.01$) above control (by about 15 sec). This last change represented an overall change of about 40 sec taking Treatment-2 levels as the starting point. Monitoring the response after the last injection shows that EAD duration was returning toward control levels.

After the last injection of diazepam (cumulated dose probably near 3.5 mg/kg) the animals became quite flaccid and were not able to rise. They were awake, but very tranquillized and relaxed. They lay down and remained that way for several hours. They were very much less responsive to a variety of sensory stimuli (tactile, auditory, visual).

Another benzodiazepine derivative tested was Ro5-4023 (Clonazepam) or 7-nitro-5-(2-chlorophenyl)-3H-1, 4 benzodiazepine-2 (1H) one, reportedly a very specific anticonvulsant (Zbinden and Randall, 1967). As previous trials with diazepam (Fig. 108) and control studies (Fig. 15) indicated an effect of the diazepam vehicle on EAD duration, this compound was solubilized in the DPH vehicle.

The DPH vehicle was injected first in 5 cats (Fig. 109) and did not change EAD duration. The first injection of clonazepam was 0.1 mg/kg which produced a very significant ($P < 0.01$) decrease in EAD

Figure 108. Effect of increasing doses of diazepam (DZ) upon EAD duration in the isolated cortex. Note decrease in EAD duration caused by low doses of diazepam, but increase in EAD duration produced by a higher (2.5 mg/kg) dose. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
* = $P < 0.05$; ** = $P < 0.01$.

Figure 109. Effect of increasing doses of Ro5-4023 (Ro5) or clonazepam upon EAD duration in the isolated cortex. Note lack of effect due to drug free DPH vehicle (VEH), which was used to solubilize the clonazepam. X refers to the pooled S.E. for all the mean differences calculated from the results of experiments on 5 cats.
** = $P < 0.01$.

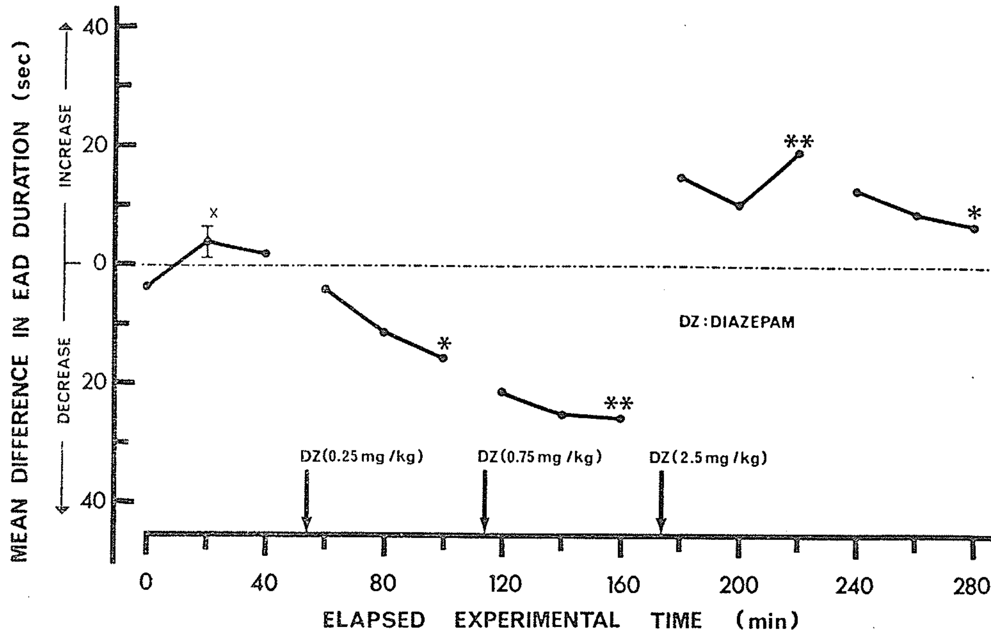


Figure 108.

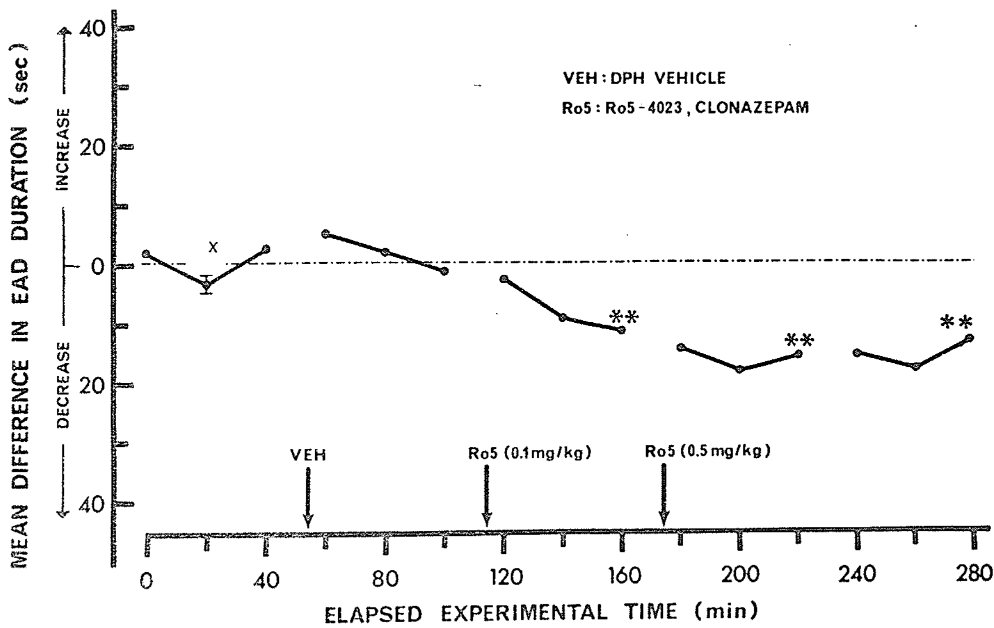


Figure 109.

duration of 10 sec. The next injection of clonazepam was 0.5 mg/kg and reduced EAD duration to about 18 sec below control, which was also a very significant ($P < 0.01$) decrease. Monitoring EAD duration further showed that it remained at this level for another 3 hours. It appears that there was some cumulation of drug.

Shortly after the second injection of clonazepam the animals went through a brief stage of apparent excitation. They showed increased exploratory behaviour and hyperactivity after which they appeared to relax and became slightly drowsy.

Another derivative in this series that was tested was chlordiazepoxide (Librium). This agent was tested only in 3 cats as it did not appear to be very potent regarding its effect on afterdischarge duration. The results of these studies are given in Table 7. The hydrochloride salt of chlordiazepoxide was used and dissolved in saline.

Table 7

Effect of Chlordiazepoxide on Afterdischarge Duration

SALINE CONTROL Mean Difference in EAD Duration (sec) ± S.E. (N)	Drug	Dose	TREATMENT-1 Mean Difference in EAD Duration (sec) ± S.E. (N)
2.7 ± 2.2 (3)	Librium	1 mg/kg	-1.8 ± 2.7 (3)
2.7 ± 2.2 (3)	Librium	4 mg/kg	-4.4 ± 3.1 (3)
0.5 ± 1.9 (3) †	Librium	10 mg/kg	-8.3 ± 2.5 (3)**

N = number of cats
** = $P < 0.01$
† = 3 different cats

It is evident that high doses of chlordiazepoxide caused very significant ($P < 0.01$) reductions in EAD duration. Monitoring the

response longer in each series of cats did not reveal any further changes. Following injection of librium 5 mg/kg the cats were moderately sedated and quite inactive. After 10 mg/kg they were well sedated and remained quietly in a prone position for several hours.

Thus, it appears that the benzodiazepine compounds diazepam, clonazepam, and chlordiazepoxide are capable of causing significant decreases in afterdischarge duration in the isolated cortex.

D. Discussion

Although my results show that benzodiazepines as a class of agents are capable of reducing EAD duration in the chronically isolated cortex, little further can be said about the specificity of this action. On the whole, these agents are relatively new and, more definitive knowledge on various aspects of their pharmacology is still lacking. As discussed earlier (section I.F.), these agents, especially diazepam, show promise in the treatment of prolonged seizure activity (Sawyer *et al.*, 1968) with little, if any, relation to etiology or seizure type. A recent monograph (Burger, 1970) mentions that chlordiazepoxide benefits more patients with *grand mal* than it does others. As a rule, these agents have to be administered systemically for their anti-epileptic effect as the results after oral administration have not been too promising, although a recent report (Elian, 1969) has recommended long-term oral administration of diazepam in epilepsy.

Potent anticonvulsant activity has been revealed for several benzodiazepine compounds (Swinyard and Castellion, 1966), especially against metrazol-induced seizures (Zbinden and Randall, 1967; Spehlman and Colley, 1968). In this regard these agents were more effective than trimethadione and also exhibited a much wider margin of safety.

Sharer and Kutt (1971) have reported that low doses of diazepam abolished peripheral "jerking" resulting from intracortical foci of penicillin. Larger doses of this agent were requested to reduce or abolish self-sustained cortical afterdischarges. These authors argued that diazepam limited seizure spread and propagation of discharges since it stopped peripheral activity at doses that altered very little the recorded cortical epileptiform potentials. However, it is known that diazepam is also a potent muscle relaxant capable of blocking spinal reflex activity and decerebrate rigidity (Schallek _et al._, 1964). Przybyla and Wang (1968) have shown that the muscle relaxant properties of diazepam are due to depression of the brain stem reticular system.

The observed convulsant effect (increased EAD duration) at high doses of diazepam (Fig. 108) is probably due to the solvent vehicle for diazepam. Testing the vehicle without any drug (Fig. 16) showed that there was an increase in EAD duration at doses of vehicle greater than 0.5 cc/kg administered intraperitoneally. A similar convulsant effect for this vehicle has been reported by Wesseling _et al._, (1971). On the other hand, Crankshaw and Raper (1971) have revealed that vehicles containing propylene glycol actually increased the potency of benzodiazepines. It is not clear whether this enhancement was due to increased solubilization of the drug or to pharmacological activity of the solvent.

Schallek and Kuehn (1963) have studied the effects of some anticonvulsants and chlordiazepoxide on after discharges in the cat brain. These investigators showed that at high doses chlordiazepoxide acted not unlike DPH in decreasing EAD durations. Others (Arrigo _et al._, 1965) have reported that diazepam is considerably more effective than chlordiazepoxide in causing the disappearance of epileptiform potentials in

cortical and subcortical sites. Hernandez-Peon *et al.*, (1964) have studied diazepam extensively in experimental animals and concluded that this agent has a generalized depressant action upon epileptogenic structures throughout the brain. These authors considered that the drug may influence the activity of the cortex independent of its actions on subcortical arousal mechanisms. An interesting finding by Hernandez-Peon and his co-workers was that atropine produced EEG effects opposite to those resulting from diazepam.

Recent crystallographic studies by Camerman and Camerman (1970) have revealed that DPH and diazepam, though not chemically related, show marked similarities in their molecular conformations. The results indicate that there is a steric basis, according to C-5 substitution of the barbiturate nucleus, for their anticonvulsant activity. Stark *et al.*, (1970) studied the seizure susceptibility of a population of baboons to intermittent photic stimulation to anticonvulsants and benzodiazepines. These investigators found that phenobarbital and benzodiazepines analogous to diazepam were effective anticonvulsants. Diphenylhydantoin was shown to exhibit slight differences in action but was still as effective as the other agents. Pentobarbital has less effective anticonvulsant actions than diazepam (Wesseling *et al.*, 1971).

Straw (1968) has investigated the anti-metrazol effects of several benzodiazepines and found that all of the agents tested significantly increased seizure threshold, but that none of them had any effect on seizure duration. On the other hand, Schallek *et al.*, (1964) reported that benzodiazepines do not affect seizure thresholds in the cortex.

In agreement with my results, Guerrero-Figueroa *et al.*, (1969) have observed that diazepam and Ro5-4023 suppressed epileptiform activity

by depressing the spread of epileptiform discharges. These authors noticed that these agents did not produce significant changes in the spontaneous epileptiform activity, nor of the local evoked potentials recorded from primary focal epileptogenic lesions.

Therefore, it can be concluded that there is good evidence for the benzodiazepine derivatives diazepam, chlordiazepoxide, and Ro5-4023 (Clonazepam) to limit the spread of epileptiform afterdischarges in the chronically neuronally isolated cortex.

XV. GENERAL DISCUSSION AND SUMMARY

From the experimental evidence presented in this thesis it becomes evident that chronic neuronal isolation of a slab of cerebral cortex results in that tissue becoming abnormally hyperexcitable. With increasing periods of isolation the propensity of deafferented cortex to produce prolonged epileptiform afterdischarges is augmented. This confirms previous observations on similar preparations by other investigators. One obvious explanation for this characteristic tendency is that cortical excitability is under the control of various subcortical structures in the brain. When the cortex is completely isolated, the afferent fibres that would exert mainly a restraining influence on cortical excitability are severed. A good deal of evidence has been reported showing the histopathomorphological and electrophysiological changes in isolated cortex and has discussed their apparent relationship to the observed generalized increase in cortical excitability. These previous studies suggest that many possible factors could be involved in the resultant lack of tonic control which presumably contribute or result from the phenomena described.

There is a paucity of firm data dealing with the possible role of chemical mediators in the processe(s) governing cortical excitability. Previous pharmacological studies have suffered from the fact that responses were too unstable and the factor of fatigue could not be ruled out. This study has succeeded in stabilizing the EAD response elicited on chronically isolated cortex, which made it possible to conduct meaningful drug studies bearing on the possible pharmacological mechanisms most likely involved in this increased epileptogenicity. It is reasonable to assume that alteration of some of the synaptic mechanisms, apparently operative

in normal cerebral cortex, may have a role in the spread and maintenance of paroxysmal electrocortical potentials.

The results of the drug studies that I have reported in this thesis suggest that certain deficiencies in the synaptic function of the isolated cortex are involved in the altered excitability of this relatively independent aggregate of neurons. My studies with autonomic agonist and antagonist agents also indicate that there is still remaining a substantial intracortical system capable, to a certain extent, of holding in abeyance paroxysmal epileptiform potentials.

It is tempting to speculate that if all inhibitory influences were removed from an area of cortical tissue, it would continuously discharge in a paroxysmal pattern. This apparently inevitable result of denervation also suggests that one of the innate properties of brain cells is that of automaticity, and that cortical activity is mainly controlled by inhibitory inputs, which regulate what would otherwise become a purposeless and random discharging of excitable elements. Of course, one factor that has not been studied adequately is, which is the smallest unit that would display this automaticity, e.g. a single neuron, two neurons in a feedback circuit, and so on. On this basis the chronically isolated cortical slab presents a reasonable group of neurons for studying pharmacological interactions at the cortical level.

The overall picture that emerges from this study is that acetylcholine, particularly through its muscarinic effects, has a predominantly inhibitory function and is, as far as the experimental method could detect, the final link in an inhibitory chain of synapses. It is assumed that the cholinergic linkage functions through muscarinic type receptors because

nicotine and anti-nicotinic agents do not show any action upon the parameters that were studied. Previous discussion has indicated that there is a great deal of evidence for muscarinic inhibitory effects in the cortex. It is possible that nicotinic agents may show pharmacological activity in the cortex only when the cholinergic system is intact. This may explain why some authors report EEG effects for nicotine.

The action of cholinergic agonists and antagonists, in every instance, prevailed over all other types of agents tested. This result leads, reasonably, to my assumption that the final synaptic linkage in the inhibitory chain modulating EAD duration in the cortex is cholinergic. Since antimuscarinic and anticholinesterase agents modified EAD duration, this is strong evidence for some endogenous cholinergic tone being present in the isolated cortical slab. None of these agents would be active if complete cholinergic structures and mechanisms were not present. The exact means by which cholinergic inhibitory neurons achieve their effect is not known. Perhaps they interrupt, through hyperpolarization, the facilitated and greatly synchronized activity which appears to represent the intrinsic augmented rhythmicity of the post-synaptic membrane of epileptogenic neurons. In this context, graded inhibitory activity would be important for setting the level of cortical discharges.

Acetylcholine could be achieving inhibitory effects upon cortical elements by other means. One possibility is that ACh is causing a persistent depolarization of neurons, spreading from a synaptic junction to other parts of the cell. Another possibility is that a curare-like effect of ACh following repolarization by means of an ACh-receptor complex. However, the relative ease with which atropinics antagonize and reverse

the effects of cholinergic agents argues against these possibilities.

As the previous discussions in this thesis have pointed out, the convulsant actions reported for ACh by other authors are perhaps due to unspecific actions owing to the method of applying the agent under study. Since many of these studies were acute experiments, it is further possible that recent surgical trauma may have played a significant role in the expression of the final result. Moreover, it is not clear at what sites ACh may have been acting since intact brains were usually investigated.

The results with serotonin agonists and antagonists also suggest that there is present in the isolated cortical slab, a certain degree of inherent serotonergic tone. This is assumed because serotonin antagonists were effective in altering EAD duration. However, as the cholinergic and anticholinergic agents studied were capable of overcoming the action of 5-HT agonists and antagonists it must be assumed in the framework of the present study that the serotonergic mechanism is subservient, to the cholinergic one.

Throughout this discussion it has been assumed that all of these agents acted upon their respective and discrete receptive structures. It is further assumed that the distinctly sensitive neurons respond with an action relevant to the proper physiological function of the cerebral cortex. On this basis, it has been possible to categorize several groups of neurons according to their observed sensitivity to given classes of drugs. It is unlikely that the doses of drugs used were excessive for it proved possible to demonstrate, with each type of agent, significant blockade and reversal of actions when using the appropriate blocking agents. Thus, there is every indication that the agents studied were acting on relevant

and pharmacologically sensitive neurons, especially in the case of cholinergic agents, because the actions of this group of agents are comparatively more specific. Moreover, it was possible to show the same effect with several different cholinergic drugs.

Unfortunately, the picture is not as well elucidated in the case of serotonin agonists and antagonists, where the choice of discrete agents is rather limited, and the action of the compounds tested in my experiments, is not specifically exerted on 5-HT receptors alone. In any case, a complex population of neurons is under study. However, as discussed at greater length previously, there is growing evidence for the presence in the cortex of neurons that respond to serotonin. Therefore, pending further developments, the present limited data suggest a role for serotonin as a pharmacological modulator of electrocortical activity.

The situation becomes even less clear with respect to the role in the cortex of adrenergic α-receptor and β-receptor agonists and antagonists. It is quite apparent from my results that there is no adrenergic tone in the isolated cortical slab. At relatively physiological doses most of the adrenergic antagonists investigated did not alter EAD duration in a consistent fashion, in spite of several of these agents having relatively specific adrenergic blocking properties. Unfortunately, much essential information concerning their central actions is presently not available and certain of them possess marked and undesirable local-anaesthetic properties. Since no clear pattern emerged from the many experiments with adrenergic receptor, α and β, blocking agents, it would seem that effects observed with some of the adrenolytic compounds are probably due to general actions on non-specific sites.

Nevertheless, adrenomimetics appear to have a role, albeit a small one, in EAD modulation as all the centrally active adrenergic drugs tested were capable of modifying EAD duration. Unhappily, the results obtained on the chronically isolated slab do not permit stating with confidence whether this action is a direct one upon α-adrenoceptive sites or whether it is an indirect one mediated by the release of serotonin in the cortex. In the previous discussion it was pointed out that there are many uncertainties concerning the central actions of sympathomimetic substances. For example, applied locally these effects appear to be depressant, whereas when studying their effects after systemic administration various types of excitatory phenomena predominate. In the latter case, it might be that systemic administration results in powerful excitatory effects at subcortical sites which predominate over depressant actions in the cortex.

Since the cell bodies of adrenergic neurons appear to be confined, for the most part, to subcortical regions, and since the isolated cortical slab studied had been denervated for a long period of time, it must be assumed that either amphetamine was acting directly upon an adrenoceptive structure, or indirectly through release of some other neurohumoural candidate, possibly serotonin. An α-adrenergic receptor blocking agent which partially antagonized the action on EAD duration of amphetamine, did not show any blockade of the action of the serotonin agonist. On the other hand, more specific and direct acting adrenergic agents (ephedrine, tyramine and phenylephrine) also decreased EAD duration. It is tempting to speculate that there are some relatively specific adrenoceptive structures. It is also possible that these adrenergic compounds were activating dopa-

minergic structures. As this latter possibility was not explored extensively in this thesis, it warrants further consideration and examination.

If for the purpose of this discussion it is assumed that in my experiments sympathomimetics are acting directly on adrenoceptor-like cortical structures, certain conclusions can be drawn. Under these conditions an adrenergic receptor must be present in the isolated cortex. Since it would be expected that the chronic denervation results in degeneration of presynaptic elements, the loss of this afferent system would be responsible in part for the lack of restraint in the isolated cortex. Neither the cholinergic or serotonergic structures seem to have been affected to the same extent in terms of their residual tonic effects. Therefore, in the trans-synaptic (series) chain that modulates EAD duration, it can be assumed that the first link in this intracortical inhibitory chain is probably adrenergic.

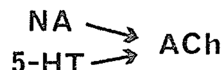
There are several possibilities for the cholinergic, serotonergic and adrenergic synaptic elements to achieve their modulatory effects in chronically isolated cortex. The vast array of cortical cell types and intracortical connections present in the cortex would provide for a multiplicity of synaptic arrangements, all of which probably do exist to varying degrees. However, it is not unreasonable that there be some preferred arrangements and the results presented in this thesis support certain synaptic systems over others.

Several different equivalent synaptic circuits can be devised in which are arranged cholinergic, serotonergic and adrenergic elements so as to achieve inhibition of electrocortical potentials:

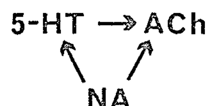
1. A series system of serotonergic-noradrenergic-cholinergic synapses respectively, i.e.

2. A parallel system of serotonergic, adrenergic and cholinergic synapses i.e. 3 independent circuits.

3. Adrenergic and serotonergic synapses in parallel to each other, but both subtended onto a cholinergic neuron



4. A basic unit of serotonergic-cholinergic linkages, i.e. 5-HT-ACh. A branching terminal activated by adrenergic agents might be in contact with both serotonergic and cholinergic structures, i.e.



5. A trans-synaptic system of adrenergic-serotonergic-cholinergic synapses respectively, i.e.

The possible synaptic arrangements in which cholinergic structures are not the final linkage in the trans-synaptic chain will be disregarded for the purposes of this discussion, because the hierarchy of the pharmacological antagonisms determined experimentally indicates that the cholinergic component is so situated as to be capable of overcoming the action of all other types of agents tested so far.

As discussed above, it is required that the adrenergic component must be the first linkage of the inhibitory neuronal chain. This rules out the first possibility where the adrenergic component is situated in an intermediate position. The second possible equivalent circuit also does not conform with the pattern of results obtained. With this arrangement it would not be possible to antagonize the action of one type of

agonist with an antagonist of another type. However as the results show, it was possible to block the action upon EAD duration of serotonergic and adrenergic agents with atropine, and furthermore, serotonin antagonists blocked the action of adrenergic agents. Also, it should be possible to show that the different types of agonists should potentiate each other. This latter possibility was not tested.

In the third proposed circuit there should be potentiation between the action of the adrenergic and the serotonergic agonists, however, this was not tested. Also, the serotonin blocking agents should not antagonize the action of adrenergic agonists. As my results show that serotonin blocking agents do antagonize the action of adrenergic drugs, this possible equivalent synaptic arrangement can be discarded.

The fourth equivalent circuit can also be discarded because with this arrangement one of its requirements would also be that serotonin blocking agents should not alter the action of adrenergic agonists. It is quite clear that the serotonin blocking agents block completely the action upon EAD duration of amphetamine.

The fifth proposed equivalent circuit appears to be the only one that is in agreement with the hierarchy of pharmacological antagonisms that have been described. In this more probable scheme it appears that the cholinergic neuron has a major role, the serotonin neuron a relatively intermediate role, and noradrenaline a comparatively minor role. The experimental results obtained do not allow a clearer definition of whether adrenergic agonists are active through release of serotonin or some other mediator substance. Until more specific agents become available it will not be possible to describe sympathomimetic effects in the cortex in

greater detail.

The series system NA - 5 - HT - ACh satisfies the pattern of experimental results wherein atropine blocks the effects of adrenergic, serotonergic and cholinergic drugs. Also explained would be the antagonism by serotonin blockers of the action of amphetamine. A sufficient number of serotonergic and cholinergic cell bodies must be present in the isolated cortex to account for the observed significant serotonergic and cholinergic tone. As mentioned previously, it was not possible to demonstrate that adrenergic tone exists in this preparation. Therefore, the experimental evidence provides considerable support for the fifth possibility of an equivalent trans-synaptic circuit.

In this attempt to construct an equivalent circuit in which all the elements are reduced to their linear components, no account is made of the infinite number of collateral intracortical connections possible in the dense cortical meshwork. The discussion is based on the working hypothesis that the given classes of agonists and antagonists used are acting at pharmacologically sensitive and specific neuronal sites. By studying their response upon application of a system of hierarchies of putative mediators it is possible to determine the approximate function and extent of physiological interaction of such neurons for drugs acting acting at the cortical level. This provides some interesting clues regarding the susceptibility of EAD duration to some types of drugs.

On first inspection it appears somewhat peculiar that all of the mimetic agents tested had the same effect, i.e. inhibition of afterdischarge duration. As discussed previously, there is much evidence for extensive inhibitory mechanisms at the cortical level. Teleologically it

would not prove to be in the best interests of the whole organism to have synaptic mechanism(s) to promote convulsions. Certainly, such a proconvulsant capacity would hardly favour the survival of a species and would serve, ostensibly, to exacerbate pathological responses. It seems to be more desirable that there are extensive pharmacological inhibitory mechanisms which would apparently assist in preserving the organism. The capability for the brain to be activated in a highly synchronous oscillatory way is inherent in its neuronal elements, with its complex circuitry, and its innumerable feedback mechanisms and interconnections. Only the presence of strong regulatory influences would prevent excitation of certain circuits from spreading explosively throughout the entire cortex. Thus, inhibitory influences would channel excitation through the appropriate pathways and modulate the activity of a given population of neurons. Disruption of this inhibitory circuitry very likely is a major factor in epileptic seizures. Therefore, it seems to be a reasonable finding that it was not possible to show conclusively an excitatory action for any of the central neurohumoural candidates investigated; acetylcholine, serotonin and noradrenaline, in prolonging EAD duration. On the contrary, this thesis shows that disruption of the function of these mediator systems with appropriate antagonist agents significantly augments EAD duration in the isolated cortex.

The effects upon EAD duration of anti-epileptic agents appear to be restricted to the class of agents known to be effective in the treatment of _grand mal_ epilepsy. Furthermore the onset of action of these agents, diphenylhydantoin and phenobarbital, is rapid, and is not directly related to the general depression of CNS function since pentobarbital which

depressed the animals does not in itself significantly alter EAD duration in the isolated slab. This suggests that the chronically isolated cortical slab might be useful as an experimental model of epilepsy for the study of agents effective in grand mal epilepsy. It is interesting that two of the agents, diphenylhydantoin and to a lesser extent, phenobarbital, show interactions with cholinergic and anticholinergic drugs. On the other hand, ethosuximide and trimethadione, two anti petit mal agents, have no effect upon EAD duration, nor do they show interactions. Since diphenylhydantoin is known to limit spread of seizure discharges in the brain, it might be that this beneficial effect is achieved, at least in part, by activation of cholinergic synapses which are part of an inhibitory chain.

It is possible that the anti-epileptic agents are acting upon other mechanisms, e.g. threshold and membrane effects. However, the present data do not allow any firm comment regarding their role in the generation, spread and maintenance of the EAD in isolated cortex. Most likely these and other neurochemical factors are acting in concert with some of the pharmacological effects observed. In any case, the results presented in my thesis indicate that synaptic mechanisms can achieve powerful inhibitory effects on the cortex, and part of the action of anti-epileptic agents effective against this paroxysmal response, may be exerted through these same synaptic pathways and mechanisms.

In summary:

1. This study has shown that it is possible to elicit consistently stable EADs of repeatable duration in chronically isolated cortex. This achievement permitted the meaningful statistical analysis of the action of diverse autonomic and anticonvulsant agents upon EAD duration.

2. Cholinergic agonists decrease EAD duration, apparently through muscarinic actions. Antimuscarinic agents, on the contrary, enhance epileptiform responses and antagonize the effects of cholinergic drugs. Cholinergic drugs without central actions or acting at nicotinic sites in the periphery are without effect on this parameter and do not influence the response to muscarinic or antimuscarinic drugs.

3. Enhancement of serotonin activity decreases EAD duration, an effect which is blocked by antiserotonin and anticholinergic agents, but not by adrenolytic agents. Antiserotonin agents on their own prolong EAD duration and block the action of adrenergic, but not cholinergic, agonists upon EAD duration.

4. Adrenergic drugs decrease EAD duration and this action is antagonized by agents known to block adrenergic receptors in peripheral structures. Adrenergic blocking agents do not have by themselves any clear effect on EAD duration. Anticholinergic, and antiserotonin drugs block the action on EAD duration of adrenergic agonists.

5. The duration of the EAD is decreased by anti-*grand mal* agents, but not by those effective in *petit mal* epilepsy. Some interactions were demonstrated between the actions upon EAD duration of anti-*grand mal* agents and cholinergic agents. These observed interactions suggest that the action of diphenylhydantoin upon EAD duration could be partially exerted through cholinergic structures. It proved possible to demonstrate a specific anticonvulsant action for phenobarbital dissociated from its well known general depressant properties, whereas pentobarbital did not behave as an 'anticonvulsant', even at almost anaesthetic doses.

6. The results presented in this thesis suggest that the chronically isolated cortical slab constitutes a suitable model of grand mal epilepsy.

7. The total evidence presented seems to support the hypothesis that there is in the cerebral cortex an inhibitory chain of neurons which function to hold in abeyance paroxysmal electrocortical activity. The experimental evidence indicates that this trans-synaptic neuronal chain is composed of adrenergic, serotonergic and cholinergic structures arranged in series respectively.

8. The hyperexcitability of the isolated cortical slab could be due in part to the denervation of adrenergic structures in this chain. Epileptiform disruption of normal electrocortical activity appears to be a property inherent to the complex neuronal network of the brain, and probably, most mediator and modulator systems present in the cortex function to prevent paroxysmal epileptiform discharges.

XVI. RECOMMENDATIONS FOR FURTHER STUDY

Although this investigation has succeeded in stabilizing the EAD elicited in chronically neuronally isolated cortex and tested the susceptibility of this response towards several classes of pharmacological agents, certain questions raised by previous authors and the present work still remain open.

More precise quantitative studies on the effects of drugs upon EAD duration are necessary to permit the construction of reliable log-dose response curves for each agent tested. A more direct route of drug administration, e.g. intravenous or intra-arterial may result in effects relatively proportional to the dose of drug injected than has been the case with the results in this thesis obtained using the intraperitoneal route. Knowing better the response to standard doses of given agents will be required when analysing the response to different agonists in animals pretreated with specific blocking agents or depleting agents. It is essential to proceed and qualify the character of the interactions between different agents in order to describe these effects definitively in terms of competitive, non-competitive, or physiological mechanisms. Moreover, these curves will be useful in determining whether certain agonists potentiate the effects of other types of agents.

Reviewing the literature makes it quite clear that there is indeed little correlation between the activity of units at given cortical depths and epileptiform at the surface. Therefore more extensive microelectrode-macroelectrode studies in chronically isolated cortex at various periods after deafferentation may bridge this important gap in our knowledge. It would prove to be more significant

if it could be shown conclusively that unitary responses are related to and affected in the same way by treatments that affect the generation of electrocortical potentials recorded with macroelectrodes. This work may provide an estimate of the minimum number of elements or portion of isolated cortex that is required to generate an EAD.

The increasing importance of biogenic amines in cerebral phenomena makes it necessary to administer more specific monoamineagic agents in order to study more precisely the role of catecholamines and serotonin in the modulation of EAD duration. These studies should be combined with those in which agents depleting cerebral stores of specified biogenic amines are also administered, keeping in mind that these substances are relatively new pharmacological tools.

Studies with a push-pull cannula that would permit localized superfusion of the cortical surface might provide useful corroborative evidence regarding the output of mediator substances. If it could be shown that drug effect on the ECoG and unitary activity was correlated with the release of neurohumoural substance(s) this would satisfy one of the criteria establishing the identity of a mediator.

Histological studies bearing on more localized histo-morphological and histochemical changes in discrete areas of isolated cortex will be necessary to strengthen interpretation of more extensive microelectrode studies. It is still not possible to state with authority which units are participating in prolonged epileptiform discharges.

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