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THE ANESTHETIC PROPERTIES OF DRUGS WHICH ACT IN THE CENTRAL NERVOUS SYSTEM

by

© KHEM JHAMANDAS

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Anesthetic Properties of Drugs which act in the Central Nervous System", submitted by Khem Jhamandas in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

It has been shown previously that both local and general anesthetics produce a depression of the central nervous system by blocking the action potential in the excitable tissue by a single mechanism. The capacity of a large number of structurally unrelated drugs to depress the central nervous system suggests that their depressant action is a manifestation of their anesthetic properties, and the mechanism of this action at the cellular level may be similar to that of the local and general anesthetics.

In the intact animals the central depressants, diphenhydramine, promethazine, chlorpromazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine and meperidine, when given alone caused sedation in small doses while they produced excitement and convulsions in high doses. Excepting chlorpromazine, they did not produce a loss of righting reflex similar to that produced by phenobarbital. But when given to mice in convulsant and nonconvulsant doses after pretreatment with subanesthetic doses of phenobarbital, they all produced a state of central depression indistinguishable from anesthesia. Meprobamate, diazepam and chlorpromazine produced a loss of righting reflex in mice when given alone, but they also enhanced the central depression induced by phenobarbital in doses which were ineffective when given singly. In contrast, the classical central stimulants such a bemegride and picrotoxin, which produced convulsions when given alone, antagonized the central depression induced by phenobarbital, while others such as caffeine, nikethamide and strychnine did not alter this depression. But none of these drugs

enhanced the action of phenobarbital.

In the neuronally isolated cortical slabs driven by single shocks, the central depressants when applied locally, or when given by injection as in the case of meprobamate and diazepam, produced only a decrease in the size and an increase in the stimulus threshold of the evoked responses of the cortical tissue. None of these agents facilitated the cortical responses even when applied to the tissue in high concentrations. In contrast local application of all stimulants to the isolated slab produced a facilitation of the responses evoked by direct stimulation and a decrease in the stimulus threshold. In addition, some of these stimulants produced a spontaneous discharge, unrelated to the applied stimulus, when they were applied to the cortical tissue.

The results indicate that a large number of drugs possess anesthetic properties that are qualitatively similar to those of local and general anesthetics, but may differ from the latter quantitatively in respect to the potency of their action in producing the various stages of anesthesia. Their action at the cellular level, which appears to be only inhibitory, may be brought about by the depression of the mechanisms responsible for the generation and the conduction of the action potential in the neuronal tissue.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

To my Father.

INTRODUCTION

In recent years the introduction of a large number of drugs for the disorders of the central nervous system is a reflection of the current interest in the field of neuro- and psychopharmacology. However, in spite of the spectacular advances made in the development of new neurotropic drugs, an adequate knowledge concerning their site and the mechanism of action is still lacking. As a consequence of this most of the therapy for the disorders of the central nervous system is largely empirical. It has become increasingly clear that various degrees of central depression such as 'sedation', 'hypnosis', 'tranquilization', 'analgesia', and 'anesthesia' can be produced by many compounds which have a broad spectrum of pharmacological activity. A rational classification of such agents, which could be responsible for an intelligent application of these drugs, is not available as yet. The present classification of drugs has a traditional rather than a scientific basis and rests mainly on the therapeutic use of the drugs. This has the disadvantage that in application of the drugs, attention is focussed only on the effects for which each drug is employed. As a consequence of this other useful properties that the drug in question may have are largely ignored because of the rigid, therapeutically based, classification. For any drug classification to be meaningful it should be based on a knowledge of the site and the mechanism of action of the drugs rather than their clinical effects, so that all the useful properties of the drugs may be fully exploited. While an advance in this direction has been made for drugs which act on the peripheral nervous system, those acting on the central nervous system still lag behind in their classification, owing to a poor knowledge of their

mechanisms of action.

The poverty of knowledge regarding the mechanism of action of drugs acting on the central nervous system can be chiefly attributed to the unrivalled complexity and the inaccessibility of this system. Its physiological and biochemical functions are poorly understood. But in spite of such a lack of information a large number of drugs currently are being used to study the cellular and the molecular organization of the nervous system. On the basis of this scant knowledge, the centrally acting drugs are being used experimentally to study fundamental functions such as central excitation and inhibition in the anticipation that such studies would yield further information.

In the course of studies on the neuropharmacology of drugs a considerable amount of data has accumulated as indicated by the voluminous literature on this subject. Such data has been chiefly derived from studies on the electrical responses of the nervous system, and also from the chemistry of the brain - chiefly the metabolic aspects. Very few systematic attempts have been made to study the basic mechanisms of drug action on the brain. On the basis of the electrical and chemical data thus available some meaningful attempts have been made to explain the mechanisms of centrally acting drugs. Among the drugs which have received most of the attention, the general anesthetics are perhaps the oldest, and rank first in the frequency of investigation. However, it is notable that in spite of extensive study and theorization, the cellular basis of general anesthesia still is not clearly understood. None of the many theories of anesthetic action proposed thus far has received a universal acceptance. It also seems likely that a

further investigation and thereby a fuller understanding of the action of general anesthetics would contribute significantly to studies of other, more specific types of drug action on the central nervous system and hopefully to a proper classification of drugs which modify the activities of the central nervous system.

I GENERAL REVIEW

I A. Theories of the anesthetic action of drugs.

The mechanism of action of anesthetic drugs has been a subject of investigation for almost a century. Various theories have been postulated as a result of investigations involving not only biological systems but also physical systems as models. Since this subject has been reviewed extensively in the past (Butler, 1950; Pittinger, 1959; Featherstone et al., 1963), the present review mainly will be confined to the most general aspects of the theories of anesthesia with particular emphasis on the major criticisms that have been raised against various theories. Like most other theories in biological sciences, the theories of anesthesia often reflect the information and the experimental methodology available at the time of proposal. With the improvement in techniques and the expansion of theoretical knowledge some of these theories have been discarded. Others that have been sustained are subjected to continuous modification in light of newer information.

Before discussing the theories of 'anesthesia' or 'narcosis', it is necessary to distinguish between these two terms. Although they often are used interchangeably, anesthesia and narcosis are not synonymous. In simplest terms narcosis can be defined as a general and reversible cellular depression produced by chemicals. Anesthesia on the other hand, is a clinical term which implies a special form of depression. General anesthesia may be defined simply as a reversible state of depression involving a loss of sensory perception and consciousness.

i) Physical theories of anesthesia.

One of the most notable features of drugs which produce general anesthesia is the great variety in chemical structure of the compounds and a great difference in the effective doses. Since the anesthetic property has not been correlated with any particular chemical grouping, attention often has been directed towards the physical properties of the anesthetic compounds. Most of the physical theories of anesthesia postulated over the last sixty years have tried to explain the mechanism of anesthesia by seeking correlations between the potency of an anesthetic on one hand and some physical property of the anesthetic molecule on the other hand.

Thus the 'Precipitation Theory' or the 'Colloidal Theory' proposed by Claude Bernard (1875) postulated a causal relationship between protein precipitation and narcosis. The 'Lipid Theory' of Meyer (1899) and Overton (1901) proposed a causal relationship between anesthetic potency and the relative solubility of anesthetic drugs in olive oil and water, expressed as the partition coefficient between the two phases. Other physical properties that have been similarly correlated with potency include the surface activity of the compounds as in 'Traube's Theory' (Traube, 1904), the thermodynamic activity as in the 'Ferguson's Hypothesis' (Ferguson, 1939), and more recently the ability to form hydrates as in the case of theories proposed by Pauling (1961) and Miller (1961).

A general weakness of most investigators who have sought the aforementioned correlations is found in the fact that they have confined their study to only a few types of narcotic agents. Very often a relationship

has been sought in a homologous series of compounds such as the alcohols rather than amongst the compounds of a diverse structure producing the same end effect. While this criticism is valid for the older theories of anesthesia, the newer theories such as those of Ferguson, Pauling, and Miller have tried to embrace a wider series of structures including such agents as the inert gases. Ferguson (1939) approached anesthesia from a thermodynamic viewpoint. He contended that maintained anesthesia is an equilibrium condition. Such being the case he argues that since the thermodynamic activity is equal in all phases of the equilibrium system, then the activity of the anesthetic in the external environment is an index of the activity of that agent at the site of action. The activity in the external phase can be measured and therefore its value in the biological phase is known. Ferguson calculated activity values for a wide variety of volatile anesthetics and found that most of these substances fell in a narrow range. He concluded that the main cause of anesthesia was the same for all drugs giving the same activity values and assumed that these agents had a non-specific action which was purely physical in nature. In contrast to these, other agents whose activity values fell outside the narrow range were assumed by him to be acting both by a physical and by a chemical mechanism to produce anesthesia. Although Ferguson's calculations are based on firm theoretical principles of thermodynamics, it can be argued that these do not offer any explanation for the actual site of action or the basic mechanisms of action of anesthetics. There is no evidence to support Ferguson's main thesis that anesthesia is the result of an action by a physical mechanism. Furthermore, his calculations do not apply to all the anesthetics

and two separate theories are required to explain the anesthetic mechanism - one based on physical effects and one on chemical effects. Recognizing the fact that thermodynamics alone cannot explain the mechanism of anesthesia, Mullins (1954) proposed that while the number of molecules of the anesthetic drug reaching a particular site is important, the size of such molecules is equally important. He suggested that the site of action of anesthetic agents is situated in a highly polar, non-aqueous phase of the cell which he considers to be the membrane. Anesthesia results when a constant fraction of the total membrane volume is occupied by the molecules of the anesthetic. He further contends that an occupation of the critical portions of the membrane might interfere with permeability to ions or molecules for cellular function.

Pauling in 1961 put forward a theory of anesthesia, which has since become known as 'Pauling's Microcrystal Hypothesis'. Briefly he found that a good correlation existed between the partial pressure of the anesthetic gases required to produce narcosis and the partial pressure necessary to form hydrate crystals of the anesthetic gases. On basis of this, Pauling suggested that anesthetics act by formation of hydrate crystals in the brain, particularly in the synaptic regions. However, Pauling recognized that anesthetic action could not be simply the result of hydrate crystals in the brain since none of the hydrates of the gases studied were stable at physiological temperatures and pressures. He therefore assumed that to fully account for the formation of such crystals in the brain other substances present in the brain fluid also take part in stabilizing the hydrate crystals. Substances that might add such

stability to hydrate crystals could be the charged side chains of proteins and the solutes of the brain fluid. Pauling further assumed that the formation of such microcrystals of gas hydrates in the synaptic region would lead to their entrapment and thus in depression of conduction in the neural networks involved in the maintenance of consciousness (Pauling, 1961).

Miller in 1961 also independently formulated a similar role for hydrates in the mechanism of anesthetic action of drugs. On the basis of his studies on the thermodynamic properties of aqueous solutions of anesthetic gases, Miller suggested that a portion of the anesthetic gas molecules orient water around themselves so that the molecules are surrounded by a shell of water. This highly structured water was termed 'iceberg' or 'ice-cover'. His calculations showed that a hypothetical surface covered with structured water in different stages of anesthesia was proportional to the pressure of anesthetic gas present. Whereas Pauling's theory could not account for the failure of production of crystalline hydrates of larger anesthetic drug molecules such as diethyl ether, Miller's proposal did embrace the anesthetic action of diethyl ether in view of its considerable solubility in water. This solubility of ether in water is indicative of an interaction between water and ether, and it would seem that ether-like, organic molecules are surrounded by an 'ice-cover' too. Miller, like Pauling assumed that structured water might lower the electrical conductance of the brain tissue, or it might "stiffen up" lipid or other membranes, or it might "plug up" the membrane pores.

Both Pauling and Miller's theories of anesthetic action give no exten-

sive explanation of the manner in which the microcrystals or the icebergs may produce anesthesia. Although between them these theories provide a possible explanation for the gaseous or volatile anesthetics, they do not appear to account for a large body of the nonvolatile anesthetics. It also may be questioned to what extent the formation of the crystals or 'ice-bergs' occurs at physiological levels of temperature and pressure. It appears that newer physical theories are more specific for a few chemical structures and essentially resemble the older theories in this respect.

This examination of the physical theories of anesthesia indicates that although anesthetic potency is associated with several physical properties of the active molecules, none of these theories allows one to determine the basic mechanism of the anesthetic action of the drugs. Many processes occur between the administration of the anesthetic and its arrival at the final site of action. Thus the physical properties may account more successfully for such processes as the transport and distribution of the various anesthetics rather than explain the basic pharmacological action of all anesthetics.

ii) Biochemical theories of anesthesia.

The basis of all biochemical theories of anesthetic action can be traced to the relationship between oxygen deprivation and cellular depression. With the introduction of anesthesia, the cellular depression caused by these drugs suggested a possible relationship between anesthetic action on one hand and a decrease in oxygen consumption on the other. On the basis of early studies in which it was shown that perfusion solutions devoid of oxygen caused a loss of the nervous function, Verworn (1903) postulated a

mechanism of narcosis. The main theme of Verworn's postulate, which has also been called the 'Asphyxial theory', is that narcosis in the nervous tissue is the result of oxygen deprivation. For some time interest in this theory was sustained by observations that the effect of anesthetics appeared to be somewhat greater than normal in the absence of oxygen. Over the years, however, considerable evidence has accumulated to show the inadequacy of the 'asphyxial theory', and related theories. Thus it was shown by Usui (1912) that concentrations of urethane and its derivatives necessary to inhibit oxidation in frog brain slices were very much higher than those producing anesthesia. As early as 1915 Winterstein demonstrated that the concentration of ethanol required to produce anesthesia in frogs in fact resulted in increased oxidation of the nervous tissue (Winterstein, 1915).

In spite of its serious limitations the asphyxial theory was the forerunner of the current theories of anesthesia based on the inhibition of the oxidative metabolism. The work of Quastel and Wheatley on the oxygen consumption of the brain tissue showed that a number of anesthetics such as barbiturates, ethyl alcohol, ether, chloroform, urethane and several others arrested the oxidative metabolism of the tissue in vitro, as reflected by oxygen utilization (Quastel and Wheatley, 1932, 1933, 1934; Quastel, 1939). The depression of oxidative enzyme systems in the brain was postulated by Quastel as the mechanism of anesthesia on basis of its correlation with the anesthetic action. He further suggested that anesthesia is the result of local effects on certain specific enzyme systems rather than hypoxia in the whole brain.

The metabolic theory of anesthesia proposed by Quastel and his group has been refuted on several grounds. Thus while Quastel *et al.* (1934) demonstrated that the oxidative inhibition produced by chlorbutanol and phenobarbitone was reversible, the effects produced by potent anesthetics such as ether (Jowet and Quastel, 1937) and ethanol (Fuhrman and Field, 1948) proved to be irreversible; a property incompatible with anesthesia. It has also become evident that the concentration of these agents required to inhibit biological oxidation *in vitro* were far beyond those tolerated in the living animal. A more serious weakness of this theory stems from the fact that the depression of oxygen consumption is an effect not uniquely confined to anesthetic drugs. It has been shown for non-anesthetic substances such as phenylethylamine, tyramine, indole, isoamylamine and others (Quastel and Wheatley, 1933), metrazol (Shideman, 1949) and picrotoxin (Klein, 1943). It is noteworthy that both metrazol and picrotoxin are potent convulsant substances. A theory of anesthesia demanding serious attention should in some measure be able to explain the differences between anesthetics which produce central depression and the convulsants which produce overt excitation of the central nervous system. So far the metabolic theory has failed to make this distinction.

More recently, attention has been focussed not so much on the oxidative processes *per se* as on the mechanisms responsible for the transfer of the energy obtained from oxidation to cellular function. Butler (1950) noted that many anesthetics can inhibit the breakdown of adenosine triphosphate (ATP). Indirect evidence obtained by Caldwell and Keynes (1957) has shown that cleavage of three high energy phosphate bonds is

necessary for the extrusion of one sodium ion from the nerve. On basis of this Hodgkin (1958) postulated that ATP is the energy source for sodium extrusion. Thus any agent inhibiting the breakdown of ATP would prevent the cell from carrying out its normal function of sodium extrusion. At present there is not sufficient data to support a hypothesis such as this. If the anesthetics did indeed inhibit the sodium transport mechanism, through inhibition of ATP breakdown, the nerve or muscle would be expected to be depolarized by anesthetics. But this does not seem to be the case (Thesleff, 1956; Yamaguchi, 1961; Inoue and Frank, 1962a).

It appears that so far there is no satisfactory biochemical theory which successfully relates a specific biochemical change in the neuronal tissue to anesthesia. It is also unknown whether the biochemical changes in the brain tissue accompanying anesthesia are really the cause of this phenomenon or merely the result of it.

iii) Membrane theories of anesthesia.

It is now universally recognised that the cell membrane is an essential unit in the control of cell permeability and the maintenance of the intracellular environment. In excitable tissues such as muscle and nerves, the cell membrane plays a fundamental role in the initiation and propagation of excitation. It would be expected, with good reason, that agents which modify excitability of the living tissue may do so by acting at the cell membrane level. Although the physical theories of narcosis have drawn attention to the membrane, none of these theories has explained in any extensive manner the way in which the membrane excitability is altered by narcotic substances. Whatever suggestions were made by the proponents of

these theories in this respect have been based on assumptions rather than on experimental evidence.

Lillie in 1923 was the first to propose what is often termed the 'Permeability Theory of Narcosis'. Many of his studies were concerned with observations of the cellular uptake or discharge of pigment substances, both of which processes seem to be inhibited by the presence of narcotic agents. In view of his own work and the preceding observation of Osterhout (1913) that the presence of a narcotic increased the electrical resistance of the cells, Lillie postulated that these agents modified the cell membrane in such a way that stimulation could not produce the normal rapid increase in permeability required for the ionic exchanges associated with depolarization. Lillie's theory escaped criticism for some time after its conception but was later questioned by those who objected to the concept of a universal change in the cell permeability (Davson and Danielli, 1943; Brooks, 1947). For the most part this criticism is untenable, since the work that refuted the theory was based on experiments carried out to show that narcotics did not always influence the cell permeability in all cells, and it is not applicable to Lillie's essential concept. The latter is essentially concerned with the very special changes in permeability that accompany excitation in the tissue. As far as general anesthesia is concerned, the only question posed by the permeability theory is whether an alteration in cell permeability occurs in neuronal membranes in the central nervous system so as to interfere with the excitability of this system. Some of the evidence in connection with this question has emerged from neurophysiological and neuropharmacological

studies in recent years and it is partially considered in the following sections.

I B. Mechanism of drug action on the central nervous system.

Although there have been many theories which attempt to explain the mechanism of general anesthesia, no theory has been completely successful in elucidating this mechanism (or mechanisms) in terms of cellular or molecular events. Various theories appearing in the past have reflected the development of newer techniques and theoretical concepts in the physical and the biological sciences. The failure of any theory to fully explain a phenomenon such as general anesthesia can in some measure be attributed to limitations imposed by techniques and concepts utilized.

The problem of cellular excitation has been a matter of considerable interest and controversy. The work of Hodgkin and Huxley (1939, 1945, 1951) on the conduction of the nervous impulse and that of Eccles (1957) on the synaptic transmission in the spinal cord has clarified some of the basic problems underlying cellular excitability of the neuronal tissue and perhaps for the first time provided a sound theoretical basis for explaining nervous function at the cellular level. In accordance with such advances attempts have been made to study drug action on both conduction and on synaptic phenomena in order to discover the site and the mechanism of action of drugs modifying excitability. In the course of such studies a variety of models of nerve conduction and synaptic transmission have been employed to overcome the technical limitations imposed by the complexity and inaccessibility of the central nervous system. Some of the work

concerning the pharmacological aspects of such studies is considered below.

i) Pharmacology of central synaptic transmission.

The present knowledge of synaptic transmission has developed over the years of investigation. The foundations of the 'Neuronal Theory' which postulated that the nervous system is composed of discrete units as opposed to a reticular network, were laid by the work of Ramon y Cajal(1909). However, Sherrington (1906) was the first to introduce the word synapse for the point of contact between two neuronal processes. He was also the first to recognize that the special properties of the synapse such as the one way transmission in the spinal reflex and the delay in transmission of the nervous impulse across a junction were due to the synaptic transmission processes between the neurones. The study of the microscopic structure of a typical synapse shows the synapse to be composed of two parts - the presynaptic and the postsynaptic. The presynaptic component is really the end of a single fibre expanded into a bulb - the bouton - which contains within it a small number of mitochondria and many synaptic vesicles. The bouton makes contact with a postsynaptic structure which is very often the soma (cell body) or dendrites of another neurone. However, the surfaces of the presynaptic and postsynaptic elements are separated by a narrow space - the synaptic cleft - which is about 200°A in width. The surface of soma and the proximal portion of the dendrites of a typical motorneurone is almost completely covered with boutons. Much of the information concerning the functional properties has been derived from studies of the extensor motorneurone in the cat spinal cord. Other synapses of greater complexity also do exist but are not considered here.

The arrival of an action potential in a nerve ending releases an appropriate chemical transmitter substance from the stores in the synaptic vesicles. The liberated transmitter diffuses across the cleft and affects the permeability of the postsynaptic membrane resulting in an electric change. Following this effect the action of the transmitter is terminated by its inactivation. It is evident that the chain of events described for a typical synapse provides a target for drug action at various sites. In terms of anesthetic action it may be reasonable to suppose that anesthesia could result from the action of drugs on i) the conduction and propagation of impulse in the pre- and postsynaptic conductile elements, ii) the release of transmitter from the presynaptic stores, iii) the postsynaptic cell membrane. The investigation of such possibilities has formed the basis for a great deal of research concerning the mechanisms of action of drugs which influence the activity of the central nervous system.

i) (a) Drug action on the postsynaptic membrane.

Studies of anesthetic drug action on synapses stems from various observations that anesthetics impair the process of synaptic transmission. One of the most widely quoted observations in this respect is that of Larabee and Posternak (1952) who showed that ether and chloroform impaired synaptic transmission in the superior cervical ganglia of the cat in concentrations which did not affect pre- or postsynaptic axonal conduction. A large number of studies along similar lines have been made in the invertebrate synapses (Grundfest, 1957, 1964; McLennan, 1963) and on synapses in the spinal cord (Eccles, 1964) but relatively few investigations have been

carried out on synapses in the brain itself.

Eccles and other workers on the basis of considerable work carried out on the motorneurones in the cat spinal cord have worked out in some detail the profile of synaptic mechanisms. This work, too extensive to be properly reviewed here, has nevertheless shown that two principal types of electrical responses in two different types of synapses can be observed from the postsynaptic membrane of a neurone in response to its presynaptic activation. On basis of this, synapses have been classified as excitatory (giving excitatory postsynaptic potential or EPSP) and inhibitory (giving inhibitory postsynaptic potential or IPSP). Activation of excitatory synapses is assumed to involve the release of a transmitter which generates an EPSP in the postsynaptic membrane (a membrane depolarization). If the EPSP attains a sufficient size, the motorneurone reacts by generation of a conducted action potential. The IPSP on the other hand is thought to be the result of the action of an inhibitory transmitter. It reflects a transient hyperpolarization of the postsynaptic membrane (the IPSP). Various degrees of interaction of excitatory and inhibitory potentials are possible. The net effect seen, either excitation or inhibition, reflects the dominance of one or the other effect.

Like most physiological processes, synaptic transmission also is subject to modification by various chemicals. Studies such as those of Larabee and Posternak (1952) in the autonomic ganglia, or Austin and Pask (1952) in the spinal cord show that drugs producing general anesthesia depress synaptic transmission in concentrations which did not impair the axonal conduction. Some of the earliest studies on the mechanism of

central synaptic blockade were carried out by Bremer and his school in Belgium (Bremer and Bonnet, 1948; Bonnet and Bremer, 1948) using the method of extracellular recording from the ventral roots in the frog spinal cord. These authors reported that the synaptic transmission in the cord is interrupted by anesthetics such as urethane and chloralose because the synaptic potential is depressed by these agents and fails to elicit a discharge. Eccles (1946) also using extracellular recordings in the cat spinal cord showed that the synaptic potential was depressed by pentobarbital only in very deep levels of anesthesia and he did not regard this as an important cause in the interruption of synaptic transmission. He concluded that in view of the elevated threshold, the blocking effect of pentobarbital is due to a stabilization of the cell membrane so that the discharge of a propagated impulse would not be initiated by synaptic potentials that are normally effective. It is noteworthy that Eccles' explanation of the action of pentobarbital is essentially in accord with Lillie's permeability theory of narcotic action. Pentobarbital was the only anesthetic studied by Eccles and no studies with volatile anesthetics on the synaptic transmission were reported by him at that time.

The introduction of intracellular recording techniques using microcapillary electrodes has provided a further impetus for the re-examination of the action of anesthetics on the synaptic transmission. Somjen and Gill (1963) have studied the mechanism of action of diethyl ether and thiopental on synaptic transmission using intracellular recordings in the cat motorneurones. These authors have found that ether suppresses the

monosynaptic reflex of the cat spinal cord mainly by reducing the postsynaptic response to an afferent stimulus, and to a lesser extent by increasing the depolarization needed to evoke an orthodromic impulse. They did not find any significant change in the resting membrane potential of the postsynaptic neurone. The rate of rise of the synaptic potential and its amplitude were also diminished. Finally Somjen and Gill concluded that by the methods available to them, they were unable to distinguish between the mechanisms of action of ether and thiopental. Up to the present time the knowledge of synaptic potentials arising within all parts of the central nervous system is essentially incomplete. Very few systematic studies of anesthetics, volatile or nonvolatile, have been undertaken and those which have been carried out are not altogether in agreement.

Synaptic mechanisms have been of greater value in elucidating the mechanisms of action of drugs that produce convulsions in animals. One such drug that has been subjected to intensive research in this respect is the alkaloid strychnine. The inhibitory synapses in the spinal cord are considered by Eccles et al. to be the site of action of strychnine and its analogs (Eccles, 1964). Tests on the cat spinal cord show that when administered intravenously or applied iontophoretically, strychnine selectively depresses the postsynaptic inhibition (the IPSP), and the resulting convulsant action of this drug has been attributed to an 'unmasking' of existing excitatory mechanisms. A direct excitatory action on the spinal cord has been excluded on the grounds that strychnine does not affect the postsynaptic membrane potential. In its rapid onset of action strychnine therefore resembles the curariform drugs which act

peripherally at the myoneural junction and a similar mechanism of action has been proposed, namely competitive occupation of the postsynaptic receptor sites normally occupied by the inhibitory transmitter. Since all the postsynaptic inhibitions that have been investigated in the cord are blocked by strychnine it has been considered likely that all are produced by the same transmitter substance. However, the identity of this chemical transmitter is not known.

In contrast to strychnine there are several convulsants which produce the same end effect but are without any detectable action on spinal postsynaptic inhibition. Such drugs include pentylenetetrazole, picrotoxin, β-methyl-β-ethylglutaramide, and meperidine (Eccles, 1965). It has been proposed that this difference accounts for the differences in the character of the convulsions induced by strychnine and other drugs such as pentylenetetrazole. The convulsions induced by the latter are flexor-extensor type, whereas strychnine produces a pure extensor convulsion resulting from the blockade of spinal inhibitory pathways. According to the present evidence pentylenetetrazole does not block presynaptic (see later) or postsynaptic inhibition. The few studies which have been conducted indicate that the stimulant action of this drug is not due to depolarization. Rather, the evidence put forward by Lewin and Esplin (1961), from their studies on the spinal cord, suggests that the excitatory effects of pentylenetetrazole may be due to a decrease in the neuronal recovery time (i.e. a decrease in the relative refractory period).

Grundfest also has studied in considerable detail the physiological and pharmacological aspects of the synaptic transmission, largely in the

invertebrates but to some extent also in vertebrates (Grundfest, 1957, 1964). He has distinguished between two types of junctions: A) Ephases - in which the junctional transmission is electrical as is seen in many invertebrate giant fibre junctions and vertebrate cardiac cells. B) Synapses - in which the transmission is chemically mediated and the postsynaptic membrane is electrically inexcitable. The latter type includes a majority of all junctions including all those of the mammalian nervous system. Grundfest's conception of synaptic transmission is essentially similar to that of Eccles and other workers. He classifies synapses roughly as depolarizing (excitatory) and hyperpolarizing (inhibitory). Against this background of synaptic types Grundfest has attempted to classify partially the drugs acting on synaptic phenomena. In his classification, drugs can be activators of excitatory synapses (I) or inhibitory synapses (II), or they can be inactivators of these two types of synapses. This scheme of classification includes drugs such as acetylcholine (activator of I and II), pentylenetetrazole (activator of I), gamma aminobutyric acid or GABA (activator of II but inactivator of I), curare (inactivator of I and II but its net effect is inhibition), and picrotoxin (inactivator of II and activator of I).

The classification of centrally active drugs as suggested by Grundfest and outlined above is attractive but is fraught with some difficulties. While not being simple it is complicated by such factors as the occurrence of several types of synapses in the same cell, the different effects of drugs on the synaptic and nonsynaptic membrane of the same cell, and the interaction between cells with different properties. The extent to which

the results obtained from several invertebrate and vertebrate species can be extrapolated to bear on the mammalian nervous system is a problem still open to question.

It often has been found that even the results from the mammalian spinal cord cannot be directly extrapolated to the brain itself without some degree of caution. This point has been aptly illustrated by investigations on the mechanism of strychnine. Large and prolonged IPSPs have been recorded from a variety of neurons in the higher centres of the mammalian brain, e.g. pyramidal cells of the motor and somatosensory cortex, hippocampus, cerebellar purkinje cells and the thalamus. It has been shown that even large doses of strychnine have no significant effect on these IPSPs, unlike its effect in the spinal cord (Crawford et al., 1963). Several theories have been advanced to explain the differences in the action of strychnine on postsynaptic inhibition in the spinal cord and in the higher centres. One of these is that the transmitter responsible for postsynaptic inhibition is different in both cases. The other possibility remains that the transmitter may be the same but the receptor site of the postsynaptic membrane may differ in two cases, there being a strong attachment of strychnine to the postsynaptic membrane in the spinal structures but a negligible attachment on the cerebral receptor sites (Eccles, 1965). The postsynaptic inhibitory potentials of the higher centres also are resistant to many other convulsants besides strychnine. It has been shown that picrotoxin, metrazol, brucine, chloralose as well as a number of other pharmacological agents such as atropine, nicotine, d-tubocurarine, dihydro-β-erythroidine, dibenamine, dichloroisoproterinol and ω-amino acids

have no effect on cortical postsynaptic inhibition (Krnjevic et al., 1964). It also has been shown that a direct electrophoretic injection of strychnine to Purkinje cells of the cerebellum causes excitation of the cells (Crawford et al., 1963). This type of evidence tends to suggest that strychnine, unlike its action in the spinal cord, may have a direct excitatory action on the neurones in the brain.

i) (b) Drug action on presynaptic inhibition.

The pharmacology of synaptic transmission has been complicated by the discovery of presynaptic inhibition, first reported by Frank and Fuortes (1957). It differs from the postsynaptic inhibition in its mechanism, in the electrical responses recorded, in its response to drugs and in its distribution in the cerebrospinal axis. In this type of inhibition, the inhibitory fibres make synaptic connection with the presynaptic terminals of excitatory fibres, the stimulation of which normally results in the generation of the EPSP. These axo-axonal terminals are histologically demonstrable by the electron microscopy. It is also thought that these fibres act by partially depolarizing the excitatory terminals on which they synapse, to the extent that the excitatory impulse cannot be transmitted in the latter. The presynaptic chemical mediator for this type of transmission also is not known. The electrical effect of the presynaptic inhibition is seen as a depolarization of the dorsal root fibres. The presynaptic inhibition differs pharmacologically from the postsynaptic counterpart thus strengthening the contention that both are mediated by two differing chemical transmitters. Convulsive doses of strychnine which strongly depress postsynaptic inhibition in the spinal

cord, have no effect on presynaptic inhibition (Eccles, 1965). But in contrast to its ineffectiveness on postsynaptic inhibition, picrotoxin depresses presynaptic inhibition. However, this action of picrotoxin is less prominent than the action of strychnine on postsynaptic inhibition. As in the case of strychnine, the selective effect of picrotoxin on one type of synaptic inhibition cannot be taken to mean that this action solely is responsible for the convulsant activity of this compound in the intact animal.

Since its discovery, presynaptic inhibition has provided a partial basis for the determination of the mechanism of action of some centrally acting drugs. According to Eccles (1964) there is evidence to suggest that the dorsal root potentials (DRP) reflect the intensity and the time course of the presynaptic inhibition. The effect of a large number of drugs has been studied on this basis. Eccles pointed out that pentobarbital and chloralose enhanced presynaptic inhibition whereas other anesthetics such as ether, paraldehyde, urethane and chloral hydrate depressed this process (Eccles, 1965). In contrast to this finding Miyahara et al.(1966), who studied the effect of several central depressants on presynaptic inhibition in the cat spinal cord, reported that eight anesthetic agents - pentobarbital, phenobarbital, ethanol, chloral hydrate, chloroform, diethyl ether, nitrous oxide, magnesium sulfate - only enhanced presynaptic inhibition in doses that produced sedation or light anesthesia. They also found that this effect on inhibition also is shared by trimethadione, a more selective drug, in anticonvulsant, nontoxic doses. Mephenesin, a central muscle relaxant, blocked this inhibition

in doses which had little effect on the monosynaptic reflex. Procaine on the other hand enhanced the presynaptic inhibition in low doses but blocked it in higher doses. In view of the findings that some anesthetics enhance presynaptic inhibition, Schmidt (1964) has suggested that this effect may contribute to the general anesthetic state produced by such drugs. However, it is considered unlikely that this is the mechanism of anesthesia, in light of the observation that although presynaptic inhibition is depressed by many agents producing anesthesia it is also depressed by others which produce little or no sedation in the intact animal. There are numerous sites in the presynaptic inhibitory pathways which may be affected by drugs. The differences in action of these drugs on presynaptic inhibition may arise from their differential effects at such sites. These different effects on the presynaptic inhibition in the spinal cord so far do not provide a concrete basis for mechanism of action of anesthetic drugs, which produce a generalized depression of the whole central nervous system.

i) (c) Drug action on transmitter substances at central synapses.

During the course of studies on synaptic transmission, many efforts have been made to prove that central synaptic transmission is chemically mediated. In addition to the perennial interest in acetylcholine as the universal transmitter, more recently other substances such as catecholamines (norepinephrine, dopamine), serotonin, histamine and various amino acids but particularly GABA and glutamic acid have emerged as possible candidates for transmitters at central synapses. Although there is evidence to suggest that transmission in the spinal cord and the cerebral

cortex is chemical in nature (McLennan, 1963; Krnjevic, 1964) there are widely differing views regarding the validity of the available evidence. Among the two schools of thought that prevail, one believes that the occurrence of transmitters in the central nervous system parallels their occurrence in the peripheral nervous system (Krnjevic, 1964). On the basis of this it is not unreasonable therefore to suppose that these chemical synapses serve as the focal point of attack for the drugs which are thought to modify the synthesis, transport, release or inactivation of transmitter substances. Among the drugs whose mechanism of action has been thought to be based on alterations of the transmitter profile are serotonin, lysergic acid diethylamide (LSD), mescaline, monoamine oxidase inhibitors, impramine, chlorpromazine, reserpine, amphetamine, and cocaine. Although all these drugs are believed to alter the monoamine picture to some extent, such alterations have not been related cohesively or coherently with the pharmacological action of these drugs.

In contrast to the above view, the school of Curtis (1962) questions whether the substances shown to affect the nerve cells need necessarily be true transmitters in the sense that they can be released by the presynaptic impulses. Curtis suggests that the behaviour of the target cells in the central nervous system may be altered by any number of substances that occur in the environment of those cells. These substances might therefore modify the behaviour of the cells, and the effects of such substances might in turn be affected by exogenous drugs without direct involvement in the transmission process. He further argues that normally occurring amino acids, particularly, cannot be true impulse-dependent transmitters and that

the transmitters at most synapses in the central nervous system are as yet unknown.

It appears that a considerable body of histochemical and biochemical evidence supports the concept of chemical transmission at central synapses. But an exclusive role for acetylcholine, serotonin, catecholamines, and the respective precursors of any of these agents (except possibly acetylcholine at the Renshaw cells in the spinal cord [Eccles, 1964]) in a particular central function has not been determined as yet. This is partly due to the fact that the drugs used in discovering the identity and function of these potential transmitters, for example as their blocking agents, depletors or enzyme inhibitors, have a rather broad spectra of action themselves and thus may introduce errors in the interpretation of the experimental results. Furthermore, the biochemical evidence for identity and the action of transmitter presents a confusing picture, since biochemical changes produced by the transmitter substances are difficult to correlate with the physiological function of these substances in a particular region of the central nervous system.

Until the true nature and the role of transmitter substances in the nervous system is fully evaluated they cannot be expected to provide a firm basis for an explanation of the cellular actions of anesthetic drugs. On the other hand, in view of the large variety of synapses in the central nervous system, these structures may provide important clues for the mechanism of drugs which produce more specific and more diverse central effects.

ii) Drug action on conduction in the central nervous system.

Although the electrical nature of the nervous impulse was recognised by physiologists by the beginning of this century, a satisfactory explanation for the generation and conduction of this event remained obscure. Julius Bernstein in 1902 was the first to attempt a reasonable explanation of this phenomenon (Bernstein, 1902). Bernstein recognized that all excitable tissue contained higher concentrations of potassium than other ions, and suggested that the cell membranes selective permeability to potassium might account for the resting membrane potential. He further proposed that during the passage of the nervous impulse the selective permeability to potassium ions is destroyed, other ions rush to the interior of the fibre and the potential across the membrane collapses to zero. The loss of selectivity was thought to spread to other parts of the fibre by the electric current generated between active and inactive sites along the membrane. Thus the impulse was made to travel by self regeneration along the entire length of the tissue.

Although Bernstein's membrane hypothesis was generally accepted at the time little direct evidence was available to support it because of the prevailing experimental difficulties. However, during the past twenty years work with the squid giant axon has produced much of the current knowledge of the initiation and propagation of the nerve impulse. The extensive work of Hodgkin and Huxley (1939, 1945, 1951) on the squid axon has shown the ionic basis for the resting membrane potential, the generation and conduction of action potential, and has provided a firm basis for the study and understanding of drug action on excitable tissues.

Hodgkin and Huxley (1939, 1945) demonstrated that the membrane potential of the squid giant axon does not merely drop to zero at the peak of the action potential as suggested earlier by Bernstein, but is actually reversed. The most satisfactory explanation for the reversal of the membrane potential was proposed by Hodgkin and Katz (1949). Their proposal, often called the 'Sodium Hypothesis', holds that the nerve membrane does not merely lose its selectivity during the rising phase of the action potential, but that it becomes highly and specifically permeable to sodium ions. They further proposed that the original internal negativity of the resting nerve is restored by a subsequent exit of the potassium ions from the intracellular fluid, and this accounts for the falling phase of the action potential. The sodium hypothesis is well supported by an earlier observation made by Overton (1902) who showed that frog muscle loses its excitability when immersed in a sodium free solution. Hodgkin and Katz (1949) in their squid axon preparation showed that both the size and the rate of rise of the action potential spike vary with ionic changes in the external fluid in a way that fits well with the theoretical predictions.

The main implication of the sodium hypothesis from the pharmacological standpoint is that any agent which interferes with the specific increase in sodium permeability of the nerve membrane would prevent the generation of the action potential. Since the latter is a fundamental unit of excitability in the neuronal tissue, it is conceivable that interference with generation and conduction of the action potential in the conductile elements of the nervous system may lead to a loss of excitability

in this organ.

If an anesthetic agent were to block the generation of an action potential, it might do so by lowering the resting membrane potential of the nerve membrane since a depolarization of the fibre below a critical level suffices to inactivate the mechanism for the rapid sodium permeability change. Most volatile and nonvolatile anesthetics however have been shown not to depolarize the nerve membrane at doses sufficient to block excitability (Thesleff, 1956; Yamaguchi, 1961; Inoue and Frank, 1962a). These workers have shown that several anesthetic agents acted by preventing the development of the high sodium permeable state that follows an adequate stimulation of the nerve or skeletal muscle fibre membrane.

In recent years a classification of nonspecifically acting drugs has been based on actions on the nerve membrane as distinct from the synaptic zones in the central nervous system. Shanes (1958) on the basis of the voluminous evidence that has accumulated from studies on the plasma membrane has classified drugs into 'stabilizers' and '_ebilizers'. His stabilization theory of anesthetic action defines anesthetics as agents which block nerve or muscle impulses without a simultaneous change in the resting membrane potential. He has attributed this effect to ability of the anesthetic, particularly the local anesthetics, to reduce the electrical effectiveness of sodium and potassium ions. He further suggested that the decrease in permeability of the membrane to these ions is due to a decrease in the size of the membrane pores through which the ions normally pass. Shanes' concept is in good

agreement with Hodgkin's theory of nerve transmission wherein a selective increase in sodium conductance produces the action potential (Hodgkin, 1958).

During the past decade there have been various attempts to study the effects of anesthetic drugs on sodium permeability during excitation. Thesleff in 1956 studied the effects of several nonvolatile anesthetics - sodium pentobarbital, urethane, chloralhydrate, chloralose, parldehyde, and tribromethanol - on the electrical activity of the frog skeletal muscle. Using the intracellular microelectrode technique, he found that all the aforementioned agents blocked the production of an action potential by suppressing the specific increase in sodium conductance which follows effective stimulation of the muscle fibre and which is responsible for the rising phase of the action potential. This effect occurred without a significant change in the resting membrane potential of the muscle fibre. Thesleff further observed that concentrations of drugs causing a reduction of sodium conductance were closely correlated, in order of potency, with doses causing a hypnotic effect in the intact frogs. An identical effect on the sodium conductance has been shown for volatile anesthetics such as ether (Inoue and Frank, 1962a), chloroform (Yamaguchi, 1961), and also ethanol (Inoue and Frank, 1967). It must not be assumed from this that such effects are only confined to the frog skeletal muscle, since a similar effect on the sodium conductance has been noted for nerve as well. Experiments of Taylor (1959) and Moore et al. (1964) using procaine and ethanol respectively showed that these substances indeed depressed the sodium conductance and therefore the inward sodium carrier mechanism in the squid giant axon too. The latter experiments were carried

out under voltage clamp conditions where it is possible to measure separately and continuously the sodium and potassium currents during depolarization and repolarization of the nerve membrane.

Inoue and Frank (1962b) found that procaine, popularly classified as a local anesthetic, blocked the production of action potentials in the frog skeletal muscle fibres by suppressing the increase in sodium conductance, without reducing the resting membrane potential of the muscle fibre membrane. In light of the fact that local and general anesthetics shared this property, they proposed that both local and general anesthetics produced anesthesia by a common mechanism of action. Frank and Sanders (1963) investigated the anesthetic properties of several local anesthetics, both in the intact mice and in neuronally isolated slabs of cerebral cortex of the cat and compared the effects of local anesthetics with the effects of already established general anesthetics. They found that while local anesthetics corresponded closely to general anesthetics in their central effects, they differed markedly in their actions from a central convulsant such a pentylenetetrazole. In view of the close correspondence between the central actions of procaine, phenobarbital and ether, and their suppressive effects on the electrical activity of the skeletal muscle cell membrane, these authors proposed that the suppression of sodium conductance in the neuronal tissue of the central nervous system represented the basic cellular mechanism for the action of anesthetic agents.

Although numerous experiments support the anesthetic blockade of the sodium conductance, it has also been shown that they all to some extent

also suppress the potassium conductance which is responsible for the repolarization of the cell membrane (Taylor, 1959; Inoue and Frank, 1962a; Moore et al., 1964). When tetrodotoxin became available as a substance which could block specifically the mechanism responsible for the primary increase in the sodium permeability without blocking the mechanism for a secondary increase in the potassium conductivity (Narahashi et al., 1964) it provided a useful tool to test the hypothesis that the central depression in anesthesia was mainly due to a suppressive effect on the primary phase of the action potential. Frank and Pinsky (1966) investigated the central action of tetrodotoxin in both intact mice and the isolated cortex. They found that tetrodotoxin induced a central depression indistinguishable from general anesthesia. This suggested to them that anesthesia was due to an action at the cellular level; namely an inactivation of the sodium carrier mechanism responsible for the increase in sodium conductance and thus the rising phase of the action potential.

I C. Pharmacological properties of central depressants and stimulants.

One of the urgent tasks for neuropharmacology is to undertake a detailed but systematic investigation of the site and the mode of action of the newly developed neurotropic agents as well as the older centrally acting drugs. Although a large body of the current literature is devoted to facts about the action of these drugs on various parameters of central nervous system activity, such facts for the most part are scattered and show a large measure of conflict. As a result, the elementary mechanism of action of drugs which modify the function of central nervous system

continues to remain a challenging problem. Moreover, due to the scarcity of the knowledge regarding mechanisms of action, the classification of neuropharmacological agents remains unsatisfactory.

On the other hand, current studies on the mechanism of action of centrally acting drugs must take into account the existing information which is useful for a meaningful interpretation of newer theories of drug action. For the purposes of the present investigation some of the pertinent data concerning some centrally depressant and stimulant agents is reviewed in the following pages. No attempt is made here to review the total literature on this subject owing to its great volume. Rather the present consideration of facts is confined to some of the important central effects of these substances, and to the place of these drugs in the current classification of neurotropic agents.

i) Depressants

i) (a) Diphenhydramine

Diphenhydramine is classified as an antihistamine drug and is regarded as a prototype of this class. It has prominent peripheral effects some of which are related to an antagonism of histamine. But it also antagonizes the action of acetylcholine and serotonin, and has a potent local anesthetic activity (Loew, 1950). In addition, diphenhydramine has both central depressant and stimulant properties. The depressant properties are seen with small doses and are responsible for its sedative and anti-motion sickness effects, whereas stimulation, which occurs with higher doses, is responsible for its convulsant activity. Although the central effects of diphenhydramine and its congeners have been known for a long

time, their mechanism of action in the central nervous system is not understood. It has been suggested that the central action, like the peripheral effects, may be related to the antagonism of histamine or even acetylcholine in the brain (Loew, 1952). The central actions of antihistamines, however, have not been explored sufficiently to support this suggestion. Recently Phillis et al. (1968) have studied the effects of iontophoretically applied histamine and antihistamines including diphenhydramine in the pre-cruciate cortex in anesthetised cats. Their results showed that antihistamines antagonized the depressant and excitant effects of applied histamine. They also antagonized the action of acetylcholine, noradrenaline and serotonin, indicating a nonspecificity of action.

i) (b) Phenothiazine drugs - Promethazine and Chlorpromazine.

A considerable amount of current literature concerns the pharmacology of the phenothiazine group of drugs of which promethazine and chlorpromazine are two prominent members. Promethazine and chlorpromazine have a broad spectrum of peripheral and central actions, and the latter drug is one of the most widely studied and used drugs in this group. In the present classification promethazine is chiefly regarded as an antihistamine and anti-motion sickness agent whereas chlorpromazine is classified as a major tranquilizer.

Both these drugs have antihistaminic, anticholinergic, antiadrenergic and anticholinesterase properties. They are also potent local anesthetics (Burns, 1954; Kopera and Armitage, 1954; Ritchie and Greengard, 1961), but are not used for this purpose. Amongst the diverse actions of these drugs, their depressant actions on locomotor activity and conditioned

behaviour are the most prominent. In mice chlorpromazine has been found to prolong sleeping times of animals treated with hexobarbital, pentobarbital and phenobarbital, and has been found to shorten the time of onset and prolong the duration of anesthesia produced by ether, nitrous oxide and ethanol (Courvoisier, 1953). In the central nervous system chlorpromazine causes changes in the electrical activity at all levels of the cerebrospinal axis. When given to cats it produces EEG effects resembling those in normal sleep with a normal arousal response following peripheral stimuli, but in higher doses such arousal response is diminished. Chlorpromazine effects also have been studied on the motor responses in decerebrate cats. Experiments have shown that in such animals the drug abolishes decerebrate rigidity and produces an inhibitory effect on motor reflexes (Dasgupta et al., 1954). Anticonvulsant studies with chlorpromazine have been carried out in animals using electroshock and convulsant drugs like strychnine, pentylenetetrazole and picrotoxin, but no significant protective effects were seen (Virtue and Jones, 1956; Fink and Swineyard, 1960). In very high doses chlorpromazine, like other phenothiazines produces extrapyramidal effects consisting of parkinsinonian syndrome, diskinesia, and acathisia.

The site and mechanism of action of chlorpromazine have been the subjects of many investigations, but no definitive explanations have emerged. Such studies for the most part have been carried out in an attempt to explain its profound action on behaviour, but some studies have also sought to explain its effects on motor activity. Experimental evidence suggests that interference with motor activity may be due primarily

to its depressant action on the brainstem reticular formation (Dasgupta and Werner, 1955). However, there is no general agreement on this point and some investigators feel that the drug may have a dual action on the brainstem, a depression at low doses and stimulation at high dose levels (Himwich and Rinaldi, 1957). In contrast to the aforementioned, it also has been suggested that the primary action of chlorpromazine may occur at sites other than the reticuar formation. The results of experiments on its effects on cortical and subcortical structures are also conflicting. It has been shown that chlorpromazine decreases the threshold for cortical afterdischarges in the intact cortex, induced by cortical or thalamic stimulation and prolongs the duration of these afterdischarges (Gangloff and Monier, 1957a). However, Preston (1956) was unable to observe any change in the threshold or amplitude of the evoked activity of the isolated or intact cortex in doses as high as 50 mg/kg given intravenously to cats. He found that the higher doses caused spike activity of a grand mal type seizure in the brainstem and in the cerebral cortex. The actions of chlorpromazine and other phenothiazines on the central system are complex and the evidence available at present is conflicting and confusing.

i) (c) Gammahydroxybutyrate and Gammabutyrolactone.

Both gammahydroxybutyrate (GHB) and Gammabutyrolactone (GBL) are structural relatives of gamma aminobutyric acid (GABA) and have been reported to be naturally distributed in the brain of rats, cats and man (Fishbein and Bessman, 1963). Interest in these compounds rests on the possibility that their concentration in the brain may be related to the

onset and the duration of the sleep. GHB, which has been investigated more than GBL, is currently classified as a hypnotic and has found some use in preanesthetic medication. Given to animals it induces a sleep-like state which is identical to natural sleep. Ban et al., (1967) reported that both GHB and GBL produced a depression of spontaneous movements, muscular relaxation, and a loss of righting reflex in mice, rats and rabbits. This action was followed by twitching convulsions in higher doses. A prolongation of sleeping time was also seen in mice pre-treated with pentobarbital. In a study on cats, Basil et al. (1964) showed that a sleep-like state in cats after GHB was characterized by a marked persistence of the righting reflex, normal respiration and an abrupt recovery. The authors also observed that when given to decerebrate and spinal cats, GHB blocked polysynaptic but not monosynaptic reflexes and concluded that the drug probably acts on the internuncial neurones in the spinal cord.

The sleep inducing effects of GHB have come under direct criticism by Winters and Spooner (1965) who, on the basis of their data on the gross behaviour, EEG, and click evoked responses in cats, have suggested that the actions of GHB are similar to an epileptoid rather than an anesthetic-like state. They have questioned the validity of the reports that GHB produces sleep and have suggested that such an effect simply may be occurring spontaneously prior to the drug administration. The mechanisms of action of the depressant and excitant effects of GHB and GBL are still unknown. In view of the structural similarity between these compounds and GABA, attempts have been made to explain the effects of GHB and GBL

in terms of a competitive interaction between these substances and GABA. However, the evidence available at present is not sufficient to warrant a general acceptance of this hypothesis.

i) (d) Hyoscine (Scopolamine)

Hyoscine is a naturally occurring alkaloid with actions that are qualitatively similar to its well known congener atropine. Relatively greater attention has been given to atropine owing to its marked anti-muscarinic properties and its wide clinical use. Hyoscine has marked central effects and in this respect it is more potent than atropine. Small doses of the drug produce sedation, amnesia and drowsiness. It is therefore used as a sedative, chiefly in the preanesthetic medication. However, small doses also can produce excitement, restlessness, hallucinations and delirium in some subjects. Given in toxic doses hyoscine produces stupor, delirium and coma in which respiration becomes rapid, shallow and finally ceases. In rats it has been found to potentiate barbiturate anesthesia (Longo, 1966).

The effects of atropine and similar drugs on the electrical activity of the brain were reviewed earlier by Toman and Davis (1949), and more recently by Longo (1966). Briefly, it has been found that both atropine and scopolamine depress the spontaneous EEG activity and the EEG arousal response to peripheral stimulation. In animals scopolamine antagonizes the EEG activation produced by hypothalamic or reticular formation stimulation (Longo, 1956; Bradley and Key, 1958). There have also been reports that atropine in low doses produces some EEG desynchronization (Beck and Goldstein, 1964) but this is not a very general observation (Longo, 1966).

Investigations on evoked activity have also been carried out. They showed that atropine and scopolamine did not influence the evoked reticular potentials (White et al., 1965) and in this respect differed from barbiturates which produce EEG synchronization and depress these evoked responses. An increase in the size of peripherally evoked responses in the intact cortex upon the topical application of atropine has been reported by Chatfield and Lord (1955).

The mechanism underlying the action of hyoscine and like drugs in the brain is not completely understood. It is not known whether the observed effects on the central nervous system are a result of a direct action on the neurone or due to an interference with the action of a central synaptic transmitter such as acetylcholine (Giarman and Pepeu, 1962). In view of the procaine-like effect of atropine on activation of spinal neurons in the cat by either cholinergic or noncholinergic stimulation, it would appear that atropine and also hyoscine may have central actions unrelated to the blocking of cholinergic synapses. Both atropine and hyoscine show some structural similarity to cocaine, and atropine also possesses a mild local anesthetic activity (Innes and Nickerson, 1965). The mechanism of their local anesthetic action may be similar to cocaine.

i) (e) Meperidine.

Meperidine is a widely used drug in the relief of pain and it is currently classified as a narcotic analgesic. Although structurally different from morphine, it has actions which are similar to the latter. In the central nervous system narcotic analgesics produce two apparently opposite effects - depressant and excitatory. The balance between these

two effects varies with the different analgesics, the species studied and the dose of the drug. Depressant effects include sedation, miosis, respiratory depression, analgesia, bradycardia, hypothermia, and diminished reflex activity, whereas stimulatory effects consist of vomiting, mydriasis, respiratory stimulation, tremors, and convulsions.

The effects of morphine-like drugs have been the subject of exhaustive biochemical and neurophysiological studies, and the subject has been reviewed at length by Wikler (1950) and by Domino (1962). However, in spite of this, the mechanism of action of analgesic drugs continues to be a problem. In the cortex, the potentials evoked by single shocks applied to the sciatic nerve of the cat or to the tooth pulp of the dog are not depressed by morphine but cortical responses to visceral afferents such as the splanchnic, phrenic and the vagus nerves are depressed. The action of topical application of morphine but not meperidine has been observed in the isolated cortex of the dog by Crepax and Infantellina (1956). They showed that the threshold of the response to single shocks was lowered and the afterdischarge in response to the repetitive stimulation was enhanced and prolonged. The combined application of physostigmine and morphine produced activity in the isolated cortex similar to that caused by physostigmine and acetylcholine. The application of atropine blocked the effects of the former but not the latter combination. Gangloff and Monier (1957b) demonstrated an elevation in the threshold, and a slight prolongation of cortical afterdischarges in the sensorimotor cortex of the unanesthetized rabbit. In anesthetized cats Fujita et al. (1954) noted a morphine-induced depression of the local cortical potential

elicited in the suprasylvian gyrus by subcortical stimulation.

Investigations on narcotic analgesics mainly have employed morphine as the model analgesic. In most animals morphine produces convulsions in high doses but in cats it produces an atypical excitatory action in analgesic doses (Jaffe, 1965). In toxic doses, meperidine also produces marked central nervous system excitation and convulsions. Deneau and Nakai (1961) have shown that in monkeys central nervous system excitation induced by meperidine depends in large part on the rate at which meperidine is metabolized. Its metabolic product, normeperidine, has actions qualitatively similar to meperidine but its excitant action is considerably greater. This convulsant action is not antagonized by nalorphine. A large number of studies have attempted to relate the action of morphine-like compounds to the processes involved in neurohumoral transmission at the synaptic junction; particularly in view of its depressant effect on the release of acetylcholine at peripheral junctions (Martin, 1963). But since the role and interrelationship of transmitters in the central nervous system are still uncertain, the mechanism of action of narcotic analgesics cannot be based firmly on this aspect of central nervous system function.

i) (f) Meprobamate.

Meprobamate is one of the most widely used drugs employed in the treatment of anxiety. It is currently classified as a tranquilizer but in some cases it is also considered under the group of central muscle relaxants of which mephenesin in the prototype. However, the muscle-relaxant property of meprobamate is regarded as a secondary effect, since sedation and antianxiety effects appear to be the most prominent clinical effects

observed. The pharmacology of meprobamate and similar drugs has been reviewed by Smith (1965) while their central effects have been reviewed by Domino (1962).

In animals the effects of meprobamate resemble those of the barbiturates more than those of tranquilizers such as the phenothiazines or rauwolfia alkaloids. Mice exhibit a decreased activity and muscle tone, ataxia and loss of the righting reflex, and a depression of respiration. In contrast to these depressant effects, meprobamate also has been noted to produce some locomotor stimulation in dogs and a hyper-reflexia in cats (Smith, 1965). The results of numerous experiments on the reflex activity in animals show that the drug depresses polysynaptic reflexes in spinal and decerebrate animals. The effects on monosynaptic reflexes are less marked and are observed mainly in large doses. In intact, curarized cats, meprobamate produces synchronization of the spontaneous EEG selectively in various thalamic nuclei (Baird et al., 1957; Hendley, et al., 1957). These changes have been claimed to be characteristic for meprobamate and different from other central depressants. Meprobamate also partially inhibits the arousal response to hypothalamic stimulation in doses which do not affect cortical or hippocampal arousal elicited by stimulation of the reticular formation. Kletzkiin and Swan (1959) have compared the effects of meprobamate to those of pentobarbital on evoked cortical, thalamic and reticular formation potentials due to click stimuli in cats under succinylcholine. Doses of 40 mg/kg given intravenously had no effect on evoked potentials in the thalamus and in the temporal cortex but the amplitude of the responses in the reticular formation was slightly

enhanced. Although there are many other isolated investigations on neurophysiological responses, sufficient information is not available to indicate a definite locus of action or mechanism of action in the central nervous system.

i) (g) Diazepam.

Diazepam and its more widely used congener chlordiazepoxide belong to the benzodiazepene group of drugs which have been variously classified as tranquilizers, central muscle relaxants or neurosedatives. These drugs are employed in the treatment of anxiety as well as for skeletal muscle relaxation. Qualitatively both drugs produce similar effects, but diazepam has a greater potency of action (Zbinden and Randall, 1967).

In mice, chlordiazepoxide and like drugs produce hypnosis, sedation, analgesia, muscle relaxation, ataxia, and a loss of the righting reflex (Zbinden and Randall, 1967). They also inhibit convulsions induced by pentylenetetrazole or strychnine in mice. Among the neurophysiological alterations produced by these drugs, they have been found to produce a slowing of spontaneous activity in the hippocampus and the amygdala but not in the cortex, which shows a slowing only in high doses (Randall et al., 1961; Schallek et al., 1962). The thresholds for EEG arousal from stimulation of reticular formation, pyriform cortex, anterior amygdala and hypothalamus is increased moderately by diazepam. The cortical effects of these drugs are not known in detail, however, it has been found that both chlordiazepoxide and diazepam can diminish or abolish the seizure discharges produced by electric or chemical stimulation (Zbinden and Randall, 1967). Diazepam also has prominent effects on the reflexes.

It depresses the rigidity induced by intercollicular decerebration or anemic decerebration (Schallek, 1966). A comparison of its effects on poly- and monosynaptic pathways in spinal and decerebrate cats demonstrated that the effect of diazepam was more prominent on polysynaptic reflexes, and that the drug was more active in decerebrate than spinal cats (Ngai et al., 1966). This suggests that the drug action is at a supraspinal level; probably the brain stem reticular formation. These drugs also have been studied on behavioural responses. In such experiments some stimulant effects have been noticed with diazepam, and these are mainly reflected as hyperactivity and agitation (Zbinden and Randall, 1967).

ii) Stimulants

A large number of drugs have a predominantly stimulant effect on the central nervous system. These stimulants are often referred to as analeptics, a term which refers to their ability to restore the functions of depressed central nervous system. They are classified traditionally on the basis of their site of action. Thus pentylenetetrazol (PTZ), bemegride, nikethamide and picrotoxin are regarded chiefly as the brain-stem stimulants, caffeine is regarded as a cerebral stimulant, while strychnine is classed as a spinal stimulant. Such a classification is not rigid or final in any sense because these drugs are not very selective in their sites of action and are capable of stimulating the cerebrospinal axis at all levels. Because of their profound stimulant and convulsant effects these drugs are used as anesthetic antagonists and as inducers of experimental seizures in animals to serve as models of the epileptic state. The actions of analeptics have been reviewed in detail by Hahn (1966).

ii) (a) Bemegride.

Bemegride shows structural similarities to derivatives of barbituric acid and was originally regarded as a specific antagonist for the latter. The drug however is an analeptic owing to its direct stimulant property, and it antagonizes not only the effects of barbiturates but other hypnotic agents such as chloral hydrate, ethchlorvynol, hydroxydione and glutethimide as well, probably by a physiological antagonism (Frey et al., 1956; Kimura and Richards, 1957; Shulman and Laycock, 1957). In experimental animals bemegride and other brainstem stimulants produce clonic convulsions characterized by a series of rapidly occurring contractions and relaxation of all muscle groups. These may be followed by tonic convulsions consisting of sustained contraction of all muscles.

Bemegride has properties similar to PTZ and both drugs seem to act on the same parts of the brain. The origin of the convulsant activity is thought to begin in the cortex and from there the action is propagated to the lower parts of the central nervous system (Dumont, 1958). The upper central nervous system is more responsive to its action than the spinal cord. In subconvulsant doses, bemegride like PTZ increases the amplitude and duration of induced potentials. With increasing doses there are EEG patterns similar to convulsive seizures. It also increases the normal arousal reaction. The seizures induced by bemegride and like drugs can be antagonized by trimethadione and the barbiturates. The effects of bemegride on evoked responses have not been investigated as much as the activity evoked by PTZ. The latter increases the voltage, frequency, duration and the rate of recovery of cortically evoked potentials by

sciatic or cortical stimulation. In the isolated cerebral cortex PTZ also increases the evoked electrical responses to direct stimulation (Preston, 1955; Frank and Sanders, 1963).

The elementary mechanism of action of bemegride or PTZ is not known. Currently available evidence excludes the action being due to a blockade of pre- or postsynaptic inhibition. Its actions in this respect have been considered above and as suggested the excitatory effects may be due to a decrease in neuronal recovery time (Lewin and Esplin, 1961).

ii) (b) Picrotoxin.

Picrotoxin is a potent convulsant which produces a spectrum of central activity similar to PTZ and bemegride. The convulsant effect is accompanied by an increase in respiration and blood pressure resulting from stimulation of respiratory and vasomotor centres respectively. Higher levels of the central nervous system are more responsive to picrotoxin than the spinal cord. In the latter, however, picrotoxin has been found to block the presynaptic inhibition (Eccles, 1963). However, this action on the presynaptic inhibition does not account for all its excitatory actions. In invertebrates, specifically crustaceans, picrotoxin blocks the activity of inhibitory synapses. This blocking action is antagonized by GABA, which is regarded to be the inhibitory transmitter at these invertebrate synapses.

There is a mutual antagonism between picrotoxin and barbiturates and non-barbiturate hypnotics such as chloral hydrate, tribromethanol, paraldehyde, urethane, ethanol and ethyl chlorovinyl ether (Barlow, 1932; Haas, 1937; Werner, 1939; Kimura and Richards, 1957). The margin between

analeptic and lethal doses is quite narrow. Like other convulsants, picrotoxin also produces a post convulsant depression.

ii) (c) Nikethamide.

Nikethamide is regarded as a respiratory and cardiac stimulant. Although some of its stimulant actions follow from its effects on the central nervous system, this drug also has a peripheral component of action. Its central actions are similar to PTZ and bemegride, but it differs from the latter in that it also has a depressant action which is particularly noticeable in lower animals. Higher animals respond predominantly with excitation and in unanesthetized animals depression is observed only as a post-convulsive paralysis (Hahn, 1960). The antagonistic effect of nikethamide against barbiturates has been studied and remains a matter of conflict. However, most workers agree that it has at most only a weak action in this respect. Barbiturates also antagonize the convulsant effect of nikethamide but only in large anesthetic doses, and death often occurs resulting from the depressant effects of nikethamide summating with those of the barbiturates (Loewe, 1955). Its effects are mutually antagonistic with other anesthetic agents, but this antagonism is quite weak (Hahn, 1960). The mechanism of the central action of nikethamide is not known.

ii) (d) Caffeine.

Caffeine is capable of producing a marked central stimulation and has important effects on mood and performance. It also has a strong peripheral component of action especially on heart and skeletal muscle. Centrally, caffeine stimulates all parts of the cortex and the medullary centres

producing general tonic and clonic seizure activity. A clear neurophysiological explanation for caffeine stimulation has not been possible as yet. It produces an EEG arousal pattern (Toman and Davis, 1949) which persists after lesions of the midbrain thereby excluding the reticular formation as the site of its action (Killam, 1962). In the isolated cortical tissue very small doses (0.25 mg/kg) were found effective in prolonging the afterdischarge induced by repetitive stimulation in dogs (Maiti and Domino, 1961). Caffeine has a weak antagonistic action against anesthetics.

ii) (e) Strychnine.

Strychnine has for a long time been regarded as a classical convulsant drug. It is primarily a stimulant of the spinal cord but is capable of activating the medulla, cerebral cortex and other parts of the central nervous system (Goodman and Gilman, 1965). In animals it produces tonic convulsions which can be precipitated by tactile or auditory stimuli after drug administration. It is thought that strychnine does not initiate convulsions but converts normal reflex activity into convulsive movement, so that simple sensory stimuli cause maximal tonic seizures. Eccles (1962) has advocated that strychnine reduces inhibitory action on the motor neurones of the spinal cord. A release from the inhibitory control leaves free the excitatory input resulting in convulsions. In the cortex strychnine reduces the stimulus threshold and the synchronized bursts of activity produced are reflected in electrocorticogram recordings. When strychnine is topically applied it produces spikes which arise from neurones whose cell body or synapse-covered dendritic processes are in

contact with the drug. The activity produced by strychnine can be detected locally or wherever the cells send their axons. This is used in strychnine neurography to map and identify pathways in the cortex (Slater, 1965). The exact mechanism for the cortical action of strychnine is not known.

The antagonistic effects of strychnine and barbiturates have been widely studied, though with contradictory results. Strychnine and barbiturates show a mutual antagonism but in this respect strychnine is much less effective than PTZ or picrotoxin (Hahn, 1966).

iii) Amino acids

A considerable amount of experimental work has been carried out on the action of simple amino acids on the activity of the central nervous system. These attempts have been mainly directed to the establishment of a physiological role of these substances as synaptic transmitters in the central nervous system. GABA and glutamic acid are brain metabolites and their actions on neuronal activity are typical of the inhibitory and excitatory amino acids respectively; two groups into which most of the centrally active amino acids have been classified. There is no marked difference in the sensitivity of the spinal cord or the higher centres as regards the action of these substances. Systemic administration of these agents is largely without effect, and this failure has been attributed to their failure to cross the blood-brain barrier. As a result, in most investigations the amino acids have been applied directly to appropriate centres or individual neurones. The results of such studies and numerous others have been reviewed by Curtis and Watkins (1965).

In the cortex and lower centres GABA produces a decrease in either spontaneous or evoked electrical activity. It also reduces the discharges produced by chemical excitants such as glutamic acid. The negative component of a variety of evoked responses induced by direct or indirect stimulation is reduced and sometimes reversed to a positive response. This has been taken to indicate a specific action of GABA on the excitatory synapses of the superficial dendrites in the cortex, but probably its action is the same on all the cortical neurones. In the spinal cord GABA reduces the amplitude of both excitatory and inhibitory synaptic potentials and also blocks the antidromic invasion of the postsynaptic membrane. The action of GABA is not affected by strychnine or picrotoxin. Intracellular recordings from motorneurones (Curtis, 1959) and cortical Betz cells (Krnjvic, 1964) show that it has no effect on the resting membrane potential. It has been suggested that GABA increases the membrane conductance by increasing the permeability to ions which are normally in electrochemical equilibrium, at or near the resting membrane potential.

Glutamic acid and some other acidic amino acids produce excitation of spinal and cortical neurones through a depolarization of the neuronal membrane (Curtis, 1960). Excitation is detectable as an increase in the firing frequency of the cells. However, glutamic acid and similar chemicals including aspartic, cystic, homocystic acids also depress cerebral cortical neurones and produce spreading depression, which is a consequence of an excessive depolarization of the nerve cells (Van Harreveld, 1960). The exact mechanism of the depolarization caused by

glutamate on the neuronal membrane is not known, but it has been suggested to be due to an increase in the permeability to sodium ions or perhaps more generally to several ions involved in the maintenance of the resting membrane potential.

The role of the amino acids in mediation of synaptic transmission, as stated in the preceding parts of this review, is a subject of controversy and uncertainty at best. No definite site of action of either depressant or excitant amino acids has yet been found. Also no substances are known whose actions specifically block the excitatory or inhibitory effects of the amino acids.

II STATEMENT OF THE PROBLEM

A large number of the centrally acting drugs currently classified according to their therapeutic use as hypnotics, sedatives, anticonvulsants, tranquilizers, analgesics and central muscle relaxants, are capable of producing nonspecific depression of the central nervous system. Their mechanism of action at the cellular level in the neuronal tissue is largely unknown. While a number of theories have attempted to explain partially the cellular depression caused by volatile and nonvolatile anesthetic agents in therapeutic use, such theories have not accounted for the action of the aforementioned drugs. The main reason for this failure is that these drugs are neither used clinically nor are they regarded as general anesthetics. It is very likely that the failure to produce anesthesia is not due to a lack of intrinsic anesthetic property but rather it is due to a lack of potency, overriding peripheral effects and acute toxicity.

The object of this study was to examine the anesthetic properties of a number of neurotropic drugs. An important objective was to see if their mechanism of action could be explained on the basis of an earlier proposal by Inoue and Frank (1962a) suggesting a common mechanism of action for local and general anesthetics. According to this hypothesis local and general anesthetics produce depression of the central nervous system by depressing the excitability in the neuronal tissues through a suppressive effect on the sodium permeability responsible for the generation and propagation of the action potential. The experiments were carried out to study the effects of drugs on the motor activity of the intact

mice and the electrical responses of neuronally isolated cerebral cortex of cats.

III EXPERIMENTAL

SECTION I.

ACTION OF DRUGS ON THE MOTOR ACTIVITY IN INTACT MICE.

METHODS.

All experiments in this section were carried out on white Swiss Albino female mice weighing between 18-30 grammes. All the drugs (except meprobamate and diazepam - see results) were prepared in normal saline and administered intraperitoneally to mice in volumes of 0.1 to 0.5 ml. Following drug treatment the animals were placed for observation in separate cages constructed to prevent mice from seeing each other but permitting observation by the experimenter. The drugs used were:

Diphenhydramine hydrochloride	Diazepam
Promethazine hydrochloride	Strychnine sulfate
Chlorpromazine hydrochloride	Bemegride
Gammahydroxybutyrate (GHB)	Nikethamide
Gammabutyrolactone (GBL)	Picrotoxin
Hyoscine hydrobromide	Caffeine
Meperidine hydrochloride	Gamma aminobutyric acid (GABA)
Meprobamate	L-Glutamic acid

After each drug treatment the animals were observed for effects on gross motor activity and behaviour. The loss of righting reflex, defined as the ability of the animal to right itself within one minute when placed on its back was used as a criterion of central depression. Two additional

criteria were the eventual recovery of the mouse and the presence of a withdrawal response to pinching of the foot indicating the persistence of the spinal reflex. The latter was also taken to exclude the presence of neuromuscular blockade. Animals meeting this criteria but without prior convulsant activity were considered to be in a state indistinguishable from general anesthesia.

Tests were carried out to determine whether the aforementioned drugs produced general anesthesia in mice primed with subanesthetic doses of phenobarbital, itself a nonvolatile general anesthetic. At first, following treatment with phenobarbitone alone the loss of righting reflex was recorded at regular time intervals. The time at which phenobarbital showed a maximal effect was determined (i.e. the time by which all animals losing righting reflex showed this response). Similar tests were conducted with each of the drugs given alone using convulsant activity, or the loss of righting reflex (where applicable) as the end point, and a maximum response time was determined for a particular effect and drug. A dose-response curve was derived for each drug and appropriate doses were selected from this curve for subsequent interaction experiments. In tests involving interaction between each drug and phenobarbitone, the two compounds were administered separately. The interval between administration was adjusted so that their maximum effects coincided with each other. The loss of righting reflex resulting from the maximal interaction was recorded. All animals which showed convulsant activity prior to the loss of righting reflex, and those which died following drug treatment were omitted from the analysis of results.

The dose-response curves were plotted and analysed by the methods of Litchfield and Wilcoxon (1949).

RESULTS.

i) Drug interaction between phenobarbital and central depressants.

The main purpose of these experiments on mice was to study the effect of various centrally acting depressant drugs on the anesthetic response produced by phenobarbital. When given alone in small doses phenobarbital produces a reversible loss of righting reflex in mice, but does not produce a loss of spinal reflexes except at higher doses. All the mice that were going to lose the righting reflex did so within 90 minutes, after which time no more animals showed the loss of this reflex. Thus the maximum response time, i.e. the time at which phenobarbital produced the peak response was 90 minutes. The central depressants, diphenhydramine, promethazine, chlorpromazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine and meperidine which produced stimulation in high doses when given alone, were given to mice pretreated with small doses of phenobarbital. The convulsant doses used and the maximum response time for the convulsant effect of each drug is shown in Table I. These drugs were given to phenobarbital treated mice in both convulsant and nonconvulsant doses. Dose-response curves were derived wherever possible for phenobarbital given alone (control curve) and for phenobarbital given in combination with a fixed dose of the other drug (test curve). The dose-responsecurves thus obtained were tested for parallelism by the methods of Litchfield and Wilcoxon (1949). Also using the latter method the dose of phenobarbital producing central depression in 50% of animals (ED 50) was derived from the test and the control curves, and tested for statistical significance. The results of individual drugs are presented below.

TABLE I

CONVULSANT DOSES INJECTED INTRAPERITONEALLY IN INTERACTION EXPERIMENTS

Drug	CD50* mg/Kg	CD99.5* mg/Kg	Maximum Response Time (Minutes)**
Diphenhydramine	60	100	20
Promethazine	130	170	30
Chlorpromazine	-	200	30
Gammahydroxybutyrate	-	1,000	30
Gammabutyrolactone	-	370	30
Hyoscine	500	750	30
Meperidine	75	-	25

* Doses producing convulsions in 50% and 99.5% of animals receiving the drug

** The time at which a peak convulsant response is produced after injection of the drug.

i) (a) Diphenhydramine.

When administered by itself to animals in doses 20-30 mg/kg diphenhydramine produced no discernible effect on the gross motor activity in mice. On injection of doses of 40 mg/kg or higher it produced cyanosis, hyperventilation tremor, and excitement, which was followed by clonic convulsions. When the drug was given to mice in convulsant dose after pretreatment with phenobarbital it potentiated the anesthetic effects of the latter as reflected in the shift of the dose response curve of phenobarbital to the left (Figure 1). The control and the test curves were parallel and the ED 50 of phenobarbital was significantly reduced in mice receiving both phenobarbital and diphenhydramine (Table III).

i) (b) Promethazine.

Promethazine given to mice by itself in doses of 100 mg/kg or less produced a slight sedative effect characterized by inactivity and a postural change (splaying of limbs). Higher doses in the range 120-200 mg/kg, caused tremor, excitement and convulsions. When given after phenobarbital it caused a loss of righting reflex in nonconvulsant dose (50 mg/kg) and a convulsant dose (130 mg/kg). The dose response curves which were shifted to the left (Figure 2) were parallel and the ED50 of phenobarbital was significantly reduced by promethazine (Table III). A large convulsant dose of promethazine (170 mg/kg) which produced convulsions in 99.5% of animals when given singly, also shifted the phenobarbital curve to smaller doses (Figure 2), but the curve obtained with the latter dose was not parallel with the control dose-response curve.

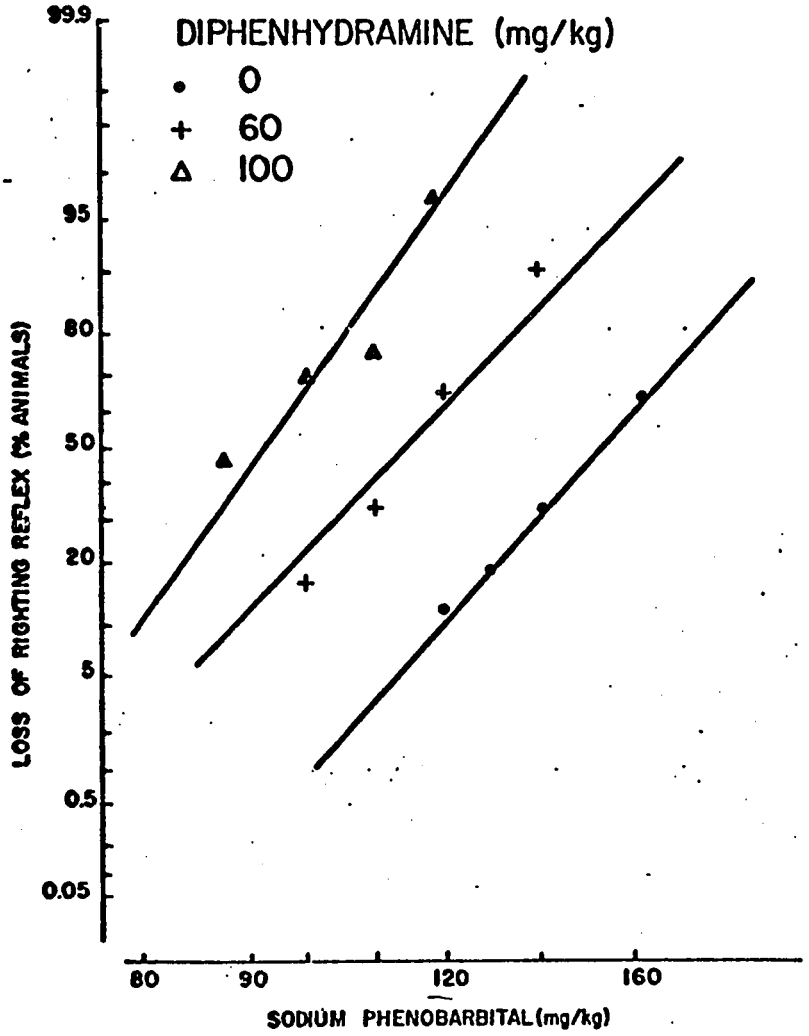

Figure 1. Central depression produced by various doses of diphenhydramine in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on graph indicate doses of diphenhydramine used.

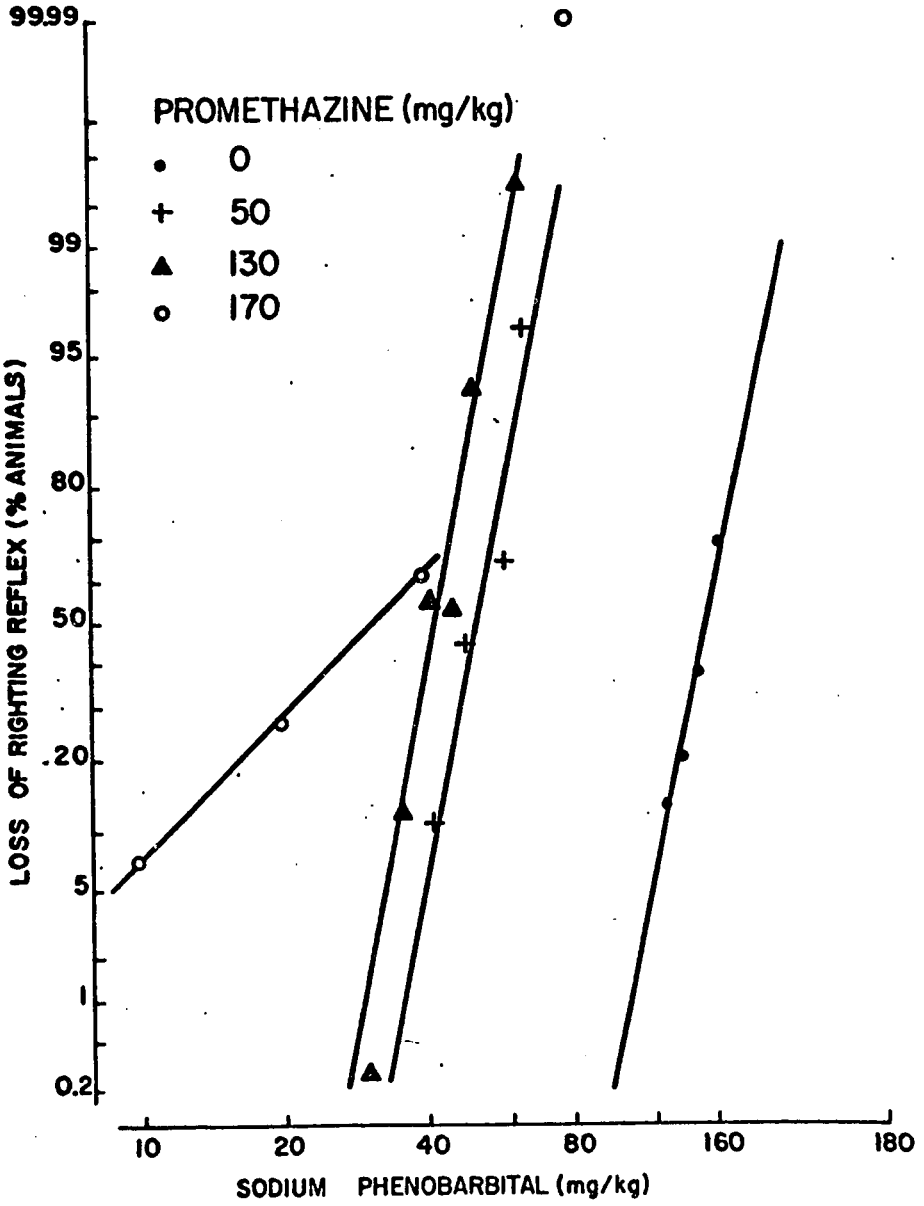

Figure 2. Central depression produced by promethazine in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on graph indicate doses of promethazine used.

i) (c) Chlorpromazine.

Chlorpromazine when given to mice in doses 5 mg/kg and lower produced only a slight sedative effect. Doses in the range 10-100 mg/kg produced a reversible loss of righting without affecting the spinal reflexes (Figure 3), while a dose of 120 mg/kg or more produced excitement and seizures similar to those produced by promethazine and diphenhydramine. A subanesthetic dose of chlorpromazine (5 mg/kg) given to mice following pretreatment with small doses of phenobarbital, potentiated the effects of the latter as shown by the shift of the phenobarbital dose-response curve to the left (Figure 4). Both dose-response curves were parallel and the ED50 of phenobarbital was reduced significantly by administration of chlorpromazine (Table III). A small dose of chlorpromazine (10 mg/kg) which produced loss of righting reflex in 8% of the animals, also shifted the dose response curve of phenobarbital to smaller doses (Figure 4). An examination of this curve showed that central depression resulting from combined effects of chlorpromazine and phenobarbital was greater than the sum of their individual effects, indicating a synergism in action. However, the dose-response curve obtained with 10 mg/kg was not parallel to the control curve. A convulsant dose of chlorpromazine (200 mg/kg) produced an even greater shift of the dose response curve to the left (Figure 4), but again it was not parallel with the control curve.

i) (d) Gammahydroxybutyrate.

Given in doses 200 mg/kg and lower gammahydroxybutyrate caused sedation in mice, while higher doses produced excitement and seizures.

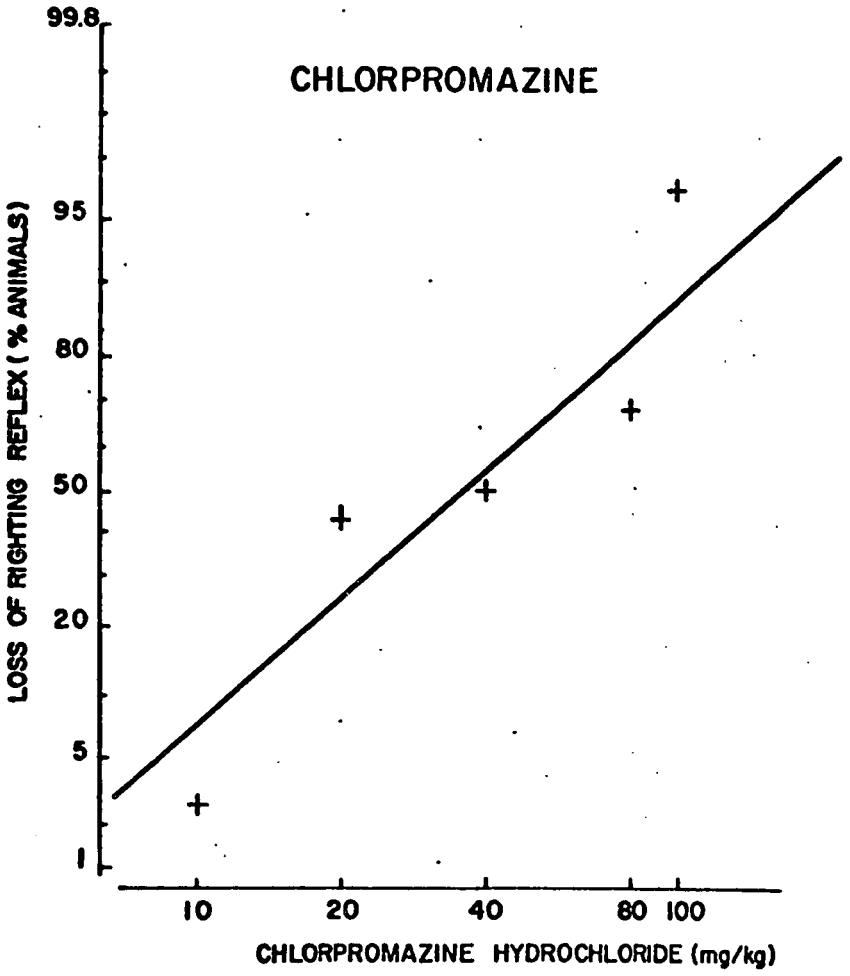

Figure 3. Central depression produced by chlorpromazine in untreated intact mice. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice.

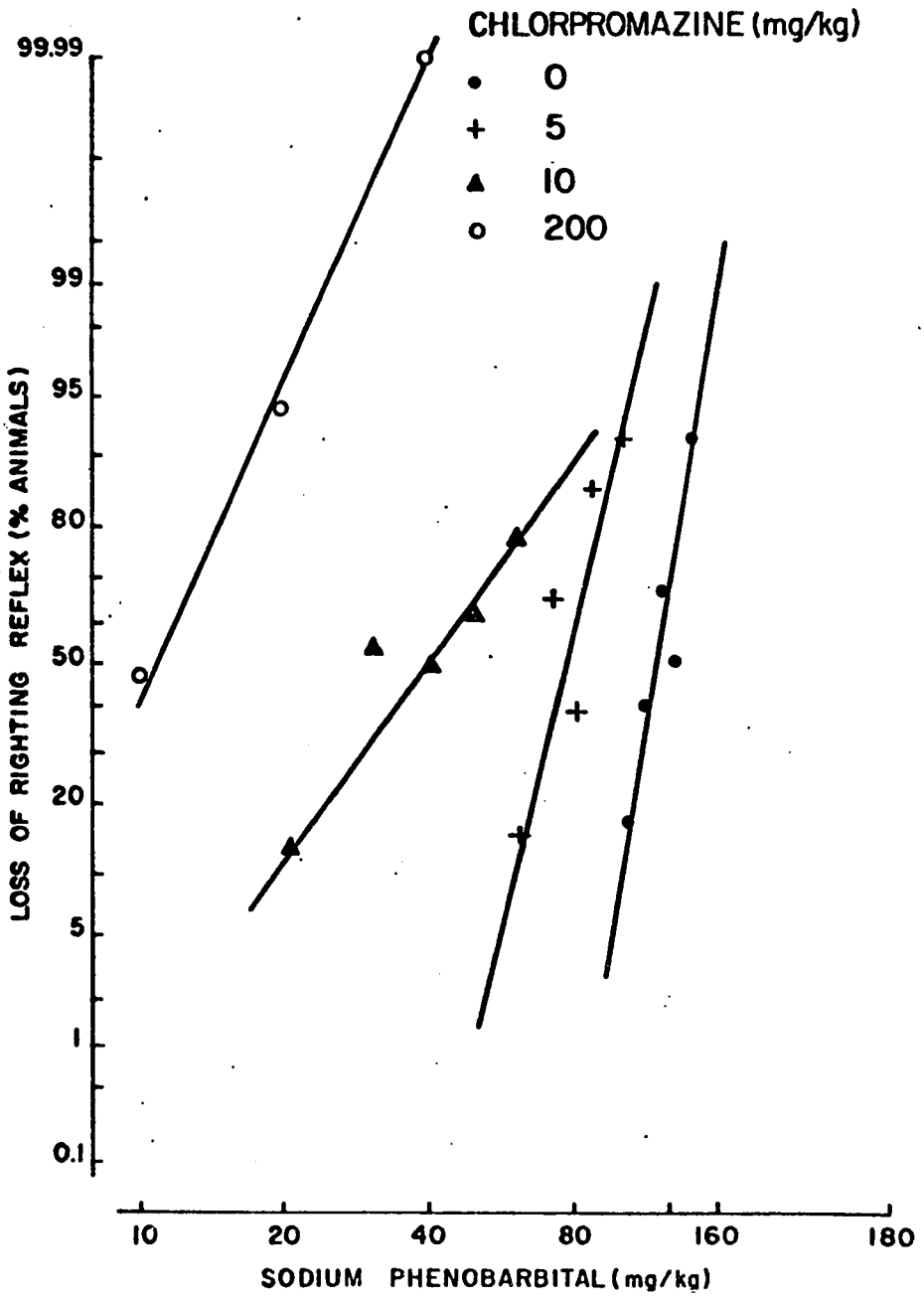

Figure 4. Central depression produced by chlorpromazine in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on the graph indicate doses of chlorpromazine used.

The convulsant action consisted of myoclonic jerks of low frequency preceded by exopthalmos, piloerection, hyperventilation, and splaying of the limbs. It potentiated the effects of phenobarbital when given after the barbiturate both in nonconvulsant doses (100, 200 mg/kg) and in a convulsant dose (1000 mg/kg) (Figure 5). The dose response curves resulting from interaction between gammahydroxybutyrate and phenobarbital were parallel and the ED50 of phenobarbital was reduced significantly by all the doses of gammahydroxybutyrate (Table III).

i) (e) Gammabutyrolactone.

Gammabutyrolactone produced effects similar to those produced by gammahydroxybutyrate but in smaller doses. Thus it produced sedation in mice in doses 100 mg/kg and lower, while higher doses produced excitement and convulsions. Given after phenobarbital it potentiated the effects of the latter in both nonconvulsant doses (50 and 100 mg/kg) and in a convulsant dose (370 mg/kg) (Figure 6). The dose response curves which were shifted to the left by gammabutyrolactone were parallel to the control curve in all cases (Figure 6) and the ED50 of phenobarbital was reduced significantly in animals receiving both drugs (Table III).

i) (f) Hyoscine.

When administered alone to mice hyoscine produced no discernible effect on the motor activity in doses 250 mg/kg and lower. On injecting higher doses, 300 mg/kg and over, it was found to produce hypermotility, exopthalmos, hyperventilation, and cyanosis followed by convulsions and death. When given after phenobarbital in a nonconvulsant dose (250 mg/kg) hyoscine potentiated the effects of phenobarbital as indicated by the shift

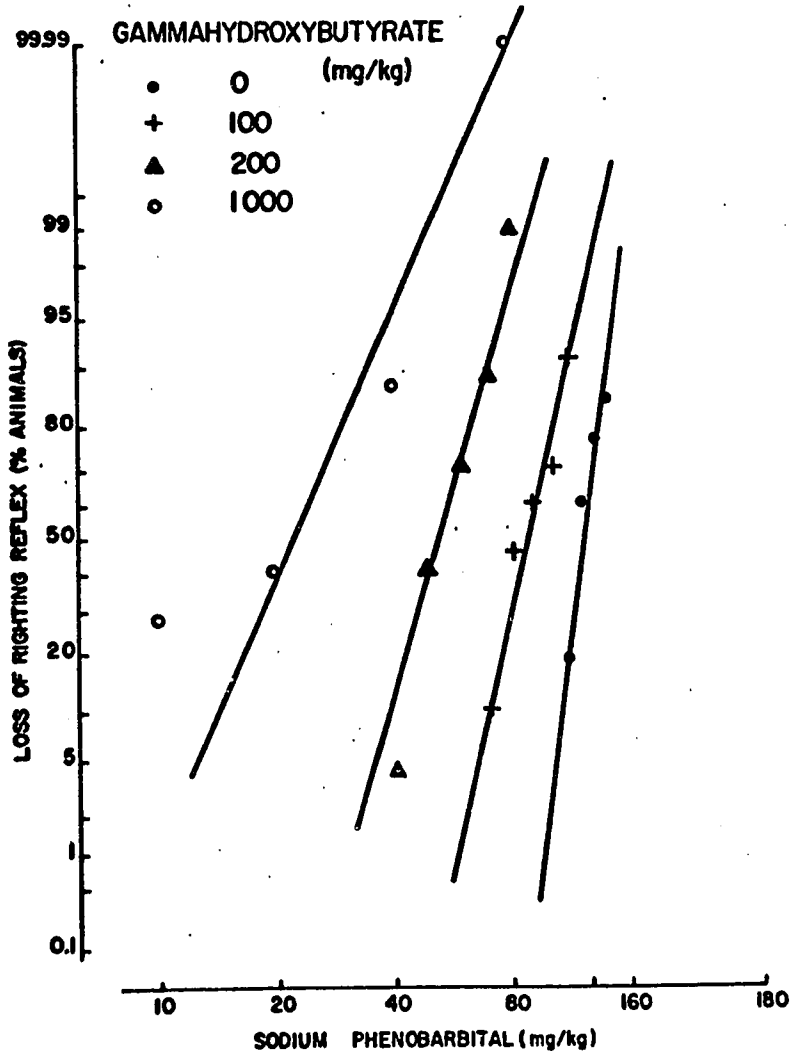

Figure 5. Central depression produced by gammahydroxybutyrate in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on the graph indicate doses of gamma-hydroxybutyrate used.

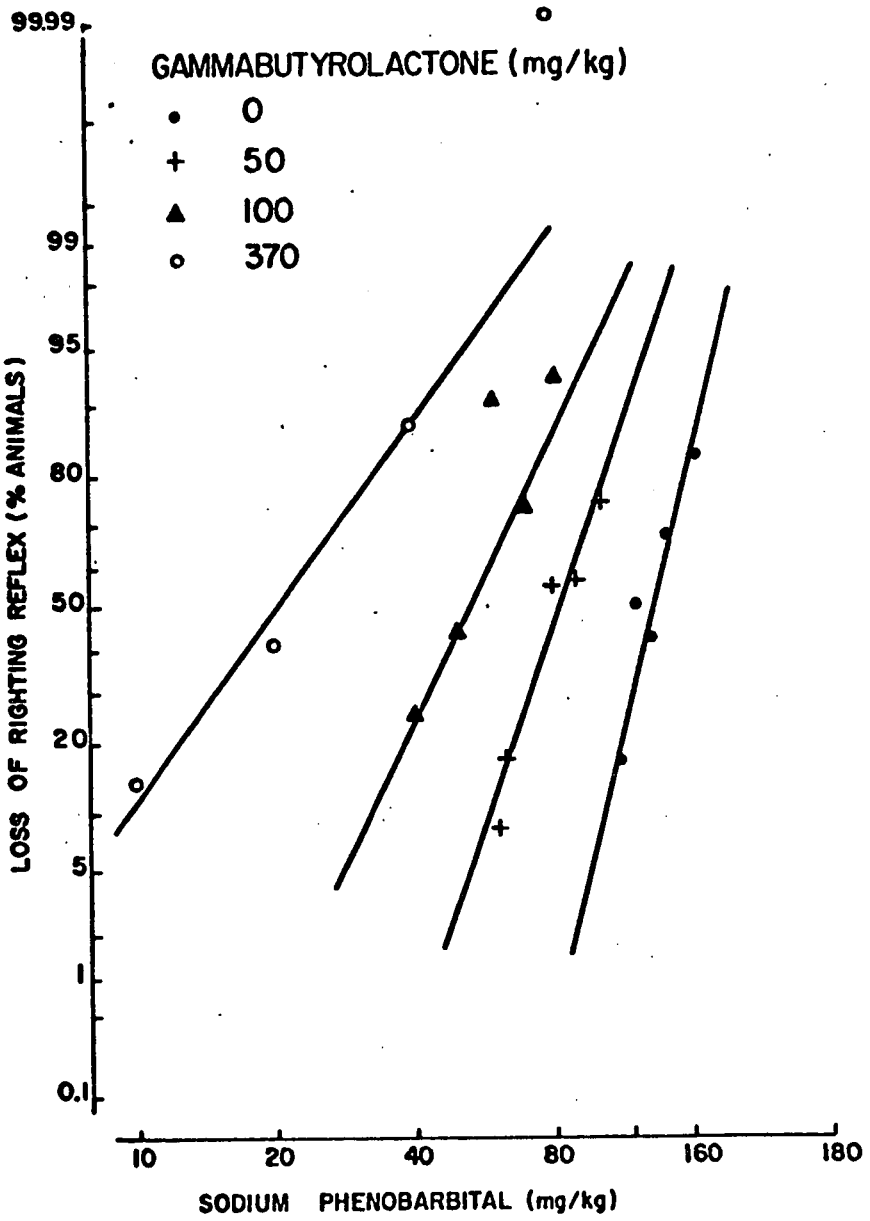

Figure 6. Central depression produced by gammabutyrolactone in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on the graph indicate doses of gamma-butyrolactone used.

of the dose response curve of the latter to the left (Figure 7). The control and the test curves were parallel and the ED50 of phenobarbital was significantly reduced by treatment with hyoscine (Table III). Convulsant doses of hyoscine, 500 mg/kg and 750 mg/kg, which produced seizures in 50% and 99.5% of the animals receiving hyoscine alone, failed to potentiate the central depression induced by phenobarbital in mice (Table II) and the animals receiving hyoscine died regardless of phenobarbital treatment. The death resulting from hyoscine would appear to be due to respiratory failure which was characterized by apnea and cyanosis and probably is the result of drug action on the peripheral tissues.

i) (g) Meperidine.

On injection of doses 50 mg/kg and lower, meperidine had no effect on the gross motor activity of the intact mice. Higher doses produced overt excitement and convulsions. In interaction experiments meperidine potentiated the loss of righting reflex induced by phenobarbital both in a convulsant dose (75 mg/kg) and a nonconvulsant dose (50 mg/kg) (Figure 8). Although both dose response curves were parallel and showed a shift to the left, and the ED50 of phenobarbital was significantly reduced (Table III), the nonconvulsant dose of meperidine lowered the ED50 more than the convulsant dose of the drug. The smaller shift produced by the latter dose may be due to a metabolic product of meperidine which may partially antagonize the effects of phenobarbital (see discussion below).

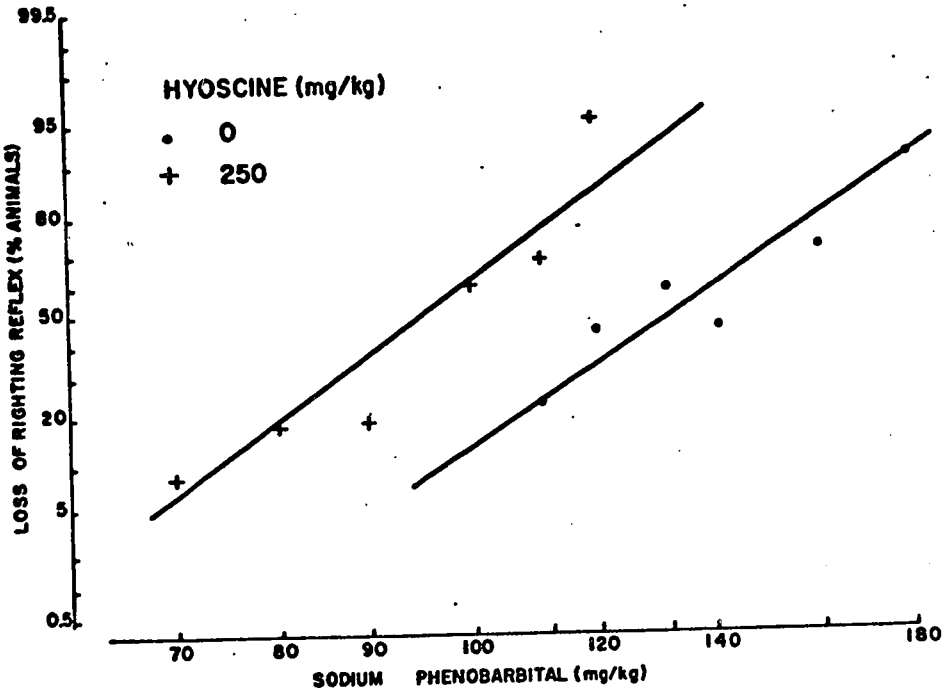

Figure 7. Central depression produced by hyoscine in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on the graph indicate doses of hyoscine used.

TABLE II

INTERACTION BETWEEN PHENOBARBITAL AND CONVULSANT DOSES OF HYOSCINE

Phenobarbital mg/kg	Hyoscine mg/kg	Animals Used	L.R.R.**	Deaths
0	500	20	0	11
135*	500	20	2	9
150*	500	20	0	12
0	750	12	0	11
135*	750	12	1	11
150*	750	12	0	12

* 135 mg/kg = ED25 150 mg/kg = ED50

** L.R.R. Number of animals showing a reversible loss of righting reflex.

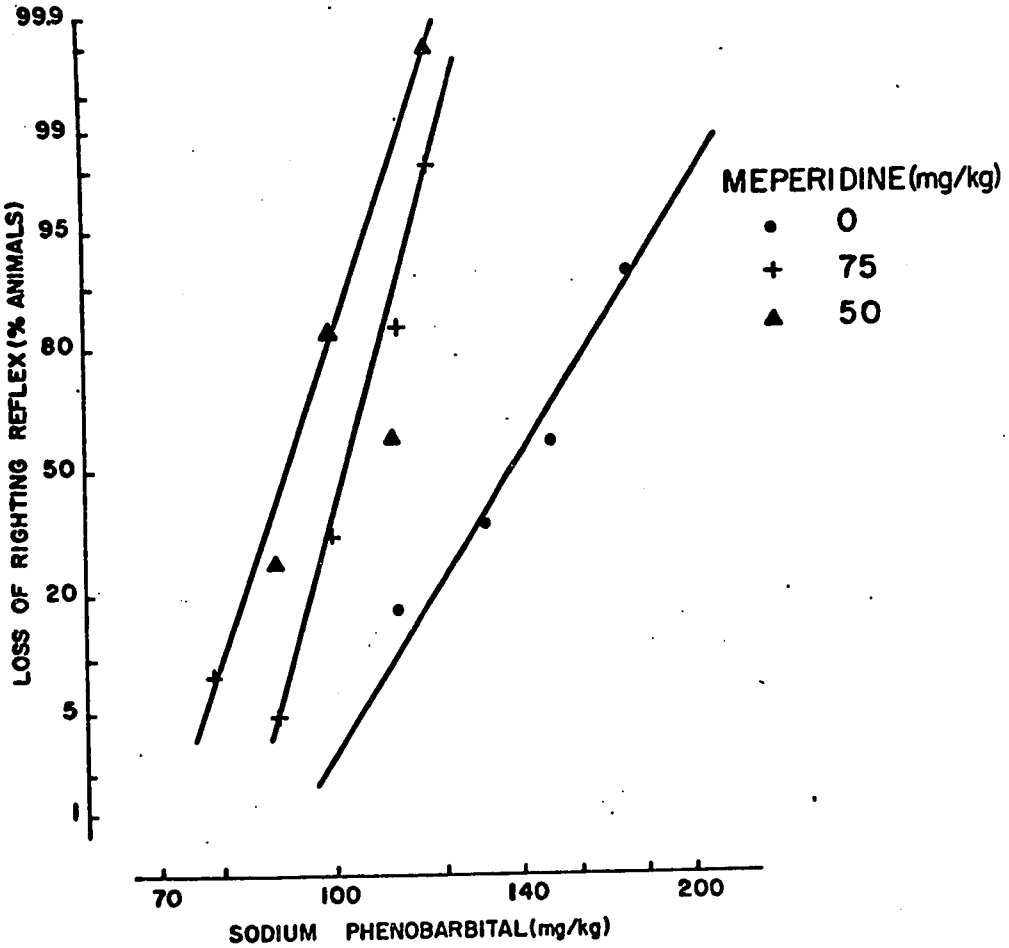

Figure 8. Central depression produced by meperidine in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on the graph indicate doses of meperidine used.

i) (h) Meprobamate.

Meprobamate being insoluble in water was given to mice in a 50% solution of polyethylene glycol. Control tests with the latter alone showed that when injected by itself in a quantity three to four times normally present in the meprobamate injection, it produced no discernible effects on the motor activity in the intact animals. When meprobamate was given alone to mice in the dose range 150-300 mg/kg it caused a reversible loss of righting reflex in these animals (Figure 9). The mice receiving the drug showed splaying of limbs, hyperventilation and occasional body jerks prior to central depression. Although all the mice receiving the drug showed a loss of righting reflex within a period of 35 minutes after the injection, none of the animals showed a loss of the spinal reflex at this time. The latter subsequently was lost also at 60-90 minutes after the injection of the drug. Higher doses of meprobamate produced a quicker loss of spinal reflexes, a flaccid paralysis and death. When given in a subanesthetic dose (100 mg/kg) to mice previously treated with small doses of phenobarbital, meprobamate potentiated the anesthetic effect of the barbiturate (Figure 10). The dose-response curves were parallel and the ED50 of phenobarbital was significantly reduced in the presence of meprobamate (Table III).

i) (i) Diazepam.

Diazepam being insoluble in water was given in the form of Diazepam Injection as supplied by Hoffman LaRoche and Company. Control tests were carried out using the Diazepam Placebo supplied in the same form. The latter when given to mice in doses equivalent to those of diazepam did not

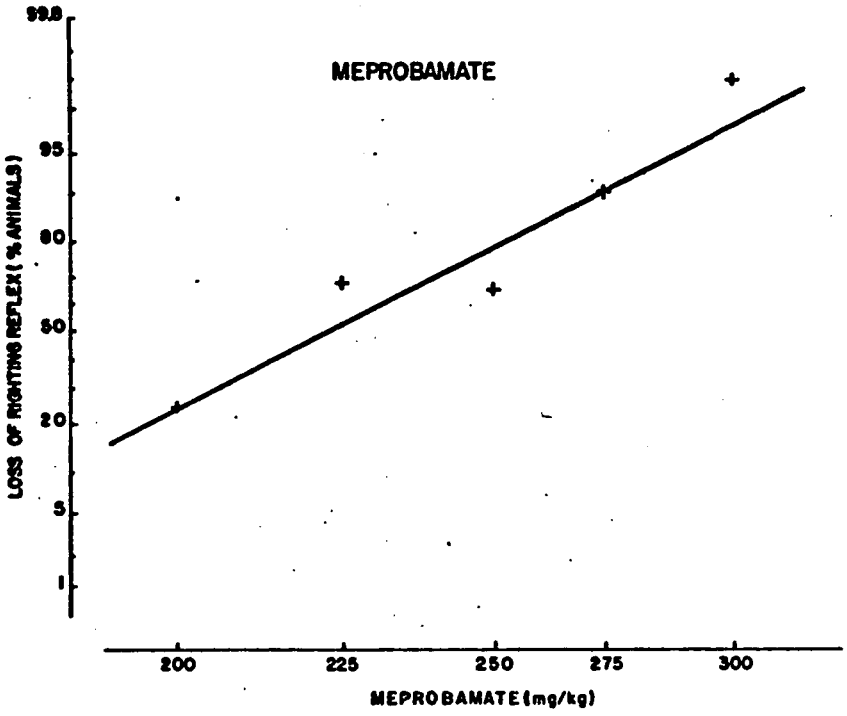

Figure 9. Central depression produced by meprobamate in untreated intact mice. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice.

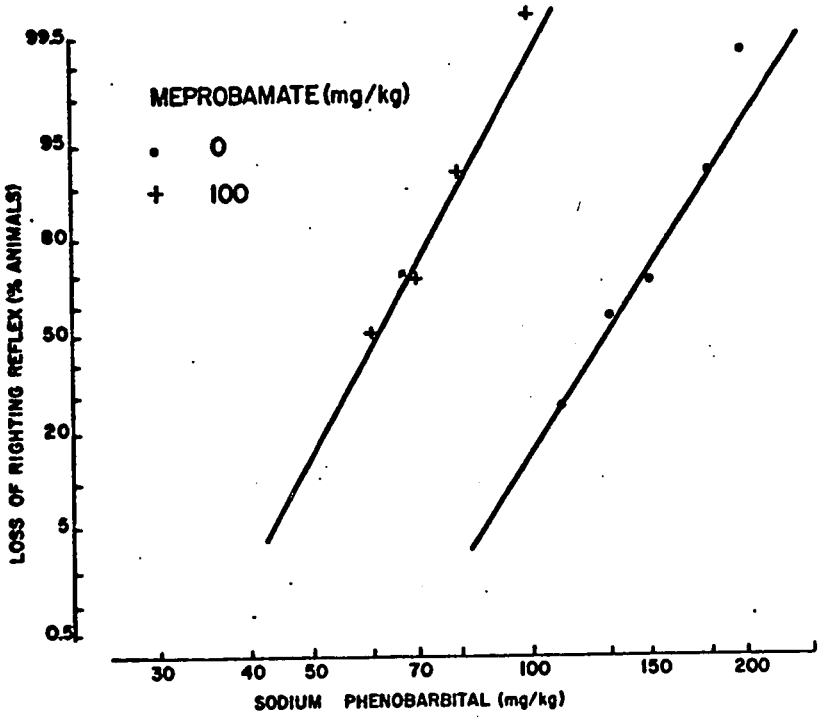

Figure 10. Central depression produced by meprobamate in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Value on the graph indicates dose of meprobamate used.

show any effect in mice. Diazepam when given to mice in the dose range 10-40 mg/kg produced a loss of righting reflex in these animals within a period of 20 minutes (Figure 11). The spinal reflex was lost soon after this (within 10 minutes). Higher doses of the drug produced a flaccid paralysis and resulted in death. When given in a subanesthetic dose (8 mg/kg) to mice which were pretreated with small doses of phenobarbital, diazepam potentiated the effects of phenobarbital as reflected in the shift of the dose-response curve of the latter drug to the left (Figure 12). The curves shown in Figure 12 were parallel and the ED50 of phenobarbital was significantly reduced in the presence of diazepam (Table III).

ii) Drug interaction between phenobarbital and central stimulants.

Experiments were carried out to study drug interaction between phenobarbital and drugs which cause a stimulation of the central nervous system. The stimulants used were: bemegride, picrotoxin, strychnine, caffeine and nikethamide. Given alone to mice in appropriate doses these drugs produced major convulsions resulting in death. The character of the convulsions varied among the drugs used. Bemegride, picrotoxin and nikethamide produced clonic convulsions, strychnine produced tonic convulsions while caffeine produced both generalized clonic and tonic convulsions. In the interaction tests between phenobarbital and these drugs, all the stimulants were given to mice in a convulsant dose following pretreatment with anesthetic doses of phenobarbital. The doses used and the maximal convulsant response time for each drug are shown in Table IV.

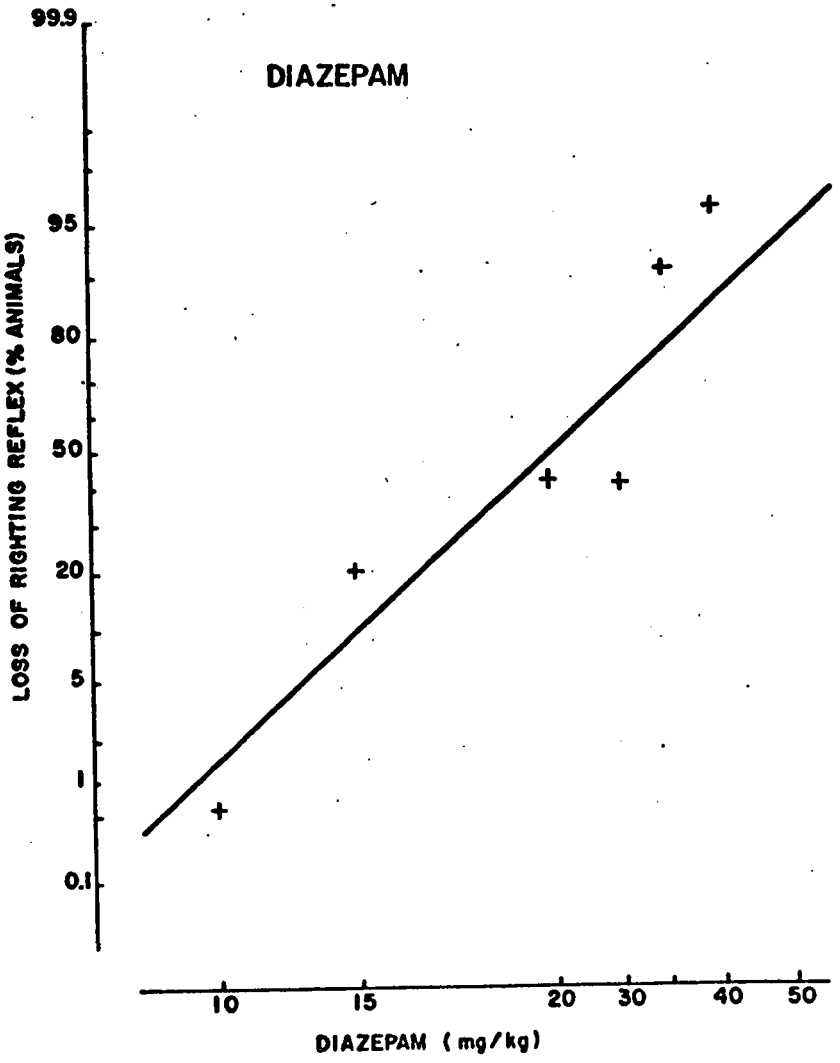

Figure 11. Central depression produced by diazepam in untreated intact mice. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice.

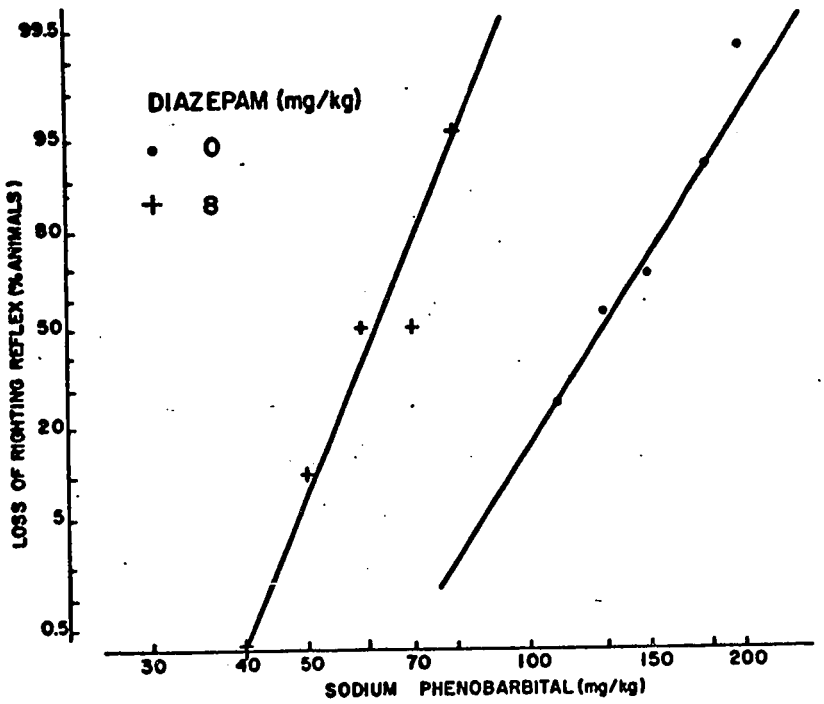

Figure 12. Central depression produced by diazepam in mice pretreated with subanesthetic doses of phenobarbital. Logarithmic probability plot. Each point is obtained from twelve to fifteen mice. Value on the graph indicates dose of diazepam used.

TABLE III

EFFECT OF SOME CENTRAL DEPRESSANTS ON ED50 OF SODIUM PHENOBARBITAL

Drug	Phenobarbital (Control)		Phenobarbital & Drug (Test)		Significance* at 95% Probability
	ED50 mg/kg	95% Confidence Limits	ED50 mg/kg	95% Confidence Limits	
Diphenhydramine					
60 mg/kg	148.0	139.6-156.9	114.0	104.1-124.8	+
100 mg/kg	148.0	139.6-156.9	93.5	87.8- 99.6	+
Promethazine					
50 mg/kg	151.0	141.1-161.5	51.3	42.8- 61.6	+
130 mg/kg	151.0	141.1-161.5	41.7	38.9- 44.7	+
Chlorpromazine					
5 mg/kg	126.0	118.9-133.6	79.4	73.4- 85.9	+
Gammahydroxybutyrate					
100 mg/kg	126.0	118.3-134.1	90.2	77.1-105.5	+
200 mg/kg	126.0	118.3-134.1	53.0	46.5- 60.4	+
1000 mg/kg	126.0	118.3-134.1	22.4	18.7- 24.9	+
Gammabutyrolactone					
50 mg/kg	135.0	125.9-144.7	85.1	76.0- 95.3	+
100 mg/kg	135.0	125.9-144.7	41.7	35.3- 49.2	+
370 mg/kg	135.0	125.9-144.7	20.0	13.3- 30.0	+
Hyoscine					
250 mg/kg	127.6	119.2-136.5	96.6	89.4-104.3	+
Meperidine					
50 mg/kg	138.0	128.3-148.3	92.3	88.3- 96.5	+
75 mg/kg	138.0	128.3-148.3	99.5	95.6-103.5	+
Meprobamate					
100 mg/kg	164.0	149.0-180.0	61.0	54.2- 68.6	+
Diazepam					
8 mg/kg	164.0	149.0-180.0	64.6	58.7- 71.1	+

* Sign (+) indicates a significant difference between control and test ED50 of phenobarbital at 95% probability level.

TABLE IV

CONVULSANT DOSES OF STIMULANT DRUGS
INJECTED INTRAPERITONEALLY AFTER PHENOBARBITAL

Drug	CD50* mg/kg	Maximum Response Time (Minutes)**
Bemegride	45	10
Picrotoxin	15	10
Strychnine	1.6	25
Caffeine	250	10
Nikethamide	300	5

* Dose producing convulsions in 50% of animals receiving the drug

** The time at which a peak convulsant response is produced after injection of the drug.

ii) (a) Bemegride and picrotoxin.

When given to mice which had been pretreated with phenobarbital, both bemegride and picrotoxin antagonized the anesthetic effects of phenobarbital. This antagonism is reflected in the shift of the dose-response curve for phenobarbital to the right (Figure 13). The control test curves were parallel. The ED50 of phenobarbital was significantly increased in the presence of bemegride and picrotoxin (Table VI). None of the animals receiving phenobarbital and one of these stimulant drugs convulsed or died as a result of the interaction.

ii) (b) Strychnine, caffeine, and nikethamide.

When administered to mice following a treatment with anesthetic doses of phenobarbital, they neither antagonized nor did they potentiate the depressant effect of phenobarbital (Table V). However, none of the animals receiving the drugs died as a result of interaction between phenobarbital and these drugs. The dose-response curves for interaction of phenobarbital with strychnine, caffeine, and nikethamide overlapped with each other. The drugs did not cause a significant change in the ED50 of phenobarbital (Table VI).

iii) Drug interaction between phenobarbital and amino acids.

iii) (a) Gamma aminobutyric acid and glutamic acid.

When given intraperitoneally to mice in doses as high as 2000-3000 mg/kg, both gamma aminobutyric acid and glutamic acid did not affect the gross motor activity or behaviour in mice. These drugs also failed to produce any change in the response of mice previously treated with anesthetic or subanesthetic doses of phenobarbital.

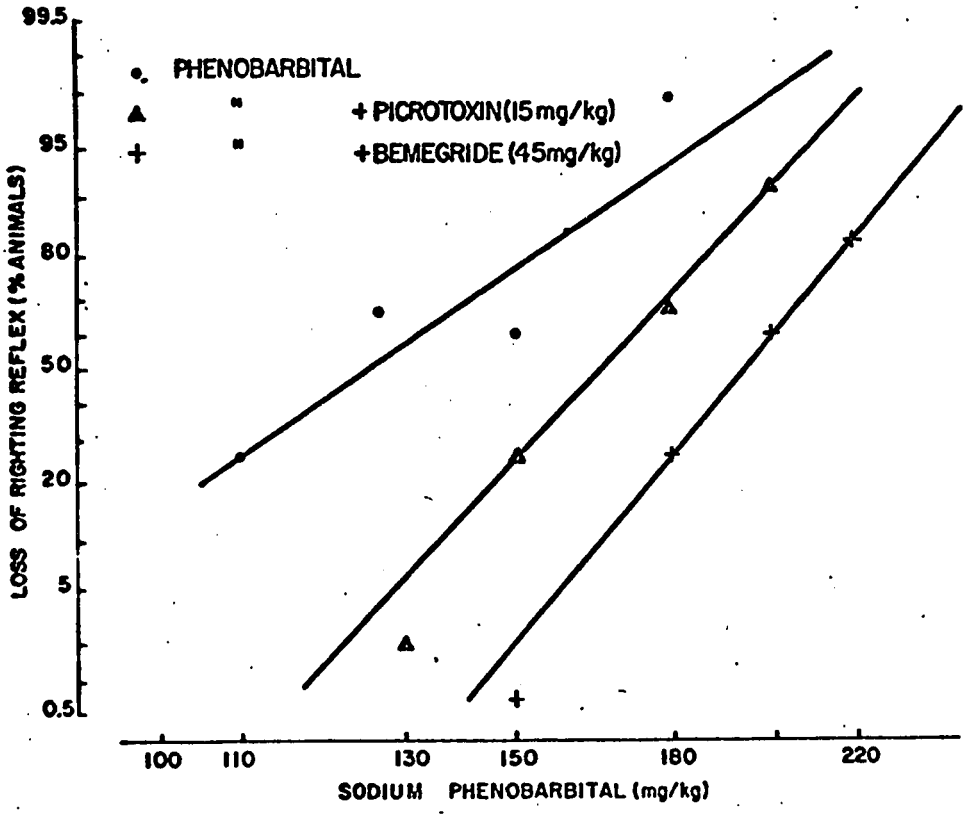

Figure 13. Central depression antagonized by bemegride and picrotoxin in mice pretreated with anesthetic doses of phenobarbital. Logarithmic probability plots. Each point is obtained from twelve to fifteen mice. Values on graph indicate doses of bemegride and picrotoxin used.

TABLE V

INTERACTION BETWEEN SODIUM PHENOBARBITAL AND CENTRAL STIMULANTS

Sodium Phenobarbital mg/kg.	% Mice losing Righting Reflex					
	Saline 0.2 ml	Bemegride 45 mg/kg*	Picrotoxin 15 mg/kg*	Caffeine 250 mg/kg*	Strychnine 1.6 mg/kg*	Nikethamide 300 mg/kg*
110	25	0	0	0	33	0
130	66	0	0	46	59	66
150	59	0	25	73	91	100
180	100	25	66	75	75	83
200	100	59	92	100	100	100
220	-	82	-	-	-	-

* Convulsant Dose (CD50)

TABLE VI.

EFFECT OF CENTRAL STIMULANTS ON ED50 OF SODIUM PHENOBARBITAL.

Drug	Phenobarbital (Control)		Phenobarbital & Drug (Test)		*Significance at 95% Probability
	ED50 mg/kg	95% Confidence Limits	ED50 mg/kg	95% Confidence Limits	
Bemegride 45 mg/kg	127.6	116.0-140.4	195.0	175.6-216.4	+
Picrotoxin 15 mg/kg	127.6	116.0-140.4	165.2	154.3-176.7	+
Caffeine 250 mg/kg	127.6	116.0-140.4	129.1	110.3-151.0	-
Strychnine 1.6 mg/kg	127.6	116.0-140.4	121.9	103.3-143.8	-
Nikethamide 300 mg/kg	127.6	116.0-140-4	117.5	98.0-131.0	-

* Sign (+) indicates presence of a significant difference; and sign (-) indicates absence of a significant difference between control and test ED50 of phenobarbital at 95% probability level.

DISCUSSION.

A number of centrally acting drugs chosen from various classes of central nervous system depressants have been examined for anesthetic properties in tests on intact mice. Such tests also have been carried out with classical stimulants of the central nervous system for a comparison between the effects of the two groups of drugs and their mechanisms of action. The depressants investigated include: a) diphenhydramine, an antihistamine and sedative; b) promethazine, an antimotion sickness drug; c) chlorpromazine, a major tranquilizer; d) gammahydroxybutyrate and gammabutyrolactone, two hypnotic agents; e) hyoscine, a sedative with anticholinergic properties; f) meperidine, a narcotic analgesic; g) meprobamate, a minor tranquilizer and central muscle relaxant; h) diazepam, a neurosedative and central muscle relaxant. The stimulant drugs used were: a) bemegride, picrotoxin, and nikethamide which are regarded as brain stem stimulants; b) caffeine, a cerebral stimulant; and c) strychnine, a spinal cord stimulant.

The results of the interaction studies between phenobarbital and the depressants showed that although all these drugs are capable of enhancing the central depression induced by phenobarbital, there are important differences among them. These differences in the interaction are a reflection of differences in the mechanisms underlying the action of these drugs. A recognition of the latter, therefore, permits one to make a rudimentary classification of these drugs on the basis of their mechanism of action. The interaction between stimulants also permits a similar rudimentary classification.

The results obtained with central depressants show that these drugs can be divided into three main groups (Table VII): A) One chief group of drugs which includes diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine, and meperidine is characterized by the observation that when administered to animals alone the drugs in this group produced excitement and convulsions in high doses. On the basis of interaction with phenobarbital, a further subdivision of this group is possible. Thus, one class of drugs which includes diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone potentiated the anesthetic effect of phenobarbital in convulsant doses. The potentiation of the response was greater in convulsant doses than in nonconvulsant doses, and in this respect their effect is similar to local anesthetic drugs which, on the basis of previous work (Frank and Sanders, 1963), can be included in this class. A second class of drugs in this group (A) includes drugs such as hyoscine, which enhanced phenobarbital effect in nonconvulsant doses but failed to interact with the latter in convulsant doses due to a lethal effect at these higher doses. In this respect, its action parallels the action of tetrodotoxin which behaved similarly in tests carried out previously (Frank and Pinsky, 1966). A third class of drugs in group A is represented by meperidine, which potentiated the phenobarbital response in a nonconvulsant dose and a convulsant dose, but the potentiation in the latter dose was less than in the former dose. The smaller degree of potentiation by a higher dose of meperidine may be due to conversion of meperidine to a metabolite in the body, which has stimulant properties antagonistic to phenobarbital. Alternatively, it

TABLE VII

A CLASSIFICATION OF CENTRALLY ACTING DRUGS BASED ON THEIR ACTION IN INTACT MICE.

I

Depressants

Shift phenobarbital dose-response curve to smaller doses.

Group A.
Convulsions in animals when given alone in high doses.
Class 1: Produce a large shift of phenobarbital curve to left in a convulsant dose.
e.g. Diphenhydramine
Promethazine
Gammahydroxybutyrate
Gammabutyrolactone
Local anesthetics*
Class 2: Do not shift phenobarbital curve in convulsant dose. Produce anoxic convulsions in higher doses.
e.g. Hyoscine
Tetrodotoxin*
Class 3: Produce a smaller shift in phenobarbital curve in convulsant dose than in a non-convulsant dose.
e.g. Meperidine

Group B.
Produce loss of righting reflex when given alone. No convulsions when given in a high dose.
e.g. Meprobamate
Diazepam
Barbiturates

Group C.
Produce loss of righting reflex when given alone, and convulsions in high doses. Shift phenobarbital curve to smaller doses in convulsant and nonconvulsant doses.
e.g. Chlorpromazine.

II

Stimulants

Convulsant when given alone. May shift phenobarbital dose-response curve to right or may not shift it.

Group A.
Antagonize effect of phenobarbital (shift the dose-response curve to higher doses) in convulsant dose.
e.g. Bemegride
Picrotoxin
Pentylenetetrazole*

Group B.
Do not antagonize phenobarbital in convulsant dose and do not potentiate it (No shift in dose-response curve)
e.g. Strychnine
Caffeine
Nikethamide

* Indicates results from previous work (See Discussion).

may also be due to a release of a substance with stimulant properties. B) A second group of drugs may be considered to include drugs such as meprobamate and diazepam. The drugs are characterized by the observation that when given alone to mice they can, unlike the drugs in the preceding group, produce a loss of righting reflex. In slightly higher doses they can also produce a loss of spinal reflexes, the dose margin between these two effects is therefore quite narrow. Also unlike other depressants, they do not produce convulsions in intact animals. However, if they do have any stimulant effect, the latter may be precluded by death resulting from muscular paralysis. The barbiturate drugs may also belong to this group since they share common properties, but the margin between doses producing loss of righting and spinal reflexes is wider in the case of barbiturates. C) The final group of central depressants is represented by chlorpromazine, and it incorporates the properties of the two preceding groups of central depressants. Thus in smaller doses chlorpromazine produces a loss of righting reflex while in higher doses it produces excitement and convulsions when given by itself to intact mice. Furthermore, in a convulsant dose it enhances the central depression due to phenobarbital when given following the latter.

In direct contrast to the central depressants which produce a shift of phenobarbital curve to smaller doses even when given in a stimulant dose, the classical central stimulants, which on their own produce only excitement and seizure activity produce either a shift of the same curve to higher doses or do not shift it at all. Drugs such as bemegride, picrotoxin, and pentylenetetrazole (previous work, Frank and Sanders, 1963)

antagonize the depressant effect of phenobarbital, while other stimulants such as strychnine, caffeine and nikethamide do not antagonize this effect. Thus, two distinct groups of stimulant drugs are recognizable on basis of their interaction with phenobarbital.

The various groups and subgroups of centrally acting drugs have been summarized in Table VII.

It appears that the central depressants have a dual action; that is their action has both depressant and stimulant components. On the basis of interaction experiments a separation is possible between the stimulant property of central depressants and that of analeptic drugs listed in column II, Table VII. It would also seem that the mechanisms which underlie the excitatory action of central depressants are fundamentally different from those underlying the actions of central stimulants. The enhancement of phenobarbital induced depression of the nervous system by convulsant doses of drugs such as diphenhydramine, promethazine and others, suggests that the mechanisms responsible for stimulant component of action are inhibitory in nature. If it is assumed that the cellular response of the central nervous system to depressants is only a depression of the neurones, it is then possible that their excitatory action at the gross level is only a result of specific depression of neurones in the inhibitory nuclei or pathways in the central nervous system. Such a release from the central inhibition may be expressed as excitation or convulsions in the whole animal. The depressant component of the dual action on the other hand may also be a consequence of cellular inhibition, but such an inhibition may involve a direct depression of the

excitatory nuclei or pathways. The threshold of the drug induced depressant responses may vary in the two types of pathways with different drugs.

In contrast to the above, the antagonism of the barbiturate induced depression by some analeptic drugs is suggestive of a direct stimulant action of these drugs, possibly resulting from excitation of neurones in the excitatory pathways in the central nervous system. The cellular mechanism of excitation, however, may be different for different analeptics. Thus, bemegride, picrotoxin and pentylenetetrazole which antagonize central depression, may differ in their mechanism of action from strychnine, caffeine and nikethamide which do not antagonize it. In order to substantiate the contention that the stimulatory effects of central depressants are indirect and those of analeptic drugs are direct, these drugs may be tested in isolated neuronal systems, which are accessible to direct application of drugs.

It appears that the enhancement of central depression produced by the depressant drugs is more than a simple addition of the individual effects of the barbiturates and these drugs. It is, in fact, a potentiation. Thus, when phenobarbital is given to mice in subthreshold doses its action results in a pharmacological stimulus which is not great enough to produce a response such as the loss of righting reflex. However, if central depressants are administered following subthreshold doses of phenobarbital the result is that a critical level of stimulus is reached and central depression is produced. It was suggested above that the depressant and stimulant effects of central nervous system

depressants are due to depression of excitatory and inhibitory neuronal pathways respectively. If this is so, one might expect to see the excitation in interaction experiments between phenobarbital and the convulsant doses of depressant drugs. But the end point in these tests which is the loss of righting reflex, expresses only the net central depression in various neuronal pathways in the central nervous system that are involved in maintaining the righting reflex and consequently any prevailing excitation is not reflected in the gross response.

In contrast to the potentiation of effect described above, central stimulants such as bemegride, which have only stimulant properties may antagonize phenobarbital induced depression through a functional or physiological antagonism. The latter may result from the possibility that depressants and antagonists interact with different receptors on a common effector system in such a way as to produce opposite effects. This type of functional interaction may be comparable to the model of labilizers (veratrine alkaloids) and stabilizers (local anesthetics) which influence the membrane potential in the opposite way acting on different receptors (Shanes, 1958). The failure of other stimulants to antagonize may be due to lack of affinity for these receptor sites.

The central excitation seen with some depressant drugs may not be central in origin but instead may result from action of drugs on the peripheral tissues. Thus, Frank and Pinsky (1966) showed that tetrodotoxin which depresses the central nervous system also produces excitement and convulsions preceding death in mice. They also showed that in such animals phenobarbital failed to modify the lethality of tetrodotoxin

and concluded that the latter resulted from the action of tetrodotoxin on the peripheral tissue. Thus, the convulsions produced by this agent are probably due to anoxia. In this study the effect of hyoscine in convulsant doses showed a similarity to tetrodotoxin effects. Like the latter the lethality due to hyoscine was not modified by phenobarbital, and it appears that the excitement and convulsions produced by hyoscine also are probably due to anoxia resulting from respiratory collapse, probably at a peripheral site.

The dose-response curves obtained as a result of interaction experiments were for the most part parallel to each other. Only dose-response curves that deviated from parallelism were those of promethazine and chlorpromazine given in high convulsant doses (Figures 2 and 4), and chlorpromazine given in a small anesthetic dose (Figure 4). The classical interpretation from parallel dose-response curves of two agents is that they are acting on the same site and probably have a common mechanism of action (Gaddum, 1937; Lewis, 1964). In view of this it is attractive to postulate that the central nervous system depressants which potentiate phenobarbital anesthesia, and central nervous system stimulant which antagonize it, are acting at the same sites as phenobarbital. But such a postulate based solely on responses of intact animals can be erroneous. The theoretical principles underlying the dose-response relationships are based on several assumptions including the one that drugs are acting on specific receptors under equilibrium conditions. Also for the most part the experimental work which supports the theoretical principles has largely been carried out on isolated organs and tissues (Ariens, 1964).

It may, therefore, be incorrect to apply the same principles to drug responses in the whole animals, in which the drug interaction is complicated by processes of absorption, transport, binding to tissues, biotransformation and the fact that many tissues contribute to the overall effect (Gaddum, 1957). Thus, conclusions regarding the site and mechanism of action based only on parallel dose-response curves from intact animal studies must be treated with caution and should be supplemented with supportive evidence at the cellular level. In interaction studies particularly, the deviations from parallelism may arise as a result of interference of one or more drugs with any of the above mentioned processes associated with drug action. A lack of deviation from parallelism in dose-response curves is useful in that a large number of central depressants and stimulants are partly acting on the same group of neurones in the central nervous system which are also responsive to phenobarbital. Parallelism also makes the statistical analysis of curves and calculation of doses and potency ratios of different drug combinations more meaningful than it would be in absence of parallelism.

One major problem in drug interaction studies of the kind described in the present investigation is the necessity of timing interval between the drugs being given to the animal. The failure to take this factor into account in the design of experiments can lead to erroneous results. Thus, although it had been shown that procaine given in convulsant doses to phenobarbital treated mice potentiated the effects of the latter drug, it was reported that procaine in similar doses fails to potentiate the effects of pentobarbital, which is a shorter acting barbiturate anesthetic

(Sanders, 1967). The failure to demonstrate interaction between procaine and pentobarbital was found to be due to the effect of pentobarbital wearing off before the effects of procaine occurred. When the time interval between two drugs was adjusted such that the two peak effects coincided, a clear potentiation of pentobarbital by procaine was seen (Frank and Jhamandas, 1969). Therefore, for a meaningful interpretation of drug interaction tests the timing is of the utmost importance. In the present study, phenobarbital was selected for priming mice for depression, since it is longer acting and free from metabolic degradation and therefore would not wear off as quickly as pentobarbital.

The anesthesia in mice in these experiments has been characterized by a reversible loss of righting reflex without a corresponding loss of the spinal reflexes. Although this criteria may not be compatible with a state of clinical anesthesia, it did establish the existence or absence of an experimental condition which was otherwise indistinguishable from general anesthesia in man. The loss of righting reflex was used in preference to the prolongation of hexobarbital sleeping time as an index of central nervous system depression on grounds of the lack of a good correlation between central depression and the prolongation of hexobarbital sleeping time. While it is true that barbiturate sleeping time is prolonged by a variety of central depressants, it is unfortunately also prolonged by a number of substances that are not central depressants (Riley and Spinks, 1958). Thus cholestrol, glucose, sucrose, glycerin, inorganic nitrates, and sodium chloride are all capable of prolonging the barbiturate sleeping time in animals (Riley and Spinks, 1958). But the

potentiation of anesthetic effects of phenobarbital as indicated by loss of righting reflex is only confined to central depressants (Maykut and Kalow, 1955; Aston and Cullumbine, 1959; Frank and Sanders, 1963). Other drugs such as neuromuscular blocking agents can also produce a reversible loss of the righting reflex but their effect is preceded by the loss of spinal reflexes owing to neuromuscular blockade.

In recent years gamma aminobutyric acid and glutamic acid have emerged as candidates for inhibitory and excitatory transmitters respectively in the central nervous system. At the cellular level GABA produces a depression of neuronal activity while glutamate acts as an excitant. When subjected to tests in this study both these compounds failed to produce any effect when given singly or after phenobarbital in very high doses. The failure of these compounds to produce an effect in intact animals has been attributed to their inability to cross the blood brain barrier (Curtis, 1965), thus our inability to demonstrate any effects on intact white mice is not surprising.

III EXPERIMENTAL

SECTION II

EFFECT OF DRUGS ON NEURONALLY ISOLATED CEREBRAL CORTEX.

METHODS.

i) Surgery.

All experiments were carried out on slabs of neuronally isolated cerebral cortex in male or female cats prepared according to the methods described by Burns (1950) and Burns and Grafstein (1952). Male or female cats weighing between 2.0 and 3.0 Kg. were anesthetized in a wooden box measuring 48 x 28 x 38 cm. The ether was administered by placing an ether soaked piece of cotton in the box. When the animal was anesthetized it was transferred to an operating table and placed on its back. The trachea was exposed by a midline incision in the neck and a metal cannula inserted into it. Ether was then administered to the animal through a vaporizer (TRIMAR) with a variable by-pass which permitted control of the concentration of ether being given to the animal. Both common carotid arteries were exposed and a loose string extending about 10 cm outside the neck was placed around each vessel in order to facilitate a rapid arrest of blood flow to the head by occlusion. An incision was made in the left flank, the femoral vein was then exposed and cannulated with a polyethylene cannula attached to a glass syringe filled with physiological saline or 5% dextrose.

The head of the animal was then fixed in a Czermak type head holder (Palmer & Co.) which allowed both rotary and vertical adjustment

of the head. The animal was turned over so that it lay on its stomach. The scalp was shaved and an incision was made to expose the temporal muscle bilaterally. The muscle overlying the cranium on one side was separated from the bone, clamped off at the point of its insertion and removed by cutting above the clamped region. A trephine hole about 12 mm in diameter was made in the side of the exposed skull. The bleeding resulting from this step was controlled with bone wax. Subsequently, the cerebral cortex was exposed by chipping the bone with a rongeur from the bony tentorium to the anterior border of the ectosylvian gyrus, and from the middle of the marginal gyrus to the inferior border of the ectosylvian gyrus. In cases of excessive bleeding during the bone removal the cat's head was raised high to control the blood loss. The dura mater overlying the exposed cortical surface was removed using blunt scissors and the bleeding from the surface vessels was controlled by sealing them off with the electricator (National Electric Instrument Co.), or by simply placing moist cotton pads on the vessels. Throughout the course of surgery the brain surface was kept moist by applying warm physiological saline from a drip bottle. Then using a small curved spatula the cortex was lifted a little anteriorly at the bony tentorium in order to obtain an access to the superior petrosal sinus. The blood vessels found along this sinus were sealed by cauterization. After this, using the edges of tentorium cerebelli as a guide for the knife, the brainstem was cut across with a blunt plastic or stainless steel knife (14 x 0.9 x 0.05 cm.). In order to preserve the vertebral and other vessels at the base of the brain the knife was pressed upon the bone in three or four places in

order to sever all nervous connections above the cut. A dragging motion across the base of the brain was particularly avoided at this stage. Some bleeding resulting from this type of midcollicular decerebration was controlled by applying pressure on vertebral artery in the neck region. The administration of ether was discontinued. Preparations in which the decerebration was successful showed normal respiration and a characteristic decerebrate rigidity, the latter appearing when the ether had been exhaled.

After decerebration, a small area on the posterior part of the suprasylvian gyrus was made avascular by cauterizing the brain surface lightly. Using suction, a small hole extending from the outer brain surface to the lateral ventricle was made carefully. This opening permitted drainage of the cerebrospinal fluid which might otherwise accumulate in the ventricle and cause swelling of the brain. The cortical slab was prepared in the suprasylvian gyrus by passing a knife (28 x 3 mm), made from a razor blade, into the hole to a depth of 4 mm and moving it anteriorly at the same depth but parallel to the surface of the gyrus. The two lateral edges and one anterior edge forming the borders of the slab were neuronally isolated by means of a piece of stainless steel wire of about 1 mm diameter bent to a right angle 4 mm from its rounded tip. The wire was inserted in the plane of the cut made previously such that it passed beneath the cortical surface. The tip of the shaft was rotated so that it could be seen beneath the pia mater but did not penetrate it. The wire tip was carefully manipulated around the borders of the intended slab, keeping it in view beneath the pial vessels at all

times. When the slab borders had been thus isolated from the surrounding cortex the preparation consisted of a neuronally isolated island of cortical tissue which was about 20 x 4 x 3 mm in size, with an intact blood supply which entered the tissue from the pia. The skin flaps resulting from the scalp incision were tied round an iron ring, 12 cm in diameter, to form the walls of a pool. The whole of the cortex and the bone sitting in this pool were covered with warm mineral oil.

The animal was maintained at 37°C by heating lamps built in the table and which were thermostatically controlled by a temperature regulator (Model 71 by Y.S.I.) and a probe (number 401 by Y.S.I.) inserted in the rectum of the cat. Wherever desirable, the blood pressure of the animal was recorded using a Statham pressure guage (Model P23AC) on a pen recorder (Dynograph Type RB). The animal was periodically given injections of physiological saline or 5% dextrose.

The relationship between the isolated cortical slab and other regions of the brain is shown in a schematic diagram in Figure 14.

ii) Stimulation and recording.

The stimulation and recording from the isolated slabs was carried out 2-3 hours after their preparation when the ether had blown off. The viable slabs showed only very small fluctuations in electrical activity and for practical purposes were electrically silent. In the few slabs which showed considerable spontaneous electrical activity, this activity was reduced either by a more complete re-isolation or occasionally by clamping both carotid arteries for 30 seconds.

The electrical activity of the viable slab stimulated directly by

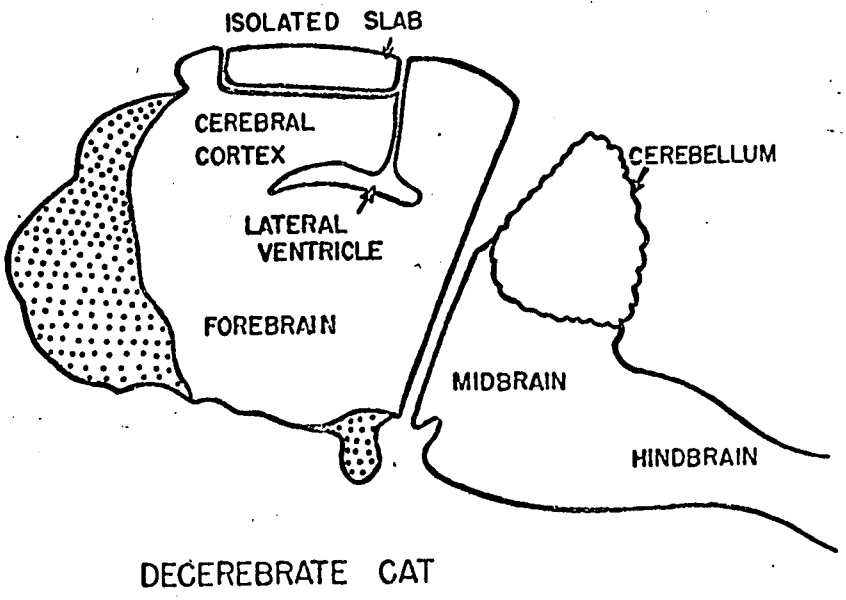

Figure 14. A diagrammatic illustration of sagittal section of the cat's brain showing the position of isolated cortical slab and the site of mid-collicular decerebration.

single shocks consisted of a surface negative and a surface positive burst response. The stimulus to the slab was delivered in the form of a rectangular pulse 5-15 volts in strength and 0.2 - 0.5 m sec in duration. The latter was generated by a combination of pulse and waveform generators (Tektronix 161 and 162) and passed through a stimulus isolator (Hewlett Packard), and through bipolar platinum electrodes with ball tips which rested about 1 to 2 mm apart on the surface of the cortical slab. The responses were recorded through electrodes consisting of saline filled glass tubing 4-5 mm in diameter with a silk thread embedded in the agar. The thread protruding from one end of the tube made contact with the slab, while the other end of the thread contacted a chlorided silver wire which made connection with the recording equipment. The monopolar recordings of the response were carried out by placing one wick electrode 1-5 mm from the stimulating electrode and the other wick electrode on the dead reference area of the slab. The responses were amplified by a differential D.C. amplifier (Model TA-2 Princeton Science Associates) and displayed on an oscilloscope (Tektronix Model 502). Measurements were made from photographic records taken off the oscilloscope with a camera (Nihon Kohden Model PC-2A). In some experiments the electrical activity was directly recorded on a pen recorder (Dynograph Type RB). The stimulation and recording arrangement is shown in Figure 15.

iii) Experimental procedures.

The drugs which were soluble in water were applied directly to the cortical slabs. A strip of filter paper 5 x 1 mm was soaked in saline

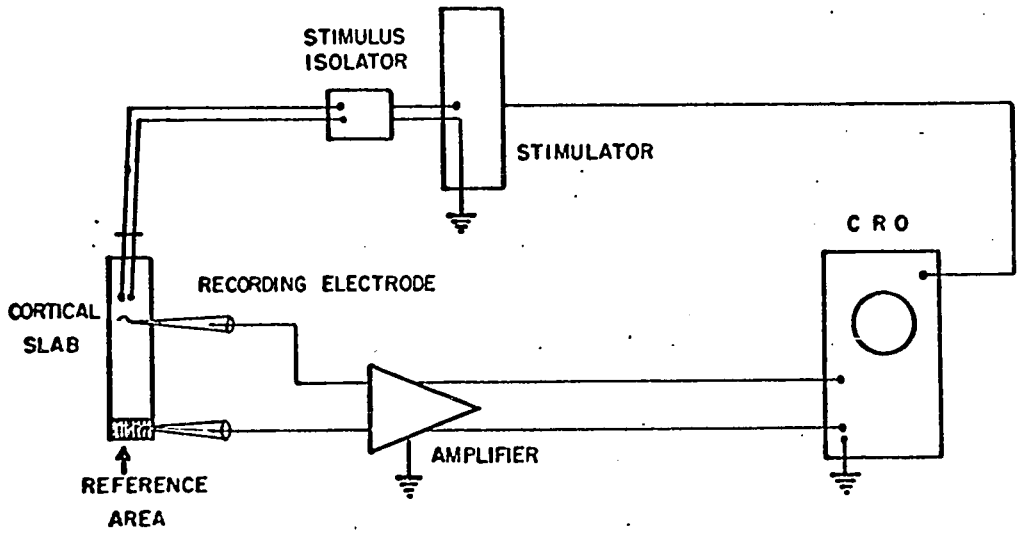

Figure 15. Arrangement of the equipment for stimulating and recording the electrical activity of the isolated cortical slab.

and placed across the width of the slab between the stimulating and recording electrodes. The recording electrode was placed either on the top or on one edge of the strip of the filter paper. A stimulus strength was chosen so that a single shock produced a surface negative response followed by a surface positive burst response. The cortical slab was stimulated every 15 seconds. Control responses were recorded for 5-10 minutes before the application of the drug in each experiment. A solution of the drug made up in physiological saline was applied to the filter paper dropwise from a tuberculin syringe using a 27 gauge needle. Approximate volume delivered by one drop was 0.015 mls. At any one concentration of the drug a maximum of 3 drops of the solution was applied If no effect was seen, the next higher concentration of the drug was then used. The filter paper was removed after the drug effect had taken place and the recording from the slab was continued until the control responses reappeared. In experiments with meprobamate and diazepam the drugs were given intravenously and in these the blood pressure of the animal was recorded simultaneously with the electrical activity.

The drugs tested in this manner have been listed previously in Section I (Page 55).

RESULTS.

i) Central depressants.

The purpose of these experiments was to investigate the effects of several central depressants on a neuronally isolated but readily accessible group of neurones. Isolated cerebral cortex was thus used and in most experiments the drugs were topically applied in order to circumvent their peripheral effects, and particularly cardiovascular alterations. A stimulus strength was chosen which produced both a surface negative and a surface positive response, and the action of various central depressants on these responses was studied in the present experiments. Each drug was applied in both low and high concentrations and tested in at least three animals. The results of such experiments are outlined in the following sections.

Since most of the drug solutions were acidic in nature, control experiments were first carried out using sodium chloride solutions whose pH had been adjusted by addition of hydrochloric acid or sodium hydroxide. Such solutions in the pH range 4-8 had no effect on the evoked responses of the isolated cortex.

i) (a) Diphenhydramine.

Diphenhydramine was applied to directly stimulated cortical slabs in solutions containing 2%, 4% and 8% of the drug. The measurements of all responses from one experiment of this type are shown in Figure 16 and the actual responses recorded from the surface are shown in Figure 17. This is one of the eight experiments carried out with diphenhydramine, and although the details of the plot varied in different animals, the qualitative effects produced by diphenhydramine on the electrical responses were consistent in all the tests. The drug in low concentrations (1-2%) reduced the amplitudes of surface negative and surface positive responses and also reduced the duration of the surface positive response. In high concentrations (4-8%) it abolished all evoked activity. After the removal of the drug soaked filter paper from the cortical surface the responses of the slab were still depressed but the activity returned to normal in 60 to 90 minutes after removal of the drug. During the early phase of the recovery while the activity was reduced, a stronger stimulus, two or three times the initial stimulus, could elicit the positive burst response when applied to the cortex, while the negative response remained depressed. Diphenhydramine did not produce any activity in the cortex by itself.

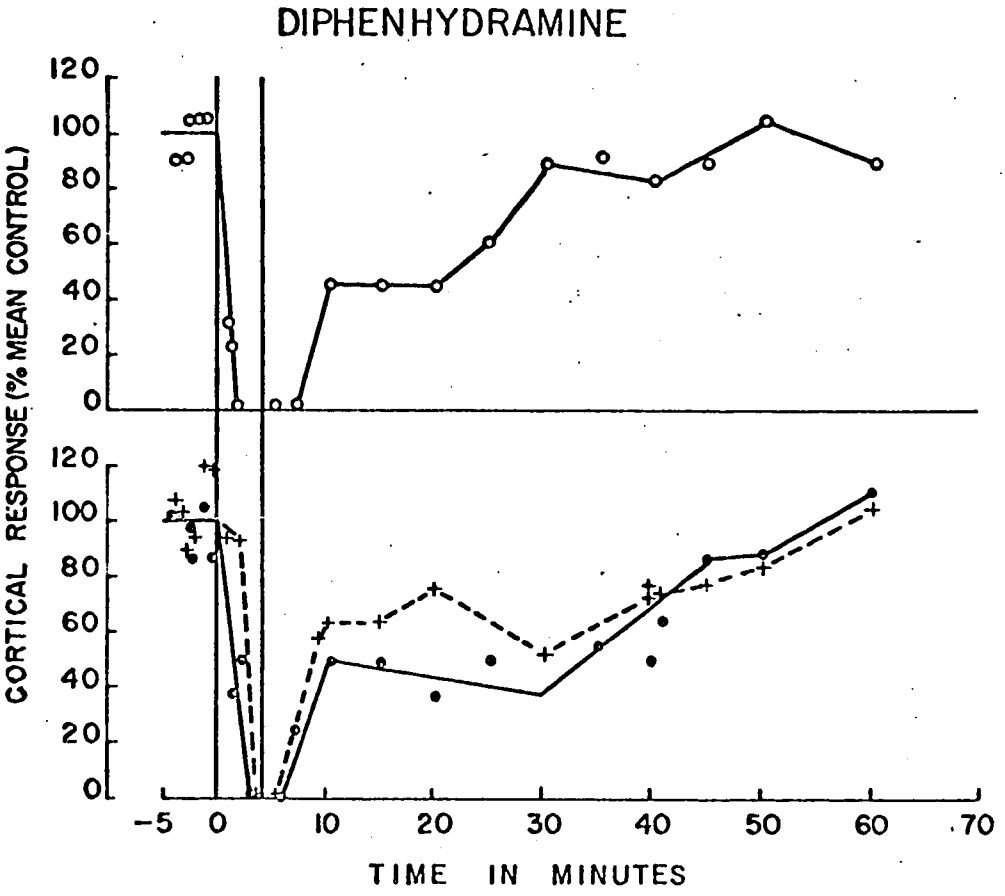

Figure 16. Effect of topical application of diphenhydramine (8% w/v) on responses of cat's isolated cerebral cortex stimulated directly by single shocks. The cortex was exposed to drug during the times between vertical lines. O, amplitude of surface negative response; ●, amplitude of surface positive response; +, duration of surface positive response.

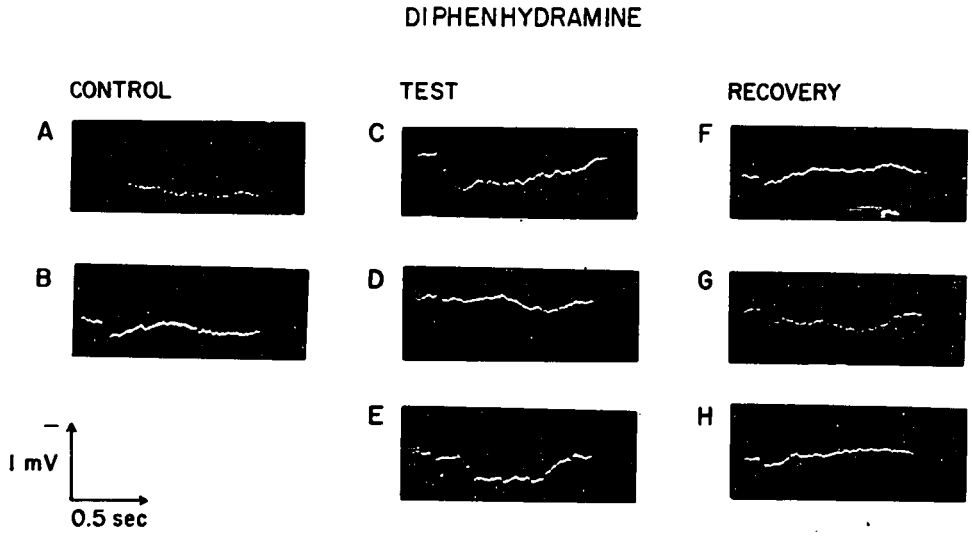

Figure 17. Responses to direct electrical stimulation recorded from the surface of isolated slab of cat's cerebral cortex before and after local application of diphenhydramine (8% w/v). A and B, control responses. Responses after drug application: C, 30 sec; D, 45 sec; E, 3 min. The filter paper containing diphenhydramine was removed from the cortex after E. Responses after removal of the drug: F, 20 min; G, 25 min; H, 60 min.

i) (b) Promethazine.

Promethazine was applied to the cortical slabs in concentrations of 1%, 2%, 5% and 8%. The results of one of the ten experiments carried out with promethazine are shown in Figure 18. Application of 2% promethazine reduced the surface negative response and abolished the surface positive response. In other experiments both surface negative and surface positive responses were abolished by promethazine both in low (1-2%) and in high (5-8%) concentrations when applied to the cortex (Figure 19). The recovery of the responses to normal levels took place in 60 to 120 minutes after the removal of the drug. The stimulus threshold of the positive response was increased during the depressed phase of the activity. Regardless of the concentration applied to the cortex, promethazine did not cause spontaneous activity in the slab by itself and did not increase the evoked activity.

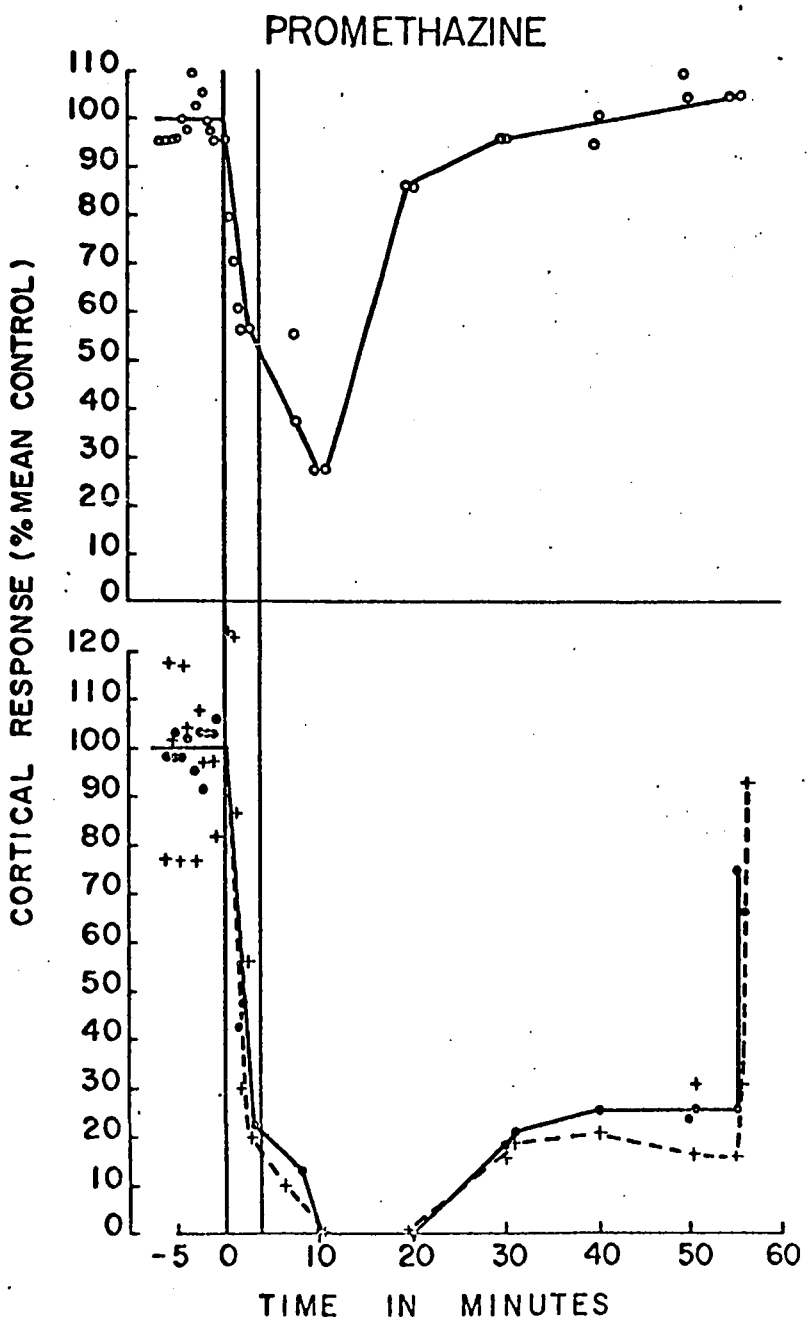

Figure 18. Effect of topical application of promethazine (2% w/v) on responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to drugs during the time between vertical lines. O, amplitude of surface negative response; ⊕, amplitude of surface positive response; +, duration of surface positive response.

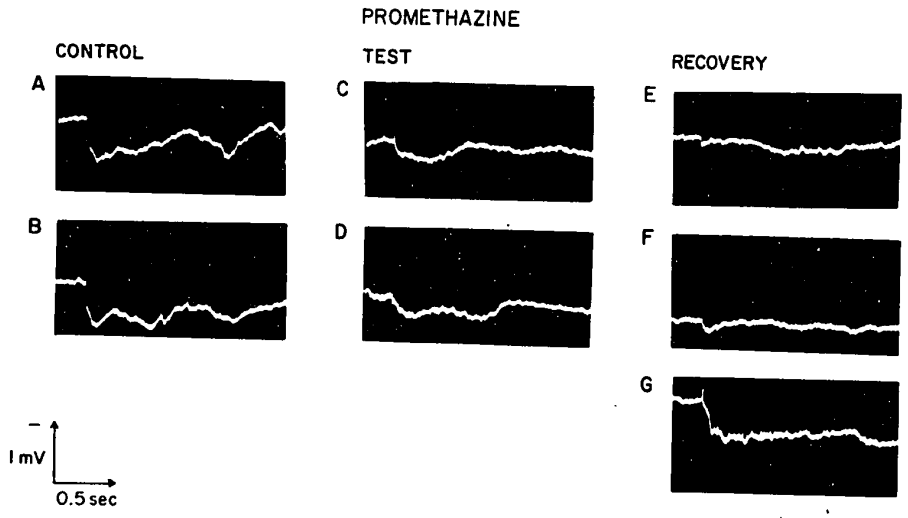

Figure 19. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after the local application of promethazine (2% w/v). Control responses: A and B. Responses after drug application: C, 75 sec; D, 4 min. The filter paper strip containing the drug was removed after D. Responses after removal of the drug: E, 5 min; F, 30 min; G, 60 min.

i) (c) Chlorpromazine.

When applied to the cortical slabs in the concentration range 1-2% chlorpromazine reduced or abolished both the surface negative and the surface positive responses. The results of one of the twelve experiments carried out with chlorpromazine are shown in Figure 20. The cortical depression produced by the drug lasted for a prolonged period and the recovery from the effects of chlorpromazine took place only after 120-240 minutes following the drug removal. During the depressed phase the stimulus threshold for the positive response showed an increase. When applied in concentrations of 5% or more, chlorpromazine produced spontaneous activity in the normally silent cortex. The stimulant activity consisted of mainly positive fluctuations in potential (Figure 21). These changes in the potential interfered with evoked responses and the latter could not be recorded satisfactorily in such experiments. Following termination of spontaneous activity the slab responses were depressed for 3-4 hours and the activity returned to normal after this period. Regardless of the concentrations of the drug applied to the isolated cortex undergoing stimulation there was no facilitation of the evoked responses by chlorpromazine.

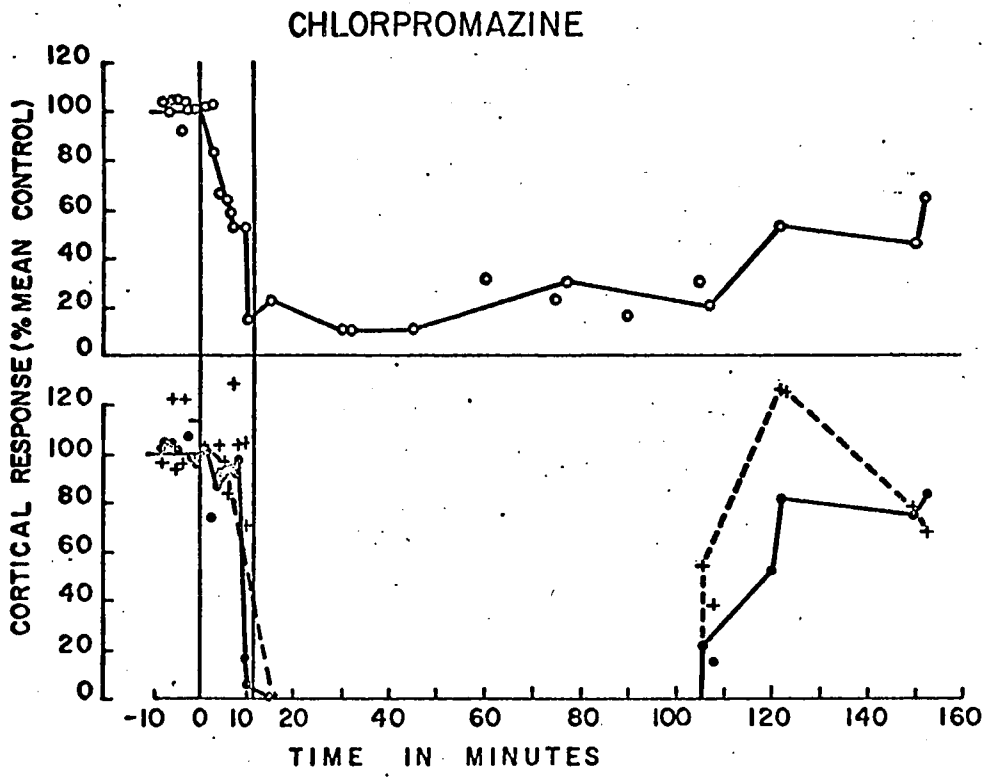

Figure 20. Effect of topical application of chlorpromazine (2% w/v) on responses of cat's isolated cerebral cortex. Stimulated directly by single shocks. The cortex was exposed to drug during times between vertical lines; O, amplitude of surface negative response; 0, amplitude of surface positive response; +, duration of surface positive response.

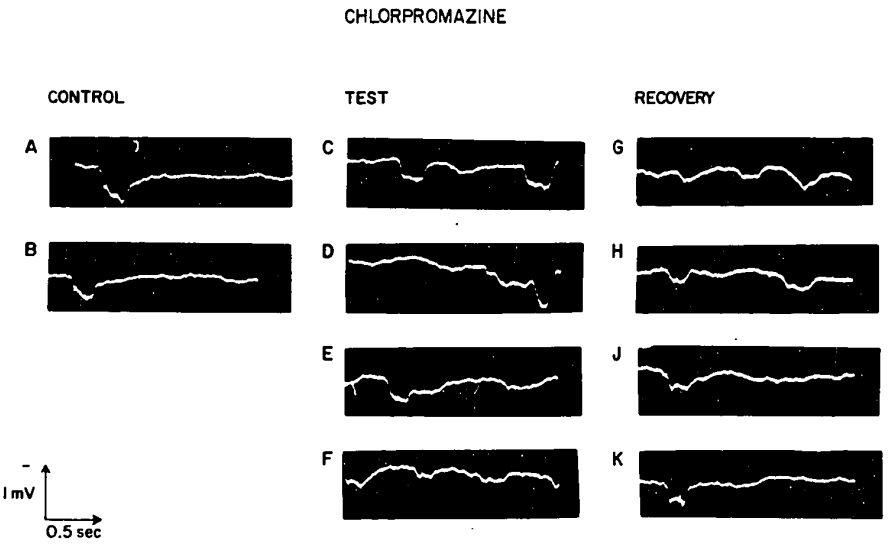

Figure 21. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of chlorpromazine (5% w/v). Control responses: A and B. Responses after drug application: C, 2 min; D, 3 min; E, 6 min; F, 10 min. The filter paper strip containing the drug was removed after F. Responses after removal of the drug: G, 15 min; H, 30 min; J, 120 min; K, 220 min.

i) (d) Gammahydroxybutyrate.

When applied to the cortex in the range 2-4% gammahydroxybutyrate reduced the amplitude of the surface negative and surface positive responses and reduced the duration of the latter. The results of one of the seven experiments carried out with gammahydroxybutyrate are shown in Figures 22 and 23. Essentially similar results were obtained in all experiments. When applied in a higher concentration (8%) in two experiments, gammahydroxybutyrate also produced depression of cortical activity. The depression of evoked activity was greater and lasted for a longer period than when using lower concentrations. The recovery from the application of smaller concentrations took place in 60-90 minutes whereas recovery from the effects of higher concentrations took place after about 120 minutes. The stimulus threshold of the positive response showed an increase during the depressed phase.

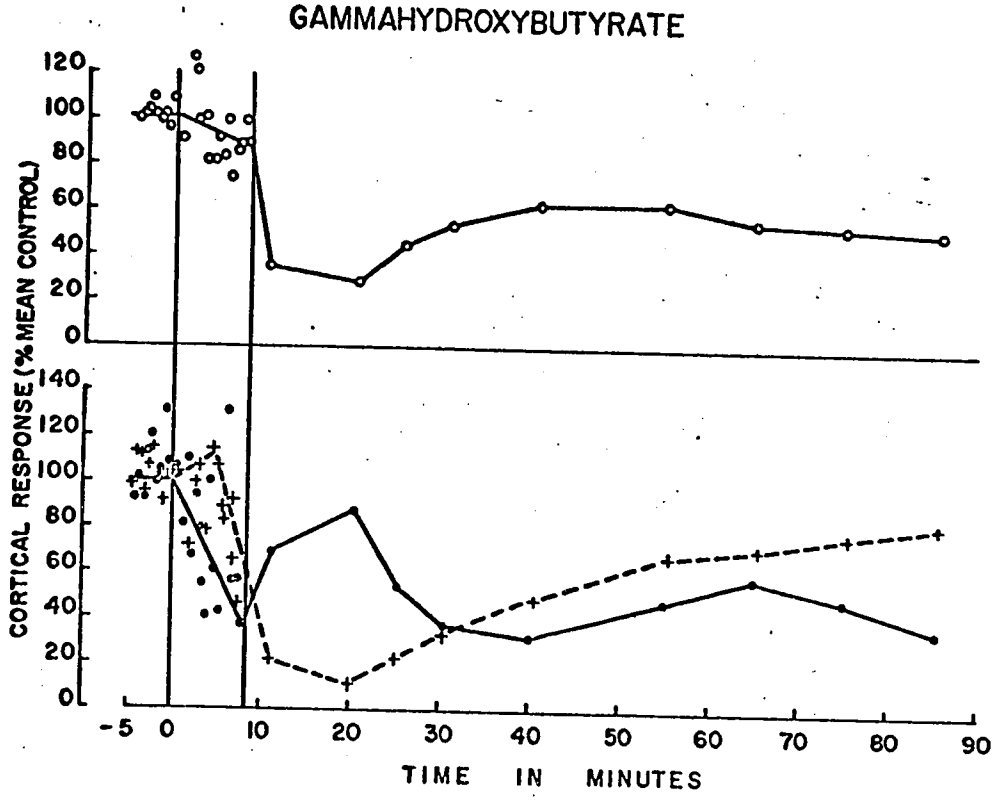

Figure 22. Effect of topical application of gammahydroxybutyrate (4% w/v) on the responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to drug during times between vertical lines; O, the amplitude of surface negative response; O, the amplitude of surface positive response; +, the duration of surface positive response.

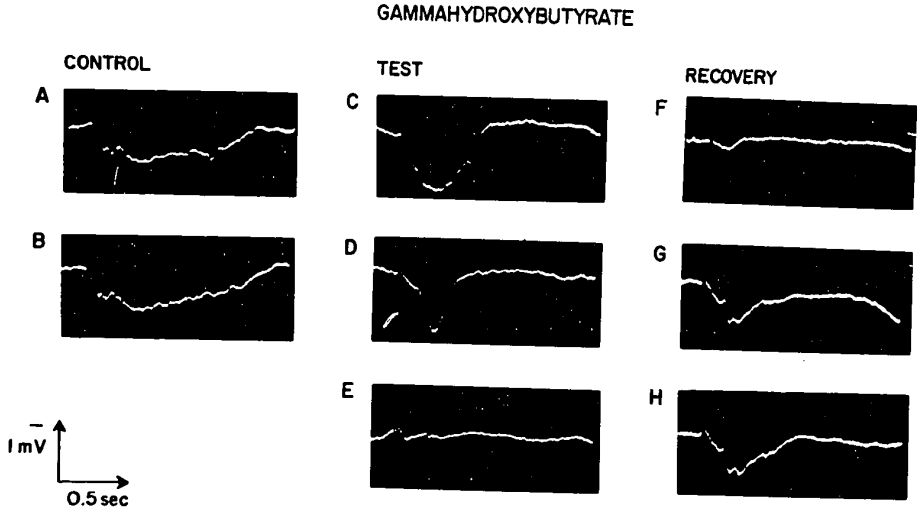

Figure 23. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of gammahydroxybutyrate (4% w/v). Control responses: A and B. Responses after drug application: C, 90 sec; D, 7 min; E, 8 min. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 10 min; G, 30 min; H, 40 min.

i) (e) Gammabutyrolactone.

A solution of gammabutyrolactone containing 2% and 4% of the drug produced depressant effects similar to those produced by gammahydroxybutyrate. The results of one of the eight experiments carried out with this drug are shown in Figure 24 while the actual responses recorded are shown in Figure 25. The amplitudes of the surface negative and the surface positive responses, and the duration were consistently reduced in all experiments. In two experiments, a higher concentration of gammabutyrolactone (8%) also reduced greatly but did not completely abolish these responses. The drug did not induce any activity by itself when applied to the cortex. The recovery from the depressant effects of gammabutyrolactone took place in 60-90 minutes. The stimulus threshold for the positive burst response showed an increase during the recovery phase when the activity of the slab was still depressed.

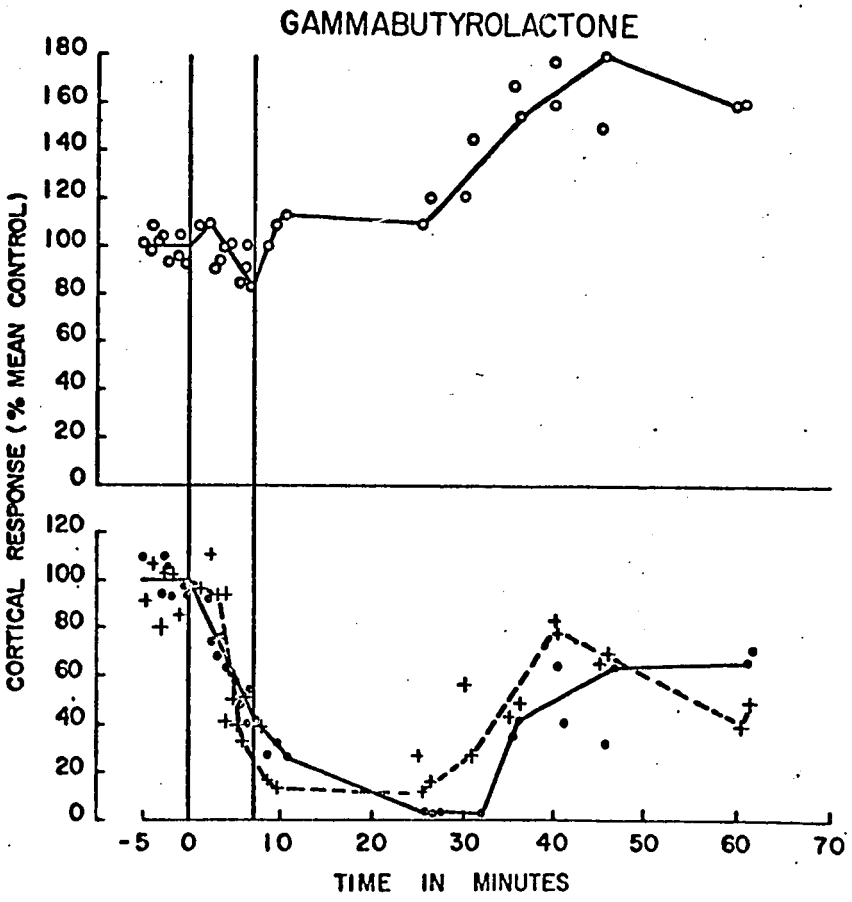

Figure 24. Effect of topical application of gammabutyrolactone (4% w/v) on the responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to drug during times between vertical lines; O, the amplitude of surface negative response; O, the amplitude of surface positive response; +, the duration of surface positive response.

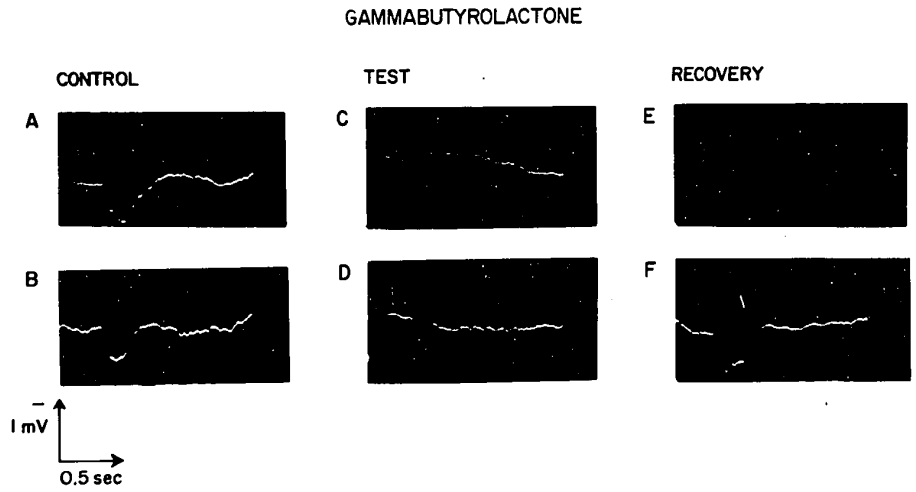

Figure 25. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of gammabutyrolactone (4% w/v). Control responses: A and B. Responses after application of the drug: C, 4 min; D, 5 min. The filter paper strip containing the drug was removed after D. Responses after removal of the drug: E, 11 min; F, 60 min.

i) (f) Gamma aminobutyric acid.

A solution containing 2% gamma aminobutyric acid depressed both the surface positive and the surface negative responses of the isolated cortex. Higher concentrations of the drug such as 4% produced a similar but a stronger depression of both responses (Figure 26). This depression was also more prolonged and the recovery from the effects of the drug took place in 60 to 90 minutes after the application of solution containing 4% of the drug. The stimulus threshold of the positive response was unchanged at the end of this period but showed an increase during the initial recovery phase when the slab was still depressed.

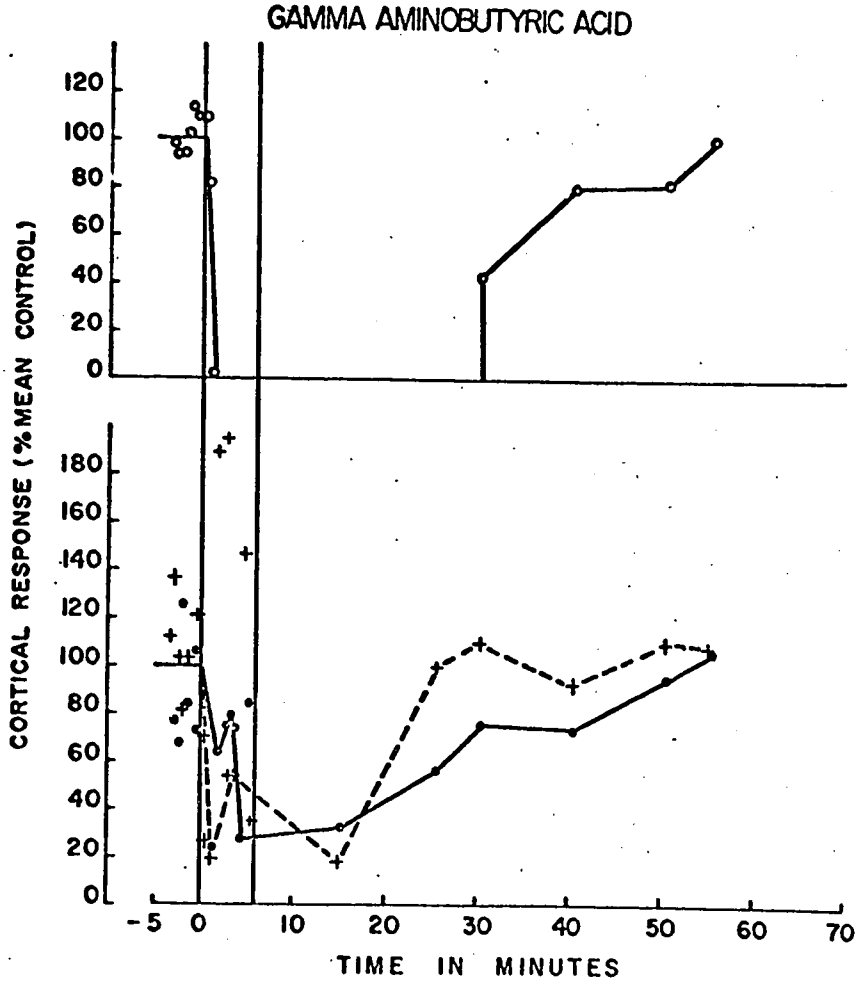

Figure 26. Effect of topical application of gamma aminobutyric acid (4% w/v) on the responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to the drug during times between vertical lines. O, the amplitude of surface negative response; O, the amplitude of surface positive response; +, the duration of surface positive response.

i) (g) Hyoscine.

Hyoscine was applied to the cortical slabs in solutions containing 1-8% of the drug in solution. The lower concentrations of the drug in the range of 1-2% produced no significant change in the activity of the preparation but a solution of 4% produced depression of surface negative and surface positive responses of the slab. A higher concentration of hyoscine (8%) produced an even greater depression of activity leading to a complete abolition of both responses. The action of hyoscine in one of the six experiments carried out with this drug is shown in Figure 27 and the responses recorded from the surface of the cortex are shown in Figure 28. The recovery from the depressant effects of hyoscine took place in 60-90 minutes, but the recovery from its effect after the high concentration of the drug took longer. Hyoscine did not produce any consistent facilitation of the responses of the cerebral cortex. The stimulus threshold for the positive response was increased in all the experiments in which the cortex was depressed as a result of hyoscine action.

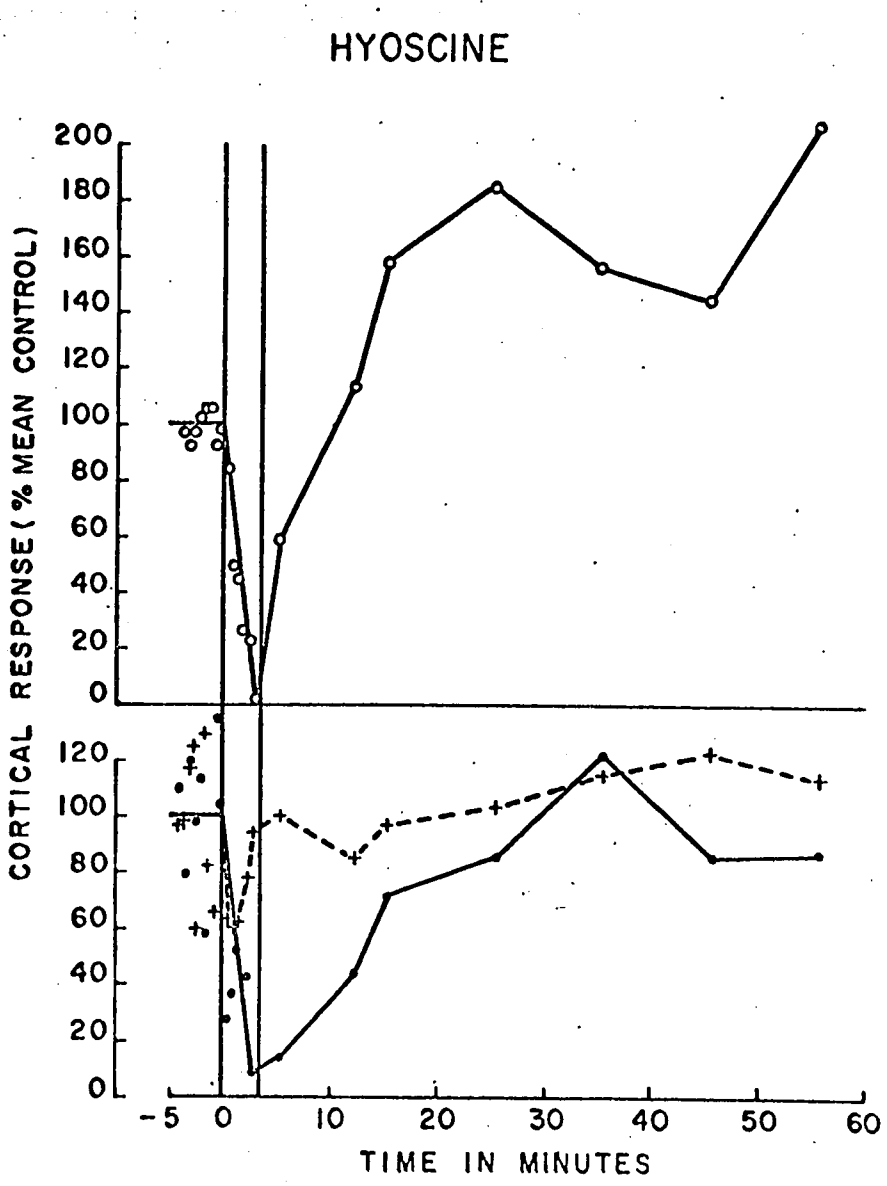

Figure 27. Effect of topical application of hyoscine (4% w/v) on the responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to drug during times between vertical lines; O, the amplitude of surface negative response; ●, amplitude of surface positive response; +, duration of surface positive response.

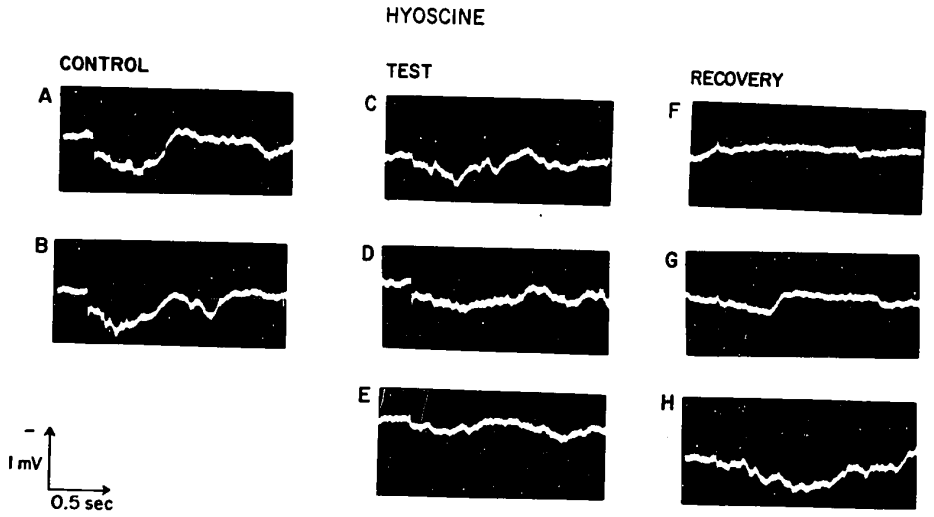

Figure 28. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of hyoscine (4% w/v). Control responses: A and B. Responses after drug application: C, 15 sec; D, 90 sec; E, 3 min. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 15 min; G, 30 min; H, 60 min.

i) (h) Meperidine.

Meperidine was applied topically to the cortical slabs in the concentration range of 0.5 - 4.0%. In the range 0.5 - 1% meperidine produced a reduction in the size of the surface negative and the surface positive response. In higher concentrations 2-4% the drug abolished both these responses. The results of one of the eleven experiments carried out with meperidine are shown in Figures 29 and 30. The recovery from smaller amounts of the drug which only reduced the activity took place in 30 to 60 minutes while recovery from application of higher amounts took place in 60-90 minutes. The stimulus threshold of the positive response was increased during the phase of depression in the slab. Topically applied meperidine did not produce any excitatory responses in the cortical slab.

In three decerebrate cats maintained on artificial respiration, meperidine was injected intravenously in a convulsant dose of 20 mg/kg. The drug caused convulsions in the decerebrate animal which were accompanied by arching of the back, extension of the limbs, tachycardia and twitching. The convulsant activity also was produced in the isolated cortical slab undergoing normal bipolar stimulation (Figure 31). The convulsant response recorded 5 minutes after the injection of meperidine consisted of spike discharges which were unrelated to the applied stimulus. The discharges lasted for 10-12 minutes, following which the cortical slab became inexcitable to stimuli previously eliciting control responses. The recovery from the effects of meperidine took place in 90-100 minutes after the injection, and normal responses could be elicited by direct stimulation after this time.

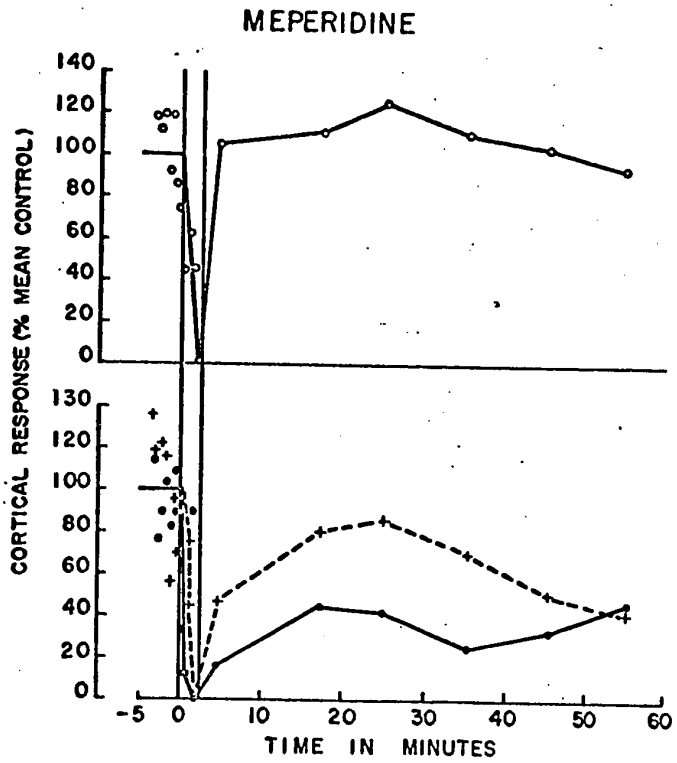

Figure 29. Effect of topical application of meperidine (2% w/v) on the responses of cat's isolated cortex stimulated directly. The cortex was exposed to drug during times between vertical lines; O, amplitude of surface negative response; ⊕, amplitude of surface positive response; +, duration of surface positive response.

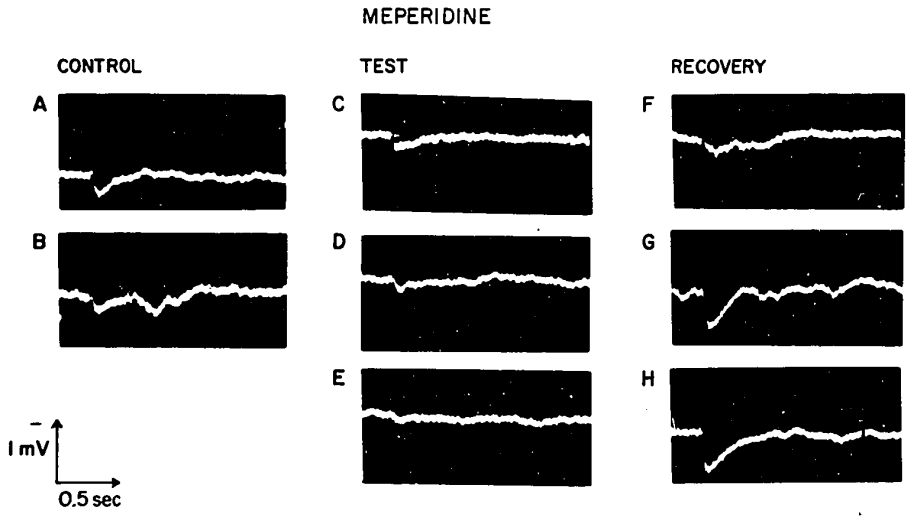

Figure 30. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of meperidine (2% w/v). Control responses: A and B. Responses after drug application: C, 60 sec; D, 75 sec; E, 120 sec. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 40 min; G, 50 min; H, 55 min.

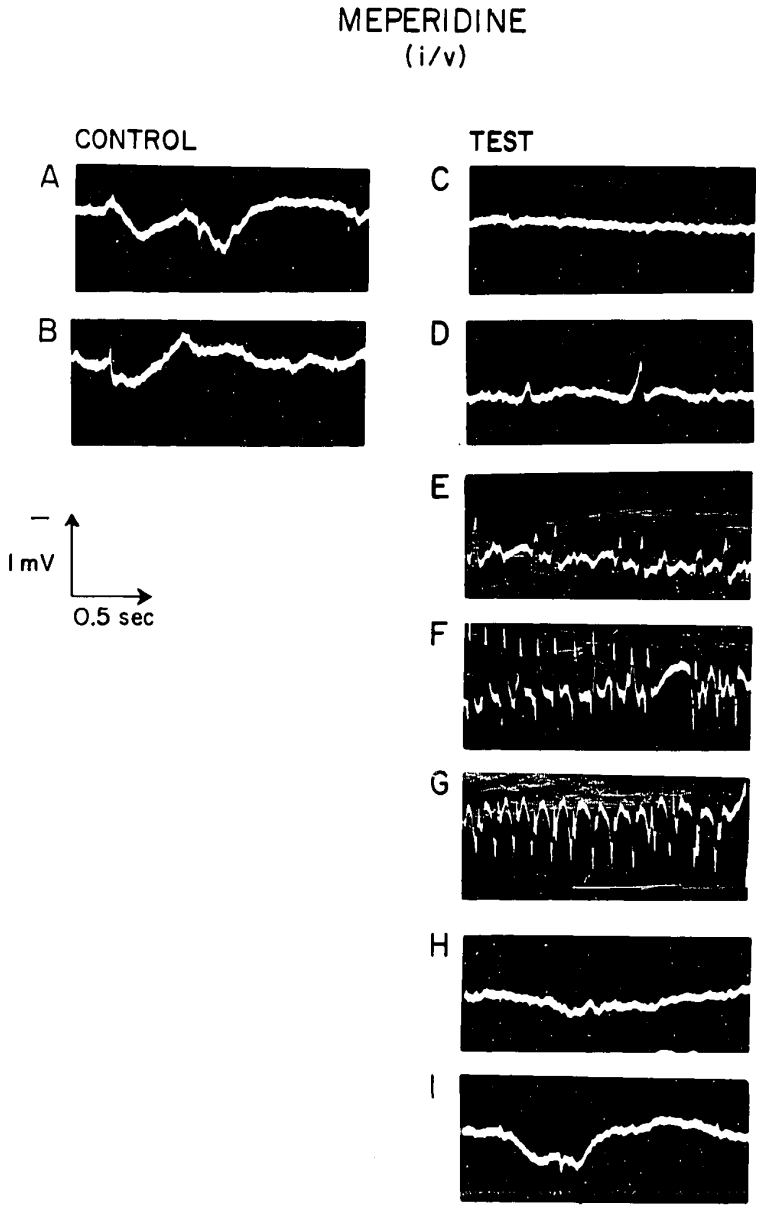

Figure 31. Responses to direct electrical stimulation of cat's isolated cortex recorded before and after a convulsant dose (20 mg/kg) of meperidine given intravenously. Control responses: A and B. Responses after injection of meperidine, C, 1 min; D, 5 min; E, 6 min; F, 8 min; G, 11 min; H, 30 min; I, 90 min.

i) (i) Meprobamate.

Meprobamate was injected intravenously in cats maintained on artificial respiration. The solution of the drug was prepared in 50% polyethylene glycol and injected slowly. A slow injection of the solvent alone did not affect the blood pressure of the animal and produced no change in the cortical responses, but a more rapid injection produced an acute drop in the blood pressure of the cat. Given slowly in this way, a dose of 20 mg/kg of meprobamate produced a depression of the cortical responses, the recovery from this effect took place in about 2 hours after the injection. The results of one of the five experiments carried out with meprobamate are shown in Figure 32. A higher dose of meprobamate, 40 mg/kg given in a similar way, produced a greater and more prolonged depression of both the surface negative and the surface positive responses of the slab undergoing normal stimulation (Figure 33). The recovery from high doses of meprobamate took place after 4-5 hours following its administration. During the depressed phase the surface positive cortical responses could be elicited by increasing the intensity of stimulation, indicating an increase in the stimulus threshold.

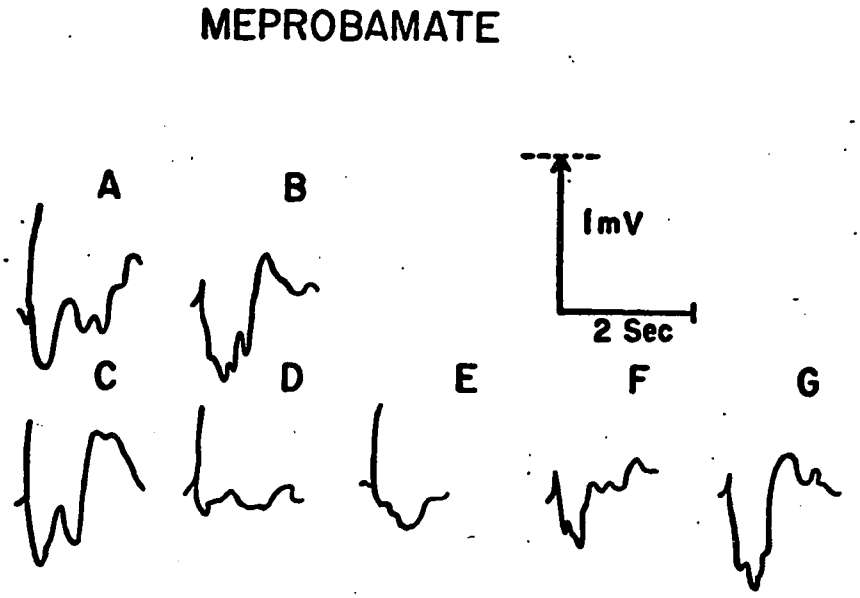

Figure 32. Responses to direct stimulation recorded from the surface of cat's isolated cerebral cortex before and after intravenous injection of mperobamate (20 mg/kg). Control responses, A. Responses after injection of drug: B, 1 min; C, 5 min; D, 45 min; E, 60 min; F, 90 min; G, 120 min.

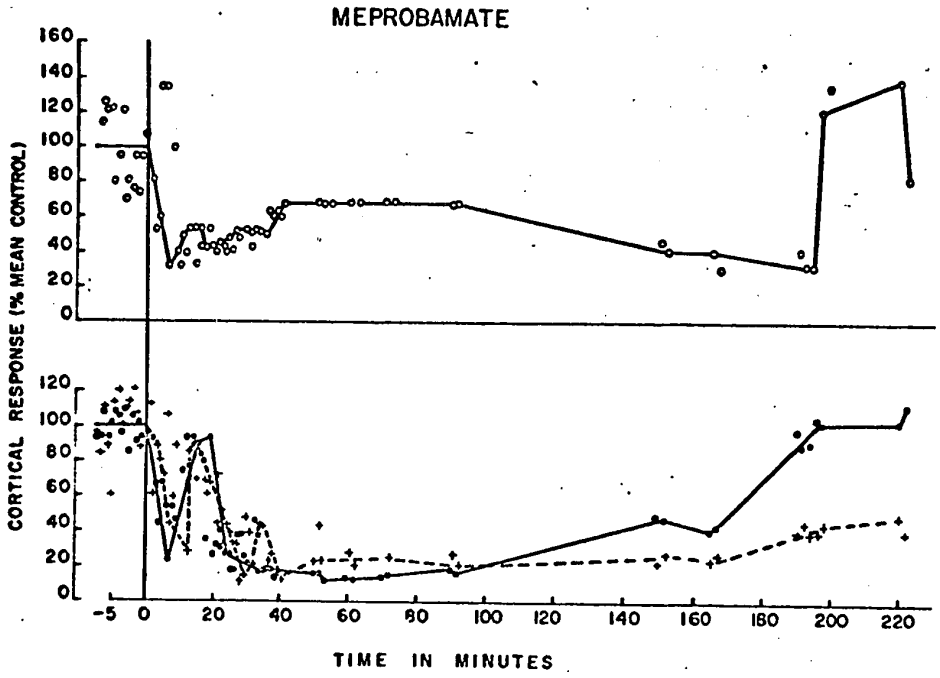

Figure 33. Effect of meprobamate (40 mg/kg) given intravenously, on the responses of cat's isolated cerebral cortex stimulated directly. The drug was administered at a time shown by the vertical line. O, amplitude of surface negative response; ⊙, amplitude of surface positive response; +, duration of surface positive response.

i) (j) Diazepam.

Effects of diazepam were investigated on the responses of the isolated slab in artificially respired animals. Diazepam was given by intravenous injection. Control tests were carried out with diazepam placebo injected in a dose of 1 and 2 mg/kg. The placebo solution had no effect on the cortical activity induced by direct stimulation. A dose of 1 mg/kg diazepam reduced the surface positive response in amplitude and duration as shown in Figure 34. This experiment was one of the five experiments carried out with diazepam. A higher dose of diazepam, 2 mg/kg also reduced the amplitude and duration of the surface positive response and depressed the amplitude of surface negative response (Figure 35). The recovery from effect of diazepam took place in 90-120 minutes after its administration. The responses could be elicited during the depressed phase by increasing the intensity of the stimulus.

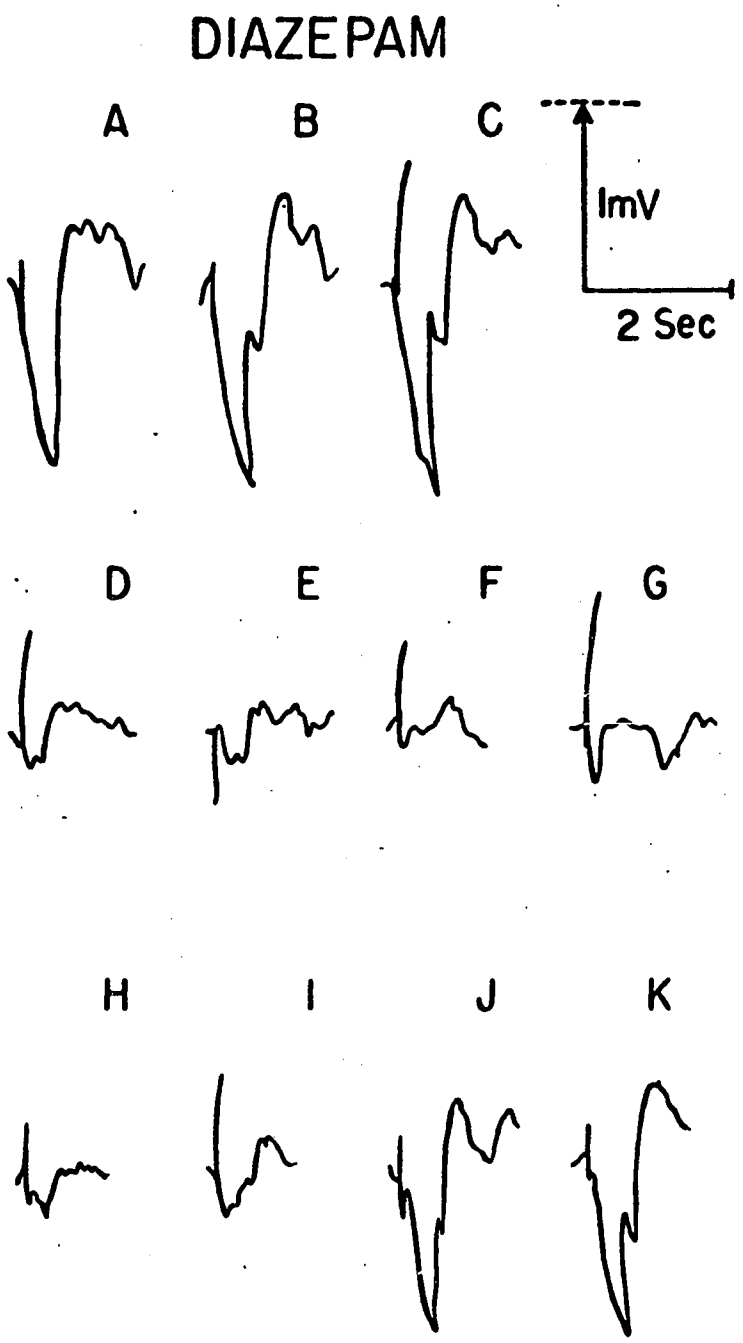

Figure 34. Responses to direct stimulation recorded from the surface of cat's isolated cerebral cortex before and after intravenous injection of diazepam (1 mg/kg). Control responses, A, B and C. Responses after injection of drug; D, 1 min; E, 5 min; F, 10 min; G, 15 min; H, 30 min; I, 45 min; J, 60 min; K, 65 min.

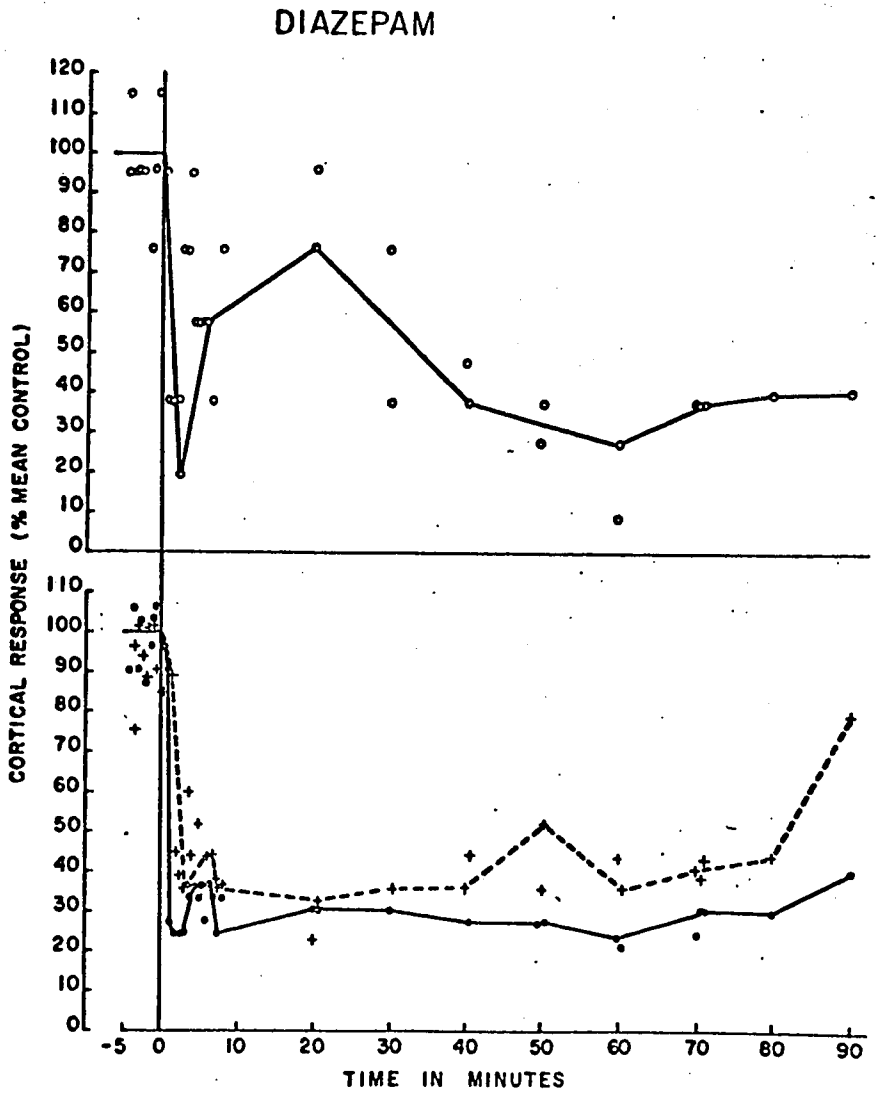

Figure 35. Effect of diazepam (2 mg/kg) given intravenously, on the responses of cat's isolated cerebral cortex stimulated directly. The drug was administered at a time shown by the vertical line. O, the amplitude of surface negative response; ●, the amplitude of surface positive response; +, the duration of surface positive response.

ii) Central stimulants.

The purpose of these experiments was to investigate the effect of some classical stimulant drugs on the electrical responses of the isolated cortex. The drugs investigated for effects on cortical activity were bemegride, picrotoxin, nikethamide, caffeine and strychnine. When applied to the cortex topically in appropriate amounts these drugs produced spontaneous electrical activity in the cortex which was unrelated to the applied stimuli. The responses produced by picrotoxin (0.15%), caffeine (3.0%) and bemegride (1.0%) are shown in Figure 36, while those produced by strychnine (1.0%) are shown in Figure 37. Since the drug-induced discharge in the isolated cortex destroyed the electrical silence of the normal slab, the evoked responses could not be recorded successfully at regular time intervals. To circumvent this problem, smaller concentrations of the various drugs were applied to the cortex in order that spontaneous activity not be produced, or if it was produced, the interference with the evoked activity was minimal. The stimulus strength used to drive the slabs was subthreshold, i.e. the stimulus used was 2.0 to 2.5 volts below the normal strength required to produce a positive burst response. The effects of topically applied stimulants on the electrical responses of the isolated cortex were investigated in this manner.

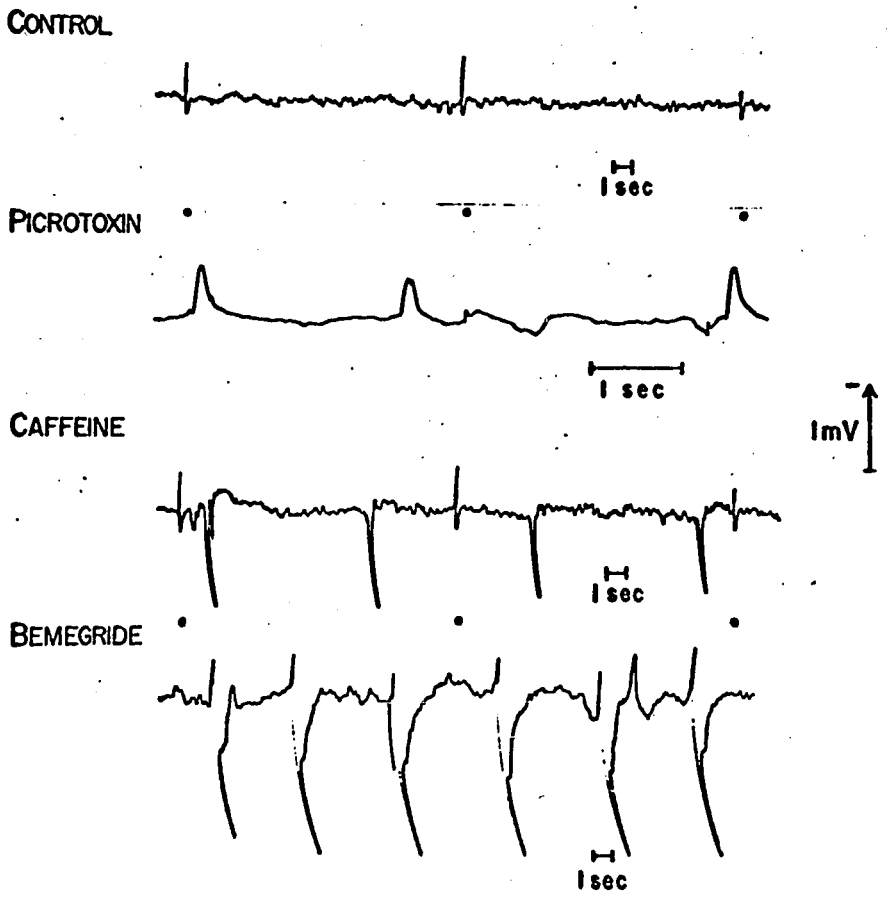

Figure 36. Responses recorded from the surface of cat's isolated cerebral cortex. A, control response to direct stimulation. B, spontaneous activity produced by picrotoxin (0.15% w/v). C, spontaneous activity produced by caffeine (3.0% w/v). D, spontaneous activity produced by bemegride (1.0% w/v). Dot indicates the stimulus applied to the cortical slab.

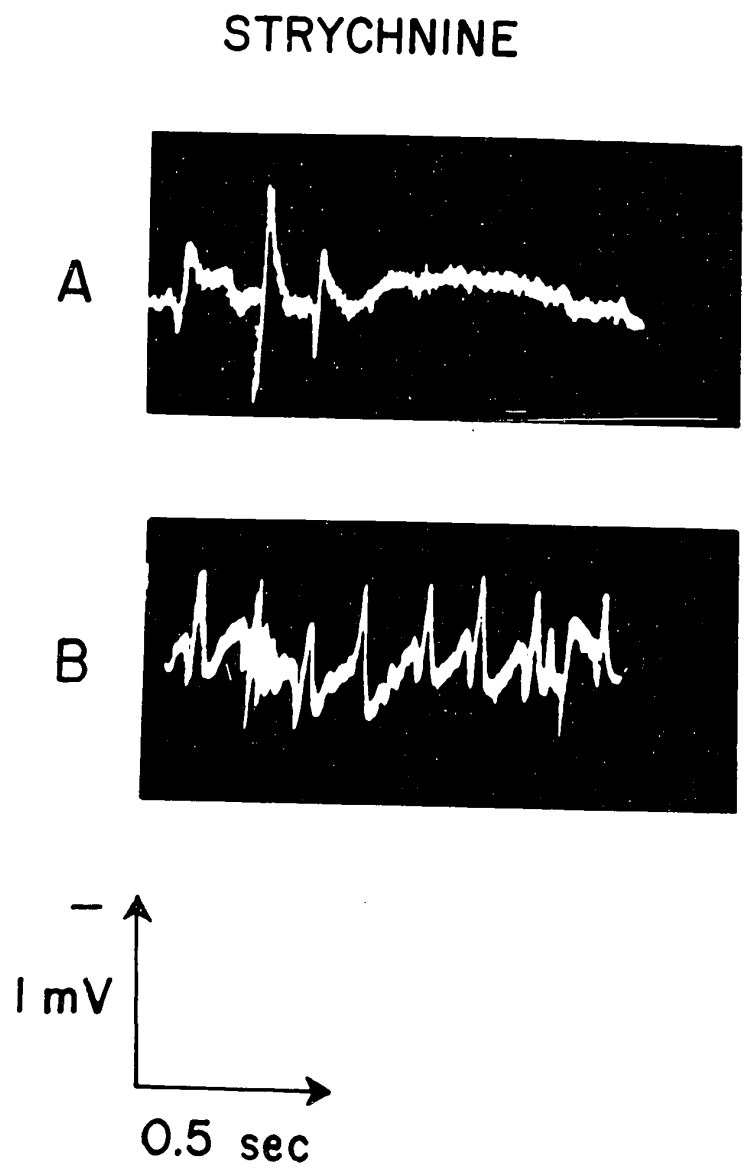

Figure 37. Spontaneous activity produced by local application of strychnine (1% w/v) to the isolated cerebral cortex of the cat. A, after 5 min; B, after 15 min.

ii) (a) Bemegride.

Bemegride was applied topically to the cortical slabs in solutions containing .025% or 0.5% of the drug. The results of one of the six experiments carried out with bemegride are shown in Figure 38. The control response showed a small surface negative response and a very small, residual surface positive response. Application of bemegride (0.5%) did not alter the surface negative response, but it increased the amplitude of the surface positive response. The burst response resulting from the action of bemegride (0.5%) was characterized by the presence of large negative spikes along its course. On removal of the drug, the recovery of the responses of the slab to their original sizes took place in 45-60 minutes. The threshold of the positive burst response remained unaltered at the end of the recovery period.
A similar result was obtained in all the other experiments with this drug, indicating an increased excitability of the cortical tissue produced by bemegride.

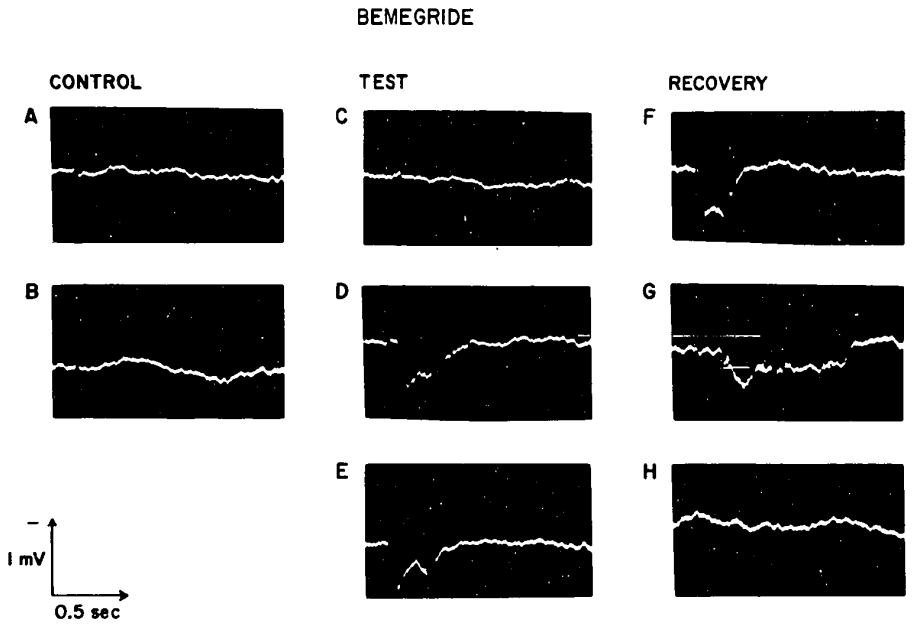

Figure 38. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after the local application of bemegride (0.5% w/v). Control responses: A and B. Responses after drug application: C, 30 sec; D, 60 sec; E, 3 min. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 10 min; G, 20 min; H, 35 min.

ii) (b) Picrotoxin.

Picrotoxin was applied to the cortical slabs stimulated with sub-threshold pulses in concentrations of 0.05% and 0.1%. The drug produced a marked increase in the excitability of the isolated cortex. The responses recorded in one of the three experiments are shown in Figure 39. The action of picrotoxin was characterized by a prolonged duration, the normal excitability returning only 4-5 hours after the initial application of the drug. The spontaneous activity produced by picrotoxin initially consisted of large negative spikes. At a later stage these were followed by positive bursts upon which epileptiform afterdischarge lasting 1-3 seconds was superimposed. The positive bursts of this type occurred even during the resting interval between two stimuli. Following this intense excitation the slab showed a depressant phase during which it became electrically inexcitable to threshold or supra threshold stimuli. The responses showed recovery to control levels 5 hours after the removal of the drug.

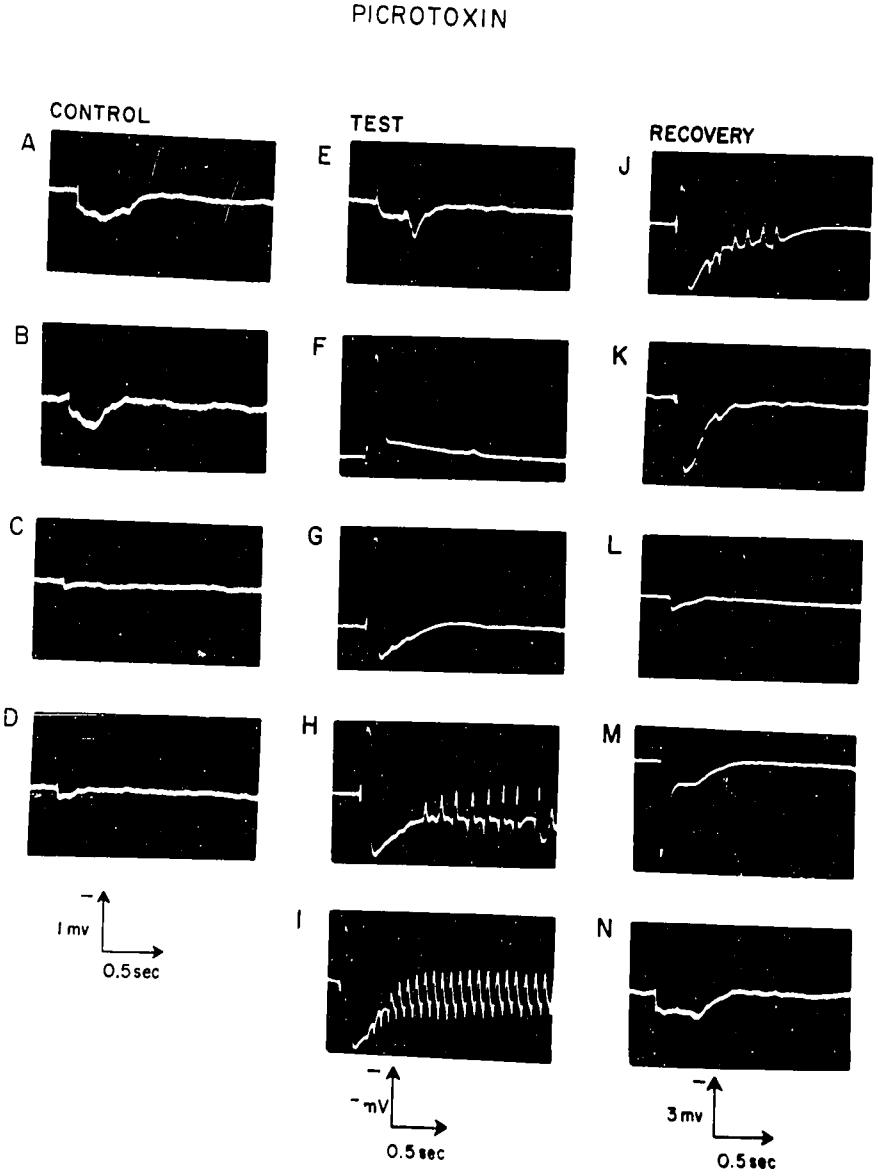

Figure 39. Responses to direct stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of picrotoxin (0.1% w/v). Control responses: A and B to supra-threshold stimulus; C and D to sub-threshold stimulus. Responses to sub-threshold stimulus after drug application: E, 5 min; F, 8 min; G, 11 min; H, 15 min; I, 20 min. The filter paper strip containing the drug was removed from cortex after I. Responses after removal of the drug: J, 60 min; K, 120 min; L, 130 min; M, 150 min; N, 5 hrs.

ii) (c) Nikethamide.

When applied in concentrations of 5% and 10% to the isolated cortex undergoing stimulation with subthreshold pulses, nikethamide increased the excitability of the tissue. The amplitude of the surface negative response was not significantly affected by the drug, but it did produce the surface positive response, which was not present before the drug application. The results of one of the six experiments carried out with nikethamide are shown in Figure 40. When applied to the slabs which were stimulated with supra-threshold stimuli, nikethamide increased the amplitude and the duration of the positive burst response but did not have a significant effect on the surface negative response (Figure 41). The recovery from the effects of nikethamide took place in 30 to 60 minutes. The stimulus threshold of the burst response was unaltered at the end of this period. Nikethamide did not produce a spontaneous discharge in the cortical slabs in concentrations up to 10%.

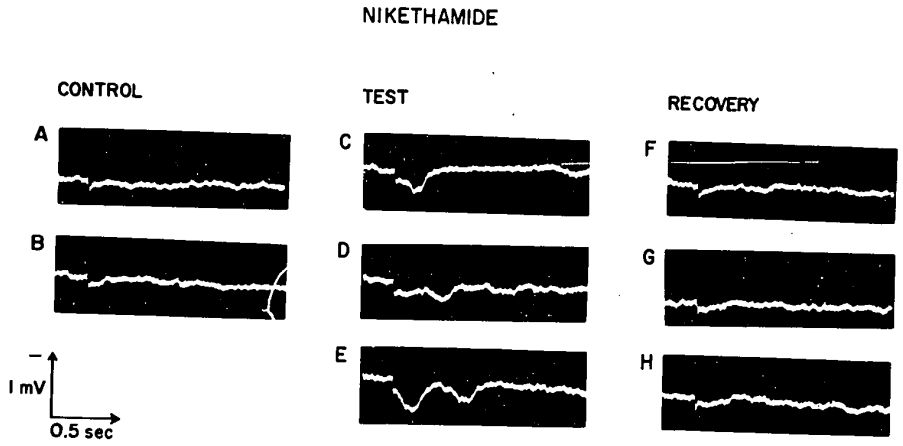

Figure 40. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after the local application of nikethamide (10% w/v). Control responses: A and B. Responses after drug application: C, 1 min; D, 2 min; E, 3 min. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 15 min; G, 20 min; H, 22 min.

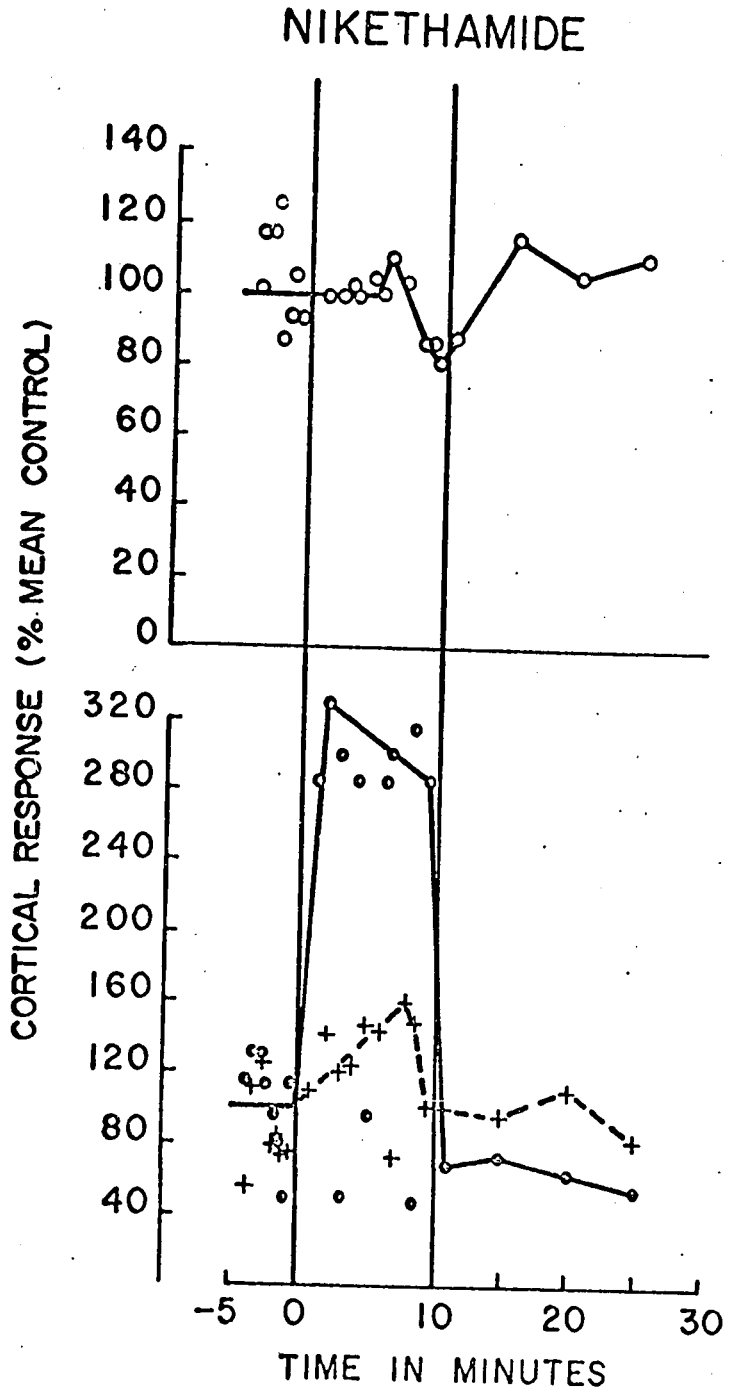

Figure 41. Effect of topical application of nikethamide (10% w/v) on the responses of cat's isolated cortex stimulated directly. The cortex was exposed to the drug during times between the vertical lines. O, the amplitude of surface negative response; ●, amplitude of surface positive response; +, the duration of surface positive response.

ii) (d) Caffeine.

Caffeine was applied directly to cortical slabs stimulated with subthreshold pulses in solutions containing 2% or 3% of the drug. The results of one of the six experiments carried out with this drug are shown in Figure 42. In such slabs caffeine produced a large surface positive response, indicating a lowering of the stimulus threshold for this response. In slabs stimulated with suprathreshold stimuli, the application of 2% caffeine increased the amplitudes of both the surface negative and the surface positive response and prolonged duration of the latter (Figure 43). The recovery from the effects of caffeine took place in 60-90 minutes after removal of the drug. The stimulus threshold was the same as control at the end of the recovery. If a large volume of 3.0% caffeine solution was applied to the cortical slabs which were being normally stimulated, caffeine caused a spontaneous discharge in these slabs. The discharge consisted of positive bursts which were unrelated to the normal stimulus (Figure 36).

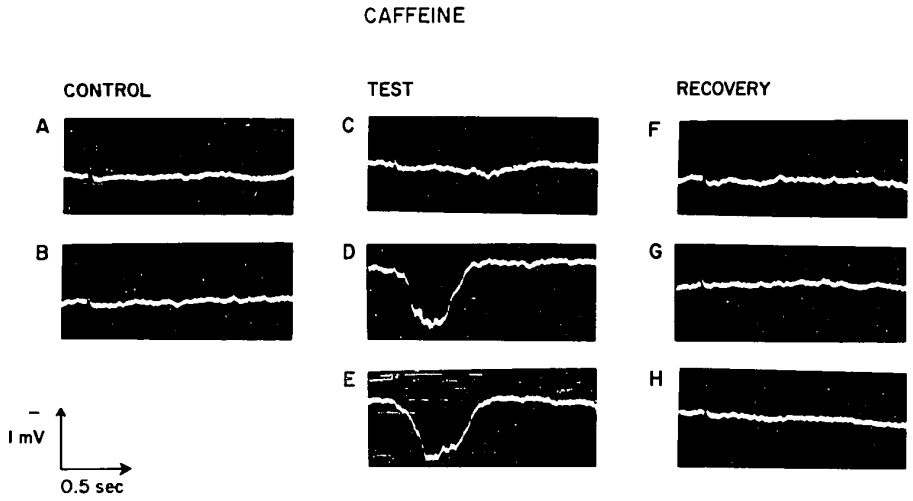

Figure 42. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after the local application of caffeine (2% w/v). Control responses: A and B. Responses after drug application: C, 3 min; D, 4 min; E, 6 min. The filter paper strip containing the drug was removed after E. Responses after removal of the drug: F, 20 min; G, 40 min; H, 50 min.

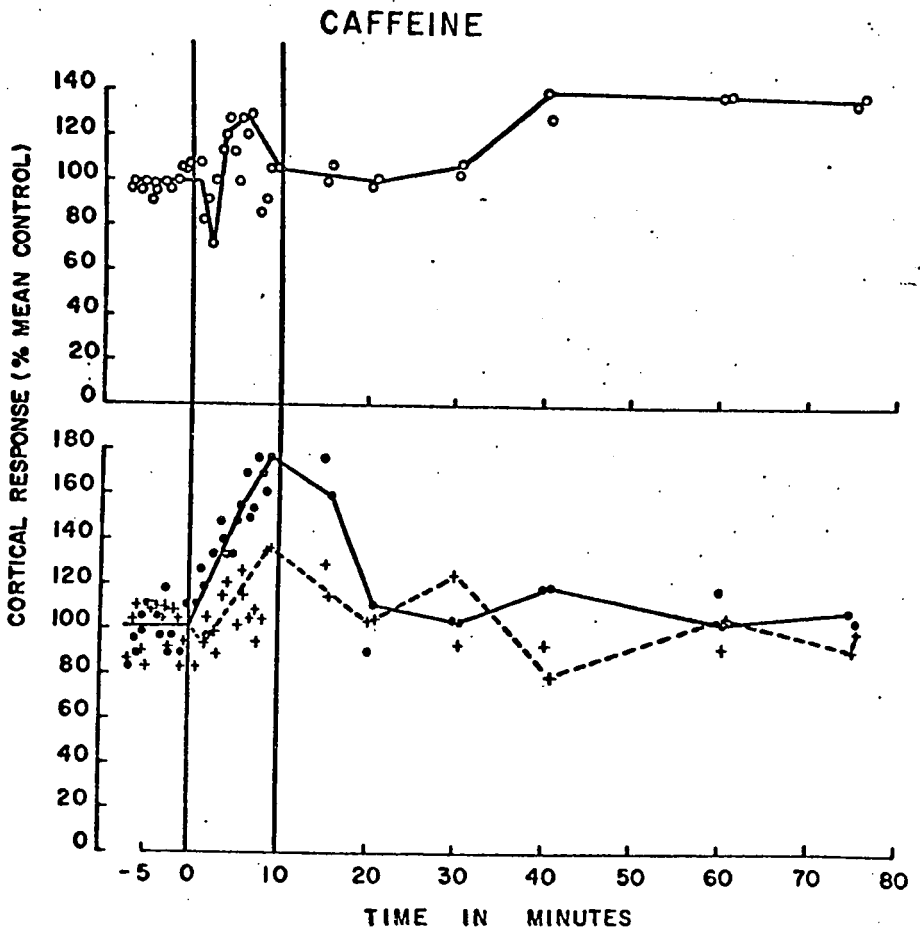

Figure 43. Effects of topical application of caffeine (2% w/v) on the responses of cat's isolated cerebral cortex stimulated directly. The cortex was exposed to drug during times between the vertical lines. O, the amplitude of surface negative response; ⊕, amplitude of surface positive response; +, duration of surface positive response.

ii) (e) Strychnine.

Strychnine was applied locally in a concentration 0.1% or 0.2% to slabs stimulated with subthreshold pulses. In these cortical slabs strychnine increased the amplitude of the surface negative response slightly and caused the appearance of a large surface positive response with a prolonged duration. The results of one of the six experiments are shown in Figure 44. During the course of the positive burst response, strychnine produced large negative spikes, similar to those also produced by bemegride under the same conditions. The recovery from effects of strychnine took place in 30 to 60 minutes, both the control responses and the stimulus threshold returned to normal at the end of this period. If higher concentrations of strychnine in the range of 1-2% were topically applied to the cortex they produced spontaneous spike discharges in the isolated slab (Figure 37) and also caused convulsions in the whole animal.

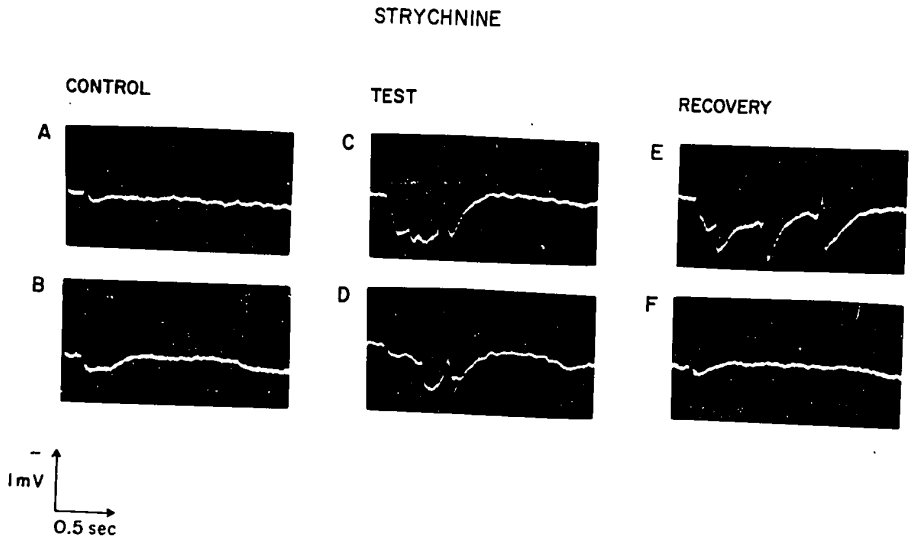

Figure 44. Responses to direct electrical stimulation recorded from the surface of cat's isolated cerebral cortex before and after local application of strychnine (0.1% w/v). Control responses: A and B. Responses after drug application: C, 15 sec; D, 30 sec. The filter paper strip containing the drug was removed after D. Responses after removal of the drug: E, 15 min; F, 20 min.

ii) (f) Glutamic acid.

The action of glutamic acid (sodium salt) was examined in the concentration range of 1-4% in eight experiments. In all these experiments glutamate induced a spreading depression characterized by total inexcitability of the cortical slab during the depressed phase. This depression was produced regardless of the stimulation of the slab. The normal responses of the slab returned to control levels 30-50 minutes after the removal of the drug. In two out of eight experiments, glutamate (2%) produced positive bursts prior to inducing the spreading depression (Figure 45). These bursts were unrelated to the applied stimulus. When applied in small concentrations which did not produce spreading depression, glutamate unlike other convulsants failed to affect the surface negative or surface positive responses of the cortical slabs.

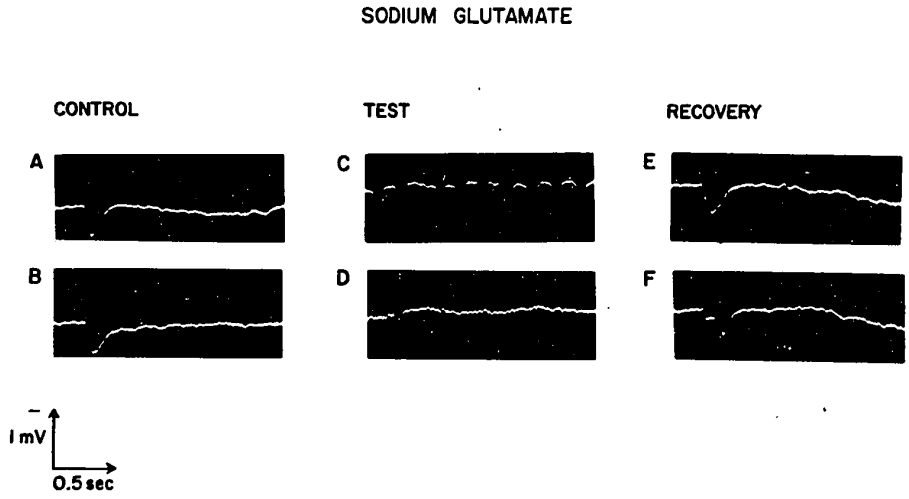

Figure 45. Responses to direct electrical stimulation recorded from the surface of isolated slab of cat's cerebral cortex before and after local application of L-glutamic acid (2% w/v). Control responses: A and B. Responses after drug application: C, 15 sec; D, 45 sec. The filter paper strip containing the drug was removed from the cortex after D. Responses after removal of the drug: F, 25 min; G, 65 min.

DISCUSSION.

The purpose of these experiments was to study the action of centrally acting drugs on a group of neurones which were isolated from the rest of the brain and were free from the effects of other anesthetic drugs. The neuronally isolated cortex in a decerebrate cat provided a convenient preparation for such a study of central depressant and stimulant drugs.

Bremer (1935) showed that when the cerebral cortex is surgically isolated from the rest of the brain at the collicular level, there is a progressive decrease in its spontaneous electrical activity. Using such decerebrate animals, Burns and Grafstein (1952) showed that small islands of tissue neuronally but not vascularly isolated from the rest of the brain were electrically silent until stimulated by a suitable pulse. Since its introduction the isolated cortex has been used in various pharmacological studies of drugs which modify the activity of the central nervous system. Its usefulness and particularly what constitutes a viable preparation has been critically reviewed by several authors (Ingvar, 1955; Domino, 1957; Sanders, 1963). When stimulated with single shocks two types of relatively uncomplicated responses are produced in the isolated slabs. These responses are subject to modification by drugs both when applied locally to the cortex or given by injection to the animal. The main advantage of the isolated cortex is that these modifications can be considered to be a direct action of the drug and not merely the reflection of its action on other neurones in the brain.

A stimulus of low intensity and 0.03 to 0.2 milliseconds in duration produces a short lasting response during which the cortical surface of the

slab becomes negative with respect to a reference electrode placed on an inactive area of the cortex. The surface negative response thus elicited is similar to the superficial response of the intact cortex first described by Adrian (1936) or the direct cortical response of the cerebral cortex (DCR). It travels in all directions from the point of stimulation at a velocity of 2 m/sec and decreases rapidly with the distance such that it cannot be recorded at a distance of 10 mm from the point of stimulation. The nature and the origin of the surface negative response are points of controversy (Bremer, 1958). Burns (1950) suggests that elements responsible for its origin lie superficially close to the brain surface and have branches which run radially downward through the grey matter. He suggested that the surface negativity is due to depolarization of these elements and the response is presynaptic in nature. His contention that its origin is presynaptic is based mainly on the observation that this response is highly resistant to the action of volatile anesthetics. Eccles (1951), on the other hand, holds that potentials generated by activity in these elements cannot be recorded at the surface and that surface negative response is a postsynaptic potential generated in elements other than those conducting this response. Frank and Pinsky (1964) have shown that an occlusion occurs when two stimuli each sufficient to produce a negative response, are applied at points 10 mm apart on the slab and the response recorded midway between them. This suggests that the negative response is generated postsynaptically. In the absence of further evidence it is considered as such to be a postsynaptic response.

When the intensity of stimulation is increased the negative response

progressively increases until a strength is reached at which another type of response is obtained. This response consists of a wave of surface positivity, 2-4 seconds in duration, which spreads radially without decrement to the borders of the isolated slab at a velocity of 10-20 cm/sec. The surface positive response or the burst response as it is often called is independent of the stimulus once the threshold has been reached. It is highly susceptible to anoxia and the action of volatile anesthetics. It is considered to represent postsynaptic activity in the network of neurones which are located more deeply than those giving rise to the negative response. The bursting obtained during the surface positive response has been attributed to a continuous activity in these neuronal chains. This contention rests on the fact that burst response can be suppressed immediately upon strong repetitive stimulation. The burst response in the isolated cortex may correspond to the deep response of Adrian (1936) seen in the intact cerebral cortex.

When a drug is topically applied to the cortical tissue the only barrier that it has to cross in order to reach the site of action is the diffusional barrier of the pial membrane. On application, the drug diffuses downward in all directions from the point of application. Hence the higher concentrations of the drug will prevail near the surface. When given by injection the drug reaches the cortical tissue by the numerous blood vessels which overlie the cortex and ramify throughout the cortical tissue, and its distribution is consequently more uniform in all areas of the tissue. The systemic administration of the drugs in the study of responses in cortical slabs has the serious disadvantage

that the changes which the drug may produce in the cardiovascular and respiratory parameters may influence the responses obtained in the slab. Local application in addition, has the advantage that the peripheral effects of the drug can be by-passed and the drugs which normally do not cross the blood brain barrier can be studied for their effects on cortical neurones by this method. But the results of topical application must be accepted with some caution since no strict correlation is possible between the dose of the drug applied and the response of the tissue, in particular the responses of those neurones located deep in the cortex.

In these experiments central depressants and the central stimulants produced opposite effects on the cortical responses induced by electrical stimulation. On the basis of the modifications produced, a partial classification of these agents is rendered possible, and this is shown in Table VIII.

The central depressants which have diverse chemical structures produced a remarkably similar pattern of activity in the cortex. As a group, all these agents only reduced the amplitude of the evoked responses and increased the stimulus threshold. Diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, gamma aminobutyric acid, hyoscine and meperidine when topically applied to the cortex reduced the amplitudes of surface negative and the surface positive response both in low and high concentrations. No consistent facilitation of either response was ever seen. Diazepam and meprobamate being insoluble in water had to be given by intravenous injection. When given in this way they too reduced reversibly both the surface negative and the surface positive responses in

TABLE VIII

A CLASSIFICATION OF DRUGS ACTING ON THE CENTRAL NERVOUS SYSTEM BASED ON THEIR EFFECT ON CORTICAL NEURONES.

I

Depressants

Depress the responses of cortical neurones and increase the stimulus threshold.

Group A.

Class 1. Reduce surface negative and surface positive response. No stimulation or facilitation of response.
e.g. Diphenhydramine
Promethazine
Gammahydroxybutyrate
Gammabutyrolactone
Gamma aminobutyric acid
Hyoscine
Diazepam
Meprobamate
Local anesthetics*
Tetrodotoxin*

Class 2. Reduce surface negative and surface positive response in low concentration. Produce spontaneous activity in high concentration.
e.g. Chlorpromazine

Class 3. Depress cortical neurones on topical application but stimulate cortical neurone on injection.
e.g. Meperidine

II

Stimulants

Facilitate the responses of cortical neurones to stimuli. Reduce the stimulus threshold.

Group A.

Class 1. Stimulate cortex directly. Increase amplitudes of surface negative and surface positive response. Produce spiking during burst activity.
e.g. Bemegride,
Strychnine

Class 2. Stimulate cortex directly. Increase amplitude of surface negative and surface positive responses. No spiking during burst activity.
e.g. Caffeine

Class 3. No direct stimulation of isolated cortex. Facilitate only surface positive response. No spiking during burst activity.
e.g. Nikethamide

Class 4. Long duration of stimulant activity. Produce large negative spikes followed by positive bursts and strong epileptiform afterdischarge.
e.g. Picrotoxin

Group B.
Initial stimulation followed by spreading depression.
e.g. Glutamate

* Results from previous work (see Discussion).

the isolated cortex. Interesting deviations from this typically depressant action of these drugs was provided by chlorpromazine and meperidine. Chlorpromazine when applied in smaller concentrations, 1-2% produced only cortical depression similar to other drugs but this depression had a very prolonged duration. In higher concentrations it produced spontaneous activity in the slab consisting of fluctuations of potentials which were mainly positive in nature. But in spite of this stimulatory effect, no facilitation of the surface negative or the surface positive responses was discernible. The stimulatory effect is peculiar to chlorpromazine itself since promethazine which is also a phenothiazine failed to show a similar effect in high concentrations. The mechanism underlying this effect of chlorpromazine is not known but this observation is similar to other isolated reports of its facilitatory action in the nervous system. Thus Rinaldi and Himwich (1955) showed that small doses of chlorpromazine (under 6 mg/kg) depress the EEG arousal in the rabbit, larger doses over 15 mg/kg produced EEG arousal; Gangloff and Monier (1957a) found that in the unanesthetized rabbit chlorpromazine elevated the threshold but prolonged the electrical afterdischarge to stimuli of the sensorimotor cortex and also the hippocampus. The tremor inducing effects of chlorpromazine and its congeners are well documented and are indicative of its stimulant action.

Meperidine when applied topically to the cortex in concentrations 0.5 - 4.0% produced only a depression of the slab undergoing stimulation. But when injected intravenously in a large dose (20 mg/kg) it produced convulsant activity both in the whole animal as well as in the isolated

slab which was being stimulated in a normal manner. In this respect its action differs from that of procaine which when injected in convulsant doses did not produce convulsant activity in the isolated slab (Sanders, 1963). It appears that the convulsant effect of injected meperidine is probably due to a metabolite. Indeed, Deneau and Nakai (1961) showed that in the monkey the degree of central excitation depends in large part on the rate at which meperidine is converted to its metabolite normeperidine. In order to substantiate further that the convulsant activity is due to normeperidine it would be necessary to test the action of the latter on the isolated cortex.

In view of the marked similarity of cortical depression produced by numerous central depressants and the depression produced by procaine, pentobarbital, and ether (Frank and Sanders, 1963) it is attractive to postulate that the cellular mechanisms underlying this depression are commonly shared by all these agents and are responsible for their anesthetic action. The nature of such mechanisms is discussed more fully in the General Discussion below.

In contrast to the central depressants, the classical central stimulants bemegride, picrotoxin, caffeine, and strychnine, produced activation of the cortex when applied locally. Nikethamide, in a concentration up to 10% failed to produce such activation. However, all these drugs (including nikethamide) greatly facilitated the surface positive response, whereas the surface negative response was relatively less affected in this respect. The threshold for the stimulus evoking a surface positive response was reduced by all these stimulants. Although the general effect of

these drugs on the cortex was stimulatory, the nature of the responses evoked after drug application varied between different drugs, but it was consistent for each drug in all the experiments. Thus, bemegride and strychnine produced large surface positive responses of long duration which were characterized by the negative spikes along its course. These spikes probably represent a depolarization of elements which are located superficially and normally give rise to the negative response of the slab. Caffeine and nikethamide also increased the amplitude and duration of the surface positive response but unlike the preceding drugs they were free of overriding negative spikes. The action of picrotoxin was characterized by its long duration of action. Very low concentrations of the drug, 0.05 - 0.1% produced stimulant activity lasting 4-5 hours. The initial effect of picrotoxin consisted of negative spikes of large amplitude, while the subsequent response consisted of positive bursting and strong epileptiform afterdischarge.

In a previous study on the action of convulsants on the isolated cortex undertaken by Grafstein (1963) the author showed that one group of drugs including strychnine, picrotoxin, penicillin, pentylenetetrazol, d-tubocurarine, and thiamine induced convulsions which were independent of stimulation while another group of drugs which included thiosemicarbazide, carbazide and isoniazid produced convulsions only if the slab was stimulated prior to drug application for 10 minutes or more. Grafstein concluded that both groups of convulsants differed in their mode of action and suggested that strychnine and presumably other drugs in the first group acted in the cortex in the same way as strychnine does in the spinal cord

by suppressing postsynaptic inhibition, whereas the hydrozides may act by blocking the synthesis of an inhibitory substance which is normally released during neuronal activity. In the present experiments, the different but consistent patterns of cortical activity induced as a result of stimulant drugs suggest that although these drugs are direct cortical stimulants, the actual mechanisms underlying the excitation probably differ among this group of drugs. Furthermore, the action of these drugs on the higher centres may differ from their mechanisms of action in the spinal cord. Thus, although it has been shown that strychnine blocks the inhibitory postsynaptic potentials (IPSP) in the cord (Eccles, 1964), it has been found that strychnine fails to block the large IPSP of cortical neurones in concentrations higher than those used to block the potentials in the spinal cord (Krnjevic et al., 1964). Moreover, Crawford et al. (1963) have shown that electrophoretic application of strychnine produces excitation of the Purkinje cell in the cerebellum. This type of evidence suggests that in the brain the excitatory action of strychnine may be exerted directly on the excitatory neurones rather than through blockade of postsynaptic inhibition. However, further experimentation of single cells would be necessary to establish the precise mechanism of action of the various convulsant drugs.

Glutamate differed from other excitatory drugs in that its application resulted mainly in cortical spreading depression. But in some experiments it was possible to detect a transient but definite stimulation consisting of positive bursts, which were quickly followed by spreading depression, during which the cortex became totally inexcitable. Spreading

depression, which is a slow moving wave of depression which travels outward from its site of evocation, is considered to result from excessive but general depolarization of all the nerve cells caused by glutamate (Van Haarveld, 1964). In this respect the action of glutamate resembles that of veratrine alkaloids (Reiffenstein, 1968) or potassium. The excitatory action of glutamate is also borne out by the increased rate of firing of single neurones when glutamate is applied iontophoretically to spinal and cortical neurones (Curtis et al., 1960; Krnjevic, 1964).

IV GENERAL DISCUSSION

The response to an anesthetic agent consists of a loss of consciousness, sensory perception and motor function. Ideally, the study of the mechanism of action of anesthetic drugs should be carried out on an isolated group of neurones of the central nervous system which are responsible for the maintenance of consciousness. But the existence of such an exclusive group of cells in the central nervous system is not known. The most generally held view in this respect is that the state of consciousness in an intact animal is maintained by activity in the reticular formation in the midbrain. Consequently, considerable attention has been devoted to the reticular formation and it has become a favourite site for the study of drugs producing anesthesia. Its isolation from the rest of the nervous system in a functional state has not been possible.

The term reticular formation has been applied to the core of the brainstem comprising cell bodies enmeshed in a network of dendrites and nerve terminals and is surrounded by long fibre tracts and nuclei of the sensory systems. The reticular formation contributes to regulatory control of respiration, vasomotor tone, gastrointestinal function and temperature regulation. It also regulates the sleep wakefulness cycle, reception, conduction and integration of sensory inflow and the extrapyramidal motor outflow. Bremer (1935) was the first to recognize that consciousness is dependent on influences ascending through the brainstem from below the level of the colliculi. In 1949 Moruzzi and Magoun showed that stimulation of reticular formation leads to awakening in a drowsy or sleeping

animal similar to that obtained by the peripheral stimulation. They also showed that lesions in the midbrain produced a sleeplike state. The sedative and the anesthetic drugs were the first group of drugs to be studied on the reticular formation. Moruzzi and Magoun (1949) showed that barbiturates blocked the reticular relays. Following this, French et al. (1953) showed that ether and pentobarbital blocked the EEG arousal to reticular stimulation and selectively depressed potentials evoked in the reticular formation by sensory stimulation. On basis of these observations French et al. (1953) proposed the depression of the reticular formation to be the basic mechanism of anesthesia. This proposal is rather limited in its scope, mainly because it heavily emphasizes the action of barbiturates and attempts to explain the site rather than the fundamental mechanism of anesthetic agents at the cellular level. It has been shown that chloralose, a general anesthetic agent, fails to produce a blockade of reticular formation in anesthetic doses (Moruzzi and Magoun, 1949). Further, Longo and Silvestrini (1958) showed that the loss of EEG arousal response occurred before depression of evoked responses to sciatic stimulation, and a blockade of reticular formation responses took place while cortical EEG desynchronization was still in evidence in rabbits receiving pentobarbital and ether respectively. Killam (1962) who has reviewed the extensive pharmacology of the reticular formation suggested that since it is the site of action which is most common to the whole series of anesthetics, the anesthetic property of a drug may depend partly on the depression of this region of the central nervous system.

The cellular mechanism of depressions of the neurons of the reticular

formation or for that matter the neurons of other regions of the central nervous system has not been explained seriously. The most popular view of drug induced depression is that it is due to a depression of the synaptic transmission through the myriad of synapses of the central nervous system, particularly the reticular formation. It is generally believed that the more synapses a conducting system contains the more readily is the transmission depressed throughout that system by an anesthetic agent (Bárány, 1947). This view has been reinforced in the past by the observations that the polysynaptic reflexes (e.g. flexor reflex) are much more susceptible to anesthetics than are the monosynaptic reflexes (e.g. the knee jerk). But more recent evidence obtained renders this view somewhat doubtful. For example, De Jong et al. (1968) showed that several volatile general anesthetics, ether, cyclopropane, chloroform, halothane, methoxyfluorane and nitrous oxide, abolish both monosynaptic and polysynaptic reflexes in the cat when given in high doses, but when all monosynaptic reflexes had been depressed a small but significant amount of transmission still persisted in some polysynaptic pathways. These authors concluded that anesthetics depress the transmission of afferent impulses as readily at single synapses in the spinal cord as at several links of a serial synaptic chain. Loying et al. (1964) have studied the action of barbiturates on the monosynaptic pathway in the cat spinal cord. They showed that a depression is brought about by sodium thiamylal, a short acting barbiturate, but that this depression is not due to an increase in the motorneurone firing threshold or to alterations in the electrical properties of the motor-neuronal membrane (postsynaptic membrane). Loying and colleagues suggest

that the reduced activity can be due either to a block of conduction of impulses into some afferent nerve terminals or to a reduction in the amplitude of the spike potential in each terminal.

The suppression of conduction in the fine nerve endings in the central pathways by a direct action of drugs on them has not been widely recognized. Hitherto, it has been widely held that the blockade in the polysynaptic pathways is due to a blockade of chemical transmission across the synapses between the neurones and not due to the blockade of the nerves themselves. This view appears to rest rather heavily on the observation of Larabee and Posternak (1952) who showed that in the isolated perfused sympathetic ganglia the concentrations of ether, chloroform, and pentobarbital required to block transmission across the ganglia were less than those required to block conduction in the axons entering and leaving the ganglia. However, this observation may not hold true for the fine nerve endings themselves, which are small in size and may be more susceptible to blockade than the larger axons. Indeed, Loying et al. (1964) showed that during sodium thiamylal anesthesia, although no appreciable change was recorded in the dorsal root (afferent nerves), negative spikes recorded in the motor nucleus which represent the activity in the afferent nerve terminals were markedly reduced, together with the synaptic potential and the ventral root potential. These authors suggested that since the electrical properties of the motorneurone itself were unaltered, the barbiturates must be acting mainly on the afferent nerve terminals, their action resulting in less transmitter release and reduced synaptic potential. This hypothesis,

however, does not seem to apply to general anesthetics, since ether has been found not to have any effect on the focally recorded presynaptic spike potential (Somjen and Gill, 1963). But whether this is also true of other volatile anesthetics and a large number of nonvolatile agents, particularly in the brain, is not known.

The blockade of synaptic transmission so often proposed for the mechanism of action of general anesthetics implies, at least in the pharmacological sense, a specific interference with the transmitter sensitive region of the postsynaptic membrane, which is comparable to the action of curare-like selective blocking agents on the neuromuscular junction. It has been shown that alcohol, a volatile depressant, in fact potentiates the neuromuscular transmission both in the frog (Inoue and Frank, 1967) and in the rat (Gage, 1965) by both presynaptic and postsynaptic mechanisms. It would seem surprising that so many general anesthetics, local anesthetics and, as this study shows, a variety of central depressants with anesthetic properties and profound diversity of chemical structure, should act identically in a specific manner on the synaptic junctions in the central nervous system to produce a nonspecific depression. A unified theory of depressant action based on the synaptic blockade in the pharmacological sense appears doubtful.

The blockade of synapses brought about by the depressants may be due partly to the same cellular mechanisms that are responsible for blocking conduction in the nerves and the nerve terminals in various areas of the brain. It has been proposed that both general and local anesthetics produce central depression by suppression of the action potentials in the

neuronal tissue, thus leading to a loss of conduction of impulses in the central nervous system (Frank and Sanders, 1963). More specifically, the blockade is brought about by a suppression of the initial increase in the sodium permeability, which is responsible for the rising phase of the action potential and normally follows an excitatory stimulus applied to the tissue. One would therefore expect that any agent which depresses sodium conductance in this manner would possess anesthetic properties and produce a central depression. Indeed, when tetrodotoxin became available as substance which produced a strong but specific blockade of the sodium conductance in the nerve, it was shown that this compound could produce in intact animals a state of central depression which was indistinguishable from anesthesia.

The present investigation has shown that the ability to produce anesthesia is not confined to the conventional anesthetics, but it is a definite property of a vast number of centrally acting drugs. This property although reflected in their therapeutic use in preanesthetic medication, has been somewhat ignored in the experimental work bearing on the mechanism of the anesthetic action of drugs. Most theories attempting to explain such mechanisms therefore have been confined to volatile and nonvolatile anesthetics. The results of this study support the contention that the central depression produced by a large number of drugs is indicative of their anesthetic action, and furthermore the cellular mechanisms which underlie this depression are the same as those postulated above for the general and the local anesthetics. In its purest but also extreme form, this mechanism of cellular depression is

represented by the action of tetrodotoxin in the neuronal tissue, i.e. a depression of the action potentials brought about by suppression of the specific increase in sodium permeability caused by a depolarizing stimulus.

Although the evidence for the elementary mechanism of all centrally acting drugs is still incomplete, one may attempt a partial classification of the drugs used in this study on the basis of evidence available from the experiments on the intact animals and the isolated neuronal tissue. Such a classification is shown in Table IX. It appears that the depressant drugs class in Group I produce a basically similar action in the isolated neuronal tissue, thus suggesting that the mechanism of action underlying their effect at the cellular level is perhaps the same for all these drugs. The differences encountered amongst these depressants are mainly seen in the intact animal studies, and the drugs have been divided into different classes on the basis of these studies. These differences in their actions could be due to several factors such as the potency of the drugs, their biotransformation in the body or their ability to reach the site of action. The stimulant drugs on the other hand show a much greater diversity in their action both in the intact animals and the isolated neuronal tissue. This suggests that the differences in their action are due to differences in the mechanism responsible for their stimulant properties. They have been divided, therefore, into groups and subgroups on the basis of different mechanisms of action as shown in Table IX.

If these drugs have an anesthetic action, one may justifiably

TABLE IX

A CLASSIFICATION OF CENTRALLY ACTING DRUGS BASED ON THEIR ACTION IN INTACT MICE AND ISOLATED CEREBRAL CORTEX

DEPRESSANTS

Group I

Depress evoked responses of the isolated cerebral cortex. Increase stimulus threshold.

Class 1. Produce loss of righting reflex (L.R.R.) in intact mice when given alone. In much higher doses cause loss of spinal reflexes.
e.g. Ether
Phenobarbital

Class 2. Produce L.R.R. in intact mice when given alone. In slightly higher doses cause loss of spinal reflexes.
e.g. Meprobamate
Diazepam

Class 3. Produce L.R.R. in intact mice in small doses, cause convulsions in high doses when given alone. Depress cortical responses in small doses, cause spontaneous activity in isolated cortex.
e.g. Chlorpromazine

Class 4. No L.R.R. when given alone to intact animals. Cause excitement and convulsions in high doses. Potentiate anesthetic action of phenobarbital in convulsant and nonconvulsant doses. Potentiation is greater in convulsant doses.
e.g. Diphenhydramine
Promethazine
Gammahydroxybutyrate
Gammabutyrolactone
Local anesthetics*

STIMULANTS

Group I

Produce convulsions in intact mice. Antagonize phenobarbital anesthesia in mice. Stimulate isolated cortex directly. Facilitate evoked responses of isolated cortex and lower stimulus threshold.

Sub-group 1. Facilitate mainly positive burst response of isolated cortex. Cause negative spikes during burst response.
e.g. Bemegride

Sub-group 2. Very long duration of action. Cause initial negative spikes followed by positive bursts and strong epileptiform after-discharge.
e.g. Picrotoxin.

Group II

Produce convulsions in intact mice. No antagonism of phenobarbital depression. Facilitate evoked responses of the isolated cortex. Lower stimulus threshold.

Sub-group 1. Stimulate cortex directly. Facilitate positive burst response and cause negative spikes during this response.
e.g. Strychnine

Sub-group 2. Stimulate cortex directly. Facilitate surface negative responses. No spiking during burst response.
e.g. Caffeine

TABLE IX - contd.

DEPRESSANTS	STIMULANTS
Class 5. As Class 4, but cause smaller potentiation of phenobarbital in convulsant than non-convulsant dose. Stimulate isolated cortex on intravenous injection of high dose. e.g. Meperidine	Sub-group 3. No direct stimulation of isolated cortex. Facilitate surface positive response. No spiking during burst response. e.g. Nikethamide
Class 6. As Class 4, but do not potentiate phenobarbital effect in convulsant doses. Cause anoxic convulsions. e.g. Hyoscine Tetrodotoxin*	Group III No effect in intact mice. Stimulate cortex directly. Produce spreading depression in isolated cortex. e.g. Glutamic acid
Class 7. No effect in mice on injection. Do not cross blood brain barrier. e.g. Gamma aminobutyric acid.	

* From previous work (See General Discussion).

question their failure to produce anesthesia when given to the intact animals. As far as some depressants such as diazepam and meprobamate, and possibly chlorpromazine, are concerned, the tests in the intact animals and isolated tissue show that they are remarkably similar to barbiturates both in producing loss of righting reflex and producing cortical depression. The major difference between the action of diazepam and meprobamate on one hand and phenobarbital on the other hand is that the margin between the dose which produces anesthetic depression and the dose producing muscular relaxation is narrower for diazepam and meprobamate than it is for phenobarbital. It is likely that their use as tranquilizing agents involve nothing more than a mild sedative action and muscular relaxation, two properties which are shared by barbiturates and other anesthetics. As regards other drugs which include diphenhydramine, promethazine, gamma-hydroxybutyrate, gammabutyrolactone, meperidine and hyoscine, they all produce excitement and convulsions when given in high doses, but no anesthesia. The stimulant action precludes their anesthetic action. This stimulant property of the depressants is also shared to the same extent by the local anesthetic drugs and to a more limited extent by the general anesthetic agents themselves. It has been suggested that the excitement produced by local anesthetics is an exaggerated form of Stage II of anesthesia, during which the general anesthetics produce excitement. The following Stage III of surgical anesthesia is not obtained by local anesthetics because in high doses they produce death in animals due to their toxic effects on the cardiovascular and respiratory systems. The action of a variety of central depressants corresponds to that of the

local anesthetics and it is possible that the excitement and convulsions produced by them are also exaggerated manifestations of the Stage II of anesthesia. Their anesthetic properties, however, are demonstrated by the fact that when given to mice pretreated with subanesthetic doses of the barbiturates they produce central depression which corresponds to anesthesia. It would appear that pretreatment with the barbiturates reduces the threshold for the depressant response. A subsequent administration of the depressant drug lowers this threshold to a critical level, which is sufficient to trigger the full response (i.e. the loss of righting reflex). The stimulant property of these drugs probably is also a consequence of depression at some inhibitory centres in the central nervous system. Their failure to produce excitement in the isolated cortex supports this type of indirect stimulant action for most depressants.

It appears that a large number of centrally acting drugs possess an anesthetic property which is reflected in their ability to produce a generalized depression of the central nervous system. But it must be recognized that these drugs also produce other effects as, for example, the analgesic effect produced by meperidine, the tranquilizer effect produced by chlorpromazine, or the muscle relaxation caused by diazepam. All these other effects also are a result of central depression, but it is probable that such depressions occur only in a few selected areas of the brain and they are, therefore, relatively specific depressions. The degree and the mechanisms of these types of depression vary among the different centrally depressant drugs. These selective mechanisms of

action may involve the blockade of conduction or the interruption of synaptic transmission at specific sites in the brain. The evidence for the existence of such sites is not available as yet. The other effects of the centrally acting drugs common to all, such as a sedative or hypnotic action, are perhaps nothing more than a weak generalized depression of the entire central nervous system; the anesthesia being a stronger form of this depression. The central stimulant properties manifested by most of the centrally depressant drugs is perhaps a strong depression of some inhibitory neuronal pathways.

Any theory of anesthesia claiming to explain the mechanism of action of a large number of drugs must distinguish their mechanisms from the drugs which antagonize the effects of the depressants. In the past the metabolic theories attempting to explain narcosis have broken down partly because the mechanisms postulated for narcosis were also shared by classical stimulants which in the intact animal antagonized the agents producing narcosis (Klein, 1943; Shideman, 1949). In this investigation it has been found that the analeptic drugs such as picrotoxin, and bemegride which antagonize the effects of anesthetics such as phenobarbital, are capable of producing a direct stimulation of central neurones. The mechanisms which are responsible for their stimulant property are clearly separable from those which underlie stimulation by central depressants. Even those drugs which did not antagonize the barbiturate anesthesia, but produced convulsions in the intact animals, i.e. caffeine, strychnine, and nikethamide, are capable of direct stimulation of the cortical elements, as indicated by their facilitatory action on the responses of the isolated

cerebral cortex. The study of convulsant agents of this type suggests that their mechanism of stimulant action although direct in nature may differ from each other at the cellular level. In the cortex all these drugs produced a decrease in the threshold of the stimulus used to produce the electrical responses. The decrease in the threshold may be the result of drug action on several parameters of cellular excitability. Thus the drug may lower the threshold by causing a partial depolarization so that a smaller stimulus strength is required to produce excitation, or it may render an increase in the number of neurones that are capable of excitation, i.e. spatial summation may occur. Also as far as synaptic transmission is concerned it may enhance the activity of an excitatory transmitter substance by causing its increased release or by preventing its destruction after it has been released, or it might block specific inhibitory transmitters. Although direct evidence is not available to assign these processes to various stimulant drugs, the possibility of their existence does demonstrate a basis for differences in the action of the stimulants.

Many interesting facts about the action of anesthetic agents and other centrally acting drugs on various functions of the central nervous system have been reported and a voluminous literature testifies to this fact. But the problem of relating these observations in a cohesive theory of depression has not been seriously attempted. In general, the data from the many chemical and neurophysiological studies reflects only various aspects of the interaction between drugs and functions of the central nervous system. Neurophysiological experiments on drug action are

only likely to produce speculative theories of drug action until the observed changes in gross activity are successfully related to the molecular events concerned with membrane excitation and synaptic transmission. The study of drugs on the parameters of the action potential, which is a fundamental unit of cellular excitation, therefore provides the beginning of such a molecular approach to the mechanism of action of drugs which modify the function of the central nervous system.

Thus far the evidence concerning the suppressive effect of drugs on the sodium conductance, the increase in which is responsible for the first phase of the action potential, is available for only some volatile agents such as ether (Inoue and Frank, 1965), chloroform Yamaguchi, 1961), ethyl alcohol (Armstrong and Binstock, 1964; Inoue and Frank, 1967), local anesthetics (Inoue and Frank, 1962b), and a few nonvolatile anesthetic agents (Thesleff, 1956). The evidence along similar lines for the central depressants used in this study is not available. However, some of the work carried out on sodium conductance by central depressants comes from Hille (1966) who showed that in the frog nerve,prochlorperazine, a close relative of chlorpromazine, decreased the sodium conductance as indicated by decreases in the threshold, rate of rise, and the amplitude of the action potential. Both xylocaine and tetrodotoxin produced similar effects in this preparation. More recently, Blaustein (1968a) showed that in the lobster axon tropine esters, tropine-p-tolylacetate, and its quartenary analog tropine-p-tolylacetate methiodide both decreased the maximum sodium and potassium conductances associated with membrane depolarization and thus blocked the nerve action

potential. This effect also is produced by sodium thiopental in the same preparation (Blaustein, 1968b). It is probable that the central depressants which form the subject of this investigation also have an inhibitory effect on the sodium permeability. Their actions on sodium conductance are currently under investigation in this laboratory.

A large number of models of the cell membrane has been proposed over the past years with a view to understanding the nature of ion permeability. In excitable tissue, the object has been to study the action of drugs on such permeability and define their mechanisms of action. Shanes (1958) suggested that 'labilizers', such as the veratrum alkaloids produce ion leaks in the membrane and cause excitation while the anesthetics which are the 'stabilizers' prevent the increase in sodium permeability required for excitation and produce depression. More specifically, the 'stabilizers' may interfere with the opening of pores or channels during excitation and consequently have little effect on the ion movements in the resting state. The 'labilizers' on the other hand, increase ion movement during rest as well as during activity, probably by enlarging these channels. The effects of such ion leaks may also be opposed by ion pumps which employ metabolic energy. It is tempting to assume that a large number of central depressants with anesthetic properties and an equally large number of stimulants are acting within the framework of this model. However, this classification of drugs into two categories is somewhat rigid. It may be that the 'stabilizers' and the 'labilizers' represent two extremes of drugs which modify excitability, and that a large number of other centrally acting drugs which combine

these properties to a varying extent fall in between these two ends of the spectrum. Such a placement of drugs would enable one to recognize the various differences in their action and enable a more rational classification of centrally acting drugs.

V SUMMARY AND CONCLUSIONS

1. When administered to intact white mice, the central depressants - diphenhydramine, promethazine, chlorpromazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine, and meperidine - produced sedation in small doses, but excitement and convulsions in higher doses. When given to mice pretreated with subanesthetic doses of phenobarbital these drugs abolished the righting reflex both in convulsant doses (hyoscine excepted) and in nonconvulsant doses. Meprobamate, diazepam and chlorpromazine produced a loss of righting reflex both when given alone and following phenobarbital.

2. The central stimulants bemegride and picrotoxin antagonized the loss of righting reflex produced by phenobarbital, but nikethamide, caffeine and strychnine did not alter the effects of phenobarbital. All these drugs produced convulsions in mice when given alone.

3. In the neuronally isolated cortex of the cat, local application of diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine and meperidine, and injection of diazepam and meprobamate depressed or abolished the surface negative and surface positive response to direct stimulation and raised the stimulus threshold of the positive burst response. Chlorpromazine produced a similar depression in small concentrations but caused spontaneous activity in higher concentrations. None of these drugs facilitated the responses of isolated cortex. Meperidine when given by intravenous injection in a high dose produced convulsant activity in the isolated cortex.

4. The stimulants, bemegride, picrotoxin, nikethamide, caffeine and strychnine, facilitated the surface positive response of the isolated cortex and lowered the stimulus threshold for this response. Excepting nikethamide, they all produced convulsive discharge in the isolated cortex unrelated to the applied stimulus.

5. Both gamma aminobutyric acid and glutamic acid were ineffective in mice when given alone intraperitoneally or after phenobarbital. In the isolated cortex gamma aminobutyric acid depressed the evoked responses. Glutamate produced a central depression, which was sometimes preceded by positive bursting.

6. The results of this investigation suggest that the central depression produced by a number of structurally unrelated drugs is indicative of an anesthetic property. It is suggested that the excitatory action of the depressants is indirect and is probably due to a selective inhibition of inhibitory neuronal pathways. The action of stimulants is more direct and is probably due to excitation of neurones by various different mechanisms. The mechanism of anesthetic action of central depressants at the cellular level appears to be similar to that of local anesthetics and general anesthetics in the neuronal tissue.

BIBLIOGRAPHY

ADRIAN, E. D. (1936). The spread of activity in the cerebral cortex. J. Physiol. 88, 127.

ARIENS, E. J. (1964). Molecular Pharmacology, Vol. I. New York: Academic Press.

ARMSTRONG, C. M. and BINSTOCK, L. (1964). The effects of several alcohols on the properties of the squid giant axon. J. gen. Physiol. 48, 265.

ASTON, R. and COLLUMBINE, H. (1959). Studies on the nature of joint action of ethanol and barbiturates. Toxicol. app. Pharmacol. 1, 65.

AUSTIN, G. M. and PASK, E. A. (1952). Effect of ether inhalation upon spinal cord and root action potentials. J. Physiol. 118, 405.

BAN, T., TAKAORI, S., SASA, M. and SHIMAMOTO, K. (1967). The central action of gammabutyrolactone and gammahydroxybutyrate. Jap. J. Pharmacol. 17, 30.

BAIRD, H. W., SZEKELY, E. G., WYCIS, H. T. and SPIEGEL, E. A. (1957). The effect of meprobamate on the basal ganglia. Ann. N.Y. Acad. Sci. 67, 873.

BÁRÁNY, E. G. (1947). A theoretical note concerning the action of drugs on the central nervous system. Arch. int. Pharmacodyn. 75, 222.

BARLOW, O. W. (1935). The sedative efficiency of a series of analeptics as antidotes to sublethal doses of pentobarbital, chlorahydrate, and tribromethanol. J. Pharmac. exp. Ther. 55, 1.

BASIL, B., BLAIR, A. M. J. N., and HOLMES, S. W. (1964). The action of sodium 4-hydroxybutyrate on spinal reflexes. Br. J. Pharmac. 22, 318.

BECK, R. A. and GOLDSTEIN, L. (1964). EEG alerting and behavioral stimulant effects of very low doses of atropine in rabbits. Fedn. Proc. 23, 561.

BERNARD, C. (1875). Lecons sur les anaesthetiques et sur l'asphyxic. Paris: Bailliere.

BERNSTEIN, J. (1902). Vontersuchvongen zur thermadynamik der bioelektrischen strome. Pflug. Arch. ges. Physiol. 92, 521.

BLAUSTEIN, M. P. (1968a). Action of certain tropine esters on voltage-clamped lobster axon. J. gen. Physiol. 51, 309.

BLAUSTEIN, M. P. (1968b). Barbiturates block sodium and potassium conductance increases in voltage clamped lobster axons. J. gen. Physiol. 51, 293.

BONNET, V. and BREMER, F. (1948). Analyse oscillographique des depressions fonctionnelles de la substance grise spinale. Archs int. Physiol. 56, 97.

BRADLEY, P. B. and KEY, B. J. (1958). The effects of drugs on arousal responses produced by electrical stimulation of reticular formation of brain. Electroen. Neurophysiol. 10, 97.

BREMER, F. (1935). Cerveau isole et physiologie du sommeil. C.R. Soc. Biol., Paris, 118, 1235.

BREMER, F. (1958). Cerebral and cerebellar potentials. Physiol. Rev. 38, 357.

BREMER, F. and BONNET, V. (1948). Action particuliere des barbituriques sur la transmission synaptique centrale. Archs int. Physiol. 56, 100.

BROOKS, S. C. (1947). Permeability and enzyme reactions. Adv. Enzymol. 7, 1.

BURNS, B. D. (1950). Some properties of the cat's isolated cerebral cortex. J. Physiol. 111, 50.

BURNS, J. H. (1954). The pharmacology of chlorpromazine and promethazine. Proc. R. Soc. Med. 47, 617.

BURNS, B. D. and GRAFSTEIN, B. (1952). The function and structure of some neurones in cat's cerebral cortex. J. Physiol. 118, 412.

BUTLER, T. C. (1950). Theories of general anesthesia. Pharmac. Rev. 2, 121.

CAJAL, R. (1909). Histologie du systéme nerveaux de l'homme et des vertébrés. Paris: Maloine.

CALDWELL, P. C. and KEYNES, R. D. (1957). The utilisation of phosphate bond energy for sodium extrusion from giant axons. J. Physiol. 137, 12P.

CHATFIELD, P. O. and Lord, J. T. (1955). Effects of atropine, prostigmine, and acetylcholine on evoked cortical potential. Electroen. Neurophysiol. 7, 553.

COURVOISIER, S., FOURNET, J., DURCOT, R., KOLSKY, M., and KOETSCHET, P. (1953). Propriétés pharmacodynamiques du chlorhydrate de chloro-3(dimethylamino-3-propyl)-10 phenothiazine (4.560 R.P.). Arch. int. Pharmacodyn. 92, 305.

CRAWFORD, J. M., CURTIS, D. R., VOORHOEVE, P. E. and WILSON, V. J. (1963). Strychnine and cortical inhibition. Nature, Lond. 200, 845.

CREPAX, P. and INFANTELLINA, F. (1956). Effetti della morfina sul lembo isolato di corteccia cerebrale di cone. Arch. sci. biol. (Bologna), 40, 147.

CURTIS, D. R. (1962). Humors, Hormones and Neurosecretions, ed. BROOKS, C. N., GILBERT, J. L., LEVIS, H. A. and CURTIS, D. R. New York: University Publishers.

CURTIS, D. R. and WATKINS, J. C. (1965). The pharmacology of amino acids related to gamma aminobutyric acid. Pharmac. Rev. 17, 347.

CURTIS, D. R., PHILLIS, J. W. and WATKINS, J. C. (1959). The depression of spinal neurones by gamma aminobutyric acid and beta-alanine. J. Physiol. 146, 185.

CURTIS, D. R., PHILLIS, J. W. and WATKINS, J. C. (1960). The chemical excitation of spinal neurons by certain acidic amino acids. J. Physiol. 150, 656.

DASGUPTA, S. R. and WERNER, M. (1955). Inhibitory action of chlorpromazine on motor activity. Archs int. Pharmacodyn. 100, 409.

DASGUPTA, S. R., MUKERJEE, K. L. and WERNER, G. (1954). The activity of some central depressant drugs in acute decorticate and diencephalic preparations. Archs int. Pharmacodyn. 97, 149.

DAVSON, H. and DANIELLI, J. F. (1943). The permeability of natural membranes. Cambridge: University Press.

DeJONG, R. H., ROBLES, R., CORBIN, R. W. and NACE, R. A. (1962). Effect of inhalation anesthetics on monosynaptic and polysynaptic transmission in spinal cord. J. Pharmac. exp. Ther. 162, 326.

DENEAU, G. A. and NAKAI, K. (1961). The toxicity of meperidine in the monkey as influenced by its rate of absorption. In Minutes of 23rd Meeting of Committee on Drug-Addiction and Narcotics, Appen. 6, NAS-NRC, Washington, D.C.

DOMINO, E. F. (1957). Pharmacological actions of a convulsant barbiturate. II. Effects compared with pentobarbital on cerebral cortex and some brain stem systems of the cat. J. Pharmac. exp. Ther. 119, 272.

DOMINO, E. F. (1962). Sites of action of some central nervous system depressants. A. Rev. Pharmacol. 2, 215.

DUMONT, J. (1958). Nouvelles rescherches sur les agents pharmacologiques antagonistes des depresseurs centraus (derives barbituriques, analgesiques). Annls. Soc. Sci. med. Brux. 11, 181.

ECCLES, J. C. (1946). Synaptic potentials of motorneurones. J. Neurophysiol. 9, 87.

ECCLES, J. C. (1951). Interpretation of action potentials evoked in the cerebral cortex. Electroen. Neurophysiol. 3, 449.

ECCLES, J. C. (1957). The physiology of nerve cells. Baltimore: John Hopkins Press.

ECCLES, J. C. (1962). Spinal neurones: Synaptic connexions in relation to chemical transmitters and pharmacological responses. Proc. of 1st International Pharmacological Meeting, Pharmacological analysis central nervous system action, vol. 8, pp.157-182. New York: Pergamon Press.

ECCLES, J. C. (1964). The physiology of synapses. Berlin: Springer.

ECCLES, J. C. (1965). Pharmacology of central inhibitory synapses. Br. med. Bull. 21, 19.

ECCLES, J. C., SCHMIDT, R. and WILLIS, W. D. (1963). Pharmacological studies of presynaptic inhibition. J. Physiol. 168, 500.

FEATHERSTONE, R. M. and MUELBAECHER, C. A. (1963). The current role of inert gases in the search for anesthesia mechanisms. Pharmacol. Rev. 15, 97.

FERGUSON, J. (1939). The use of chemical potentials as indices of toxicity. Proc. R. Soc. B. 127, 387.

FINK, G. B. and SWINYARD, E. A. (1960). Effects of psychopharmacologic agents on experimentally induced seizures. J. Amer. Pharm. Ass. [Sci.] 49, 510.

FRANK, K. and FUORTES, M. G. F. (1957). Presynaptic and postsynaptic inhibition of monosynaptic reflexes. Fedn Proc. 16, 39.

FRANK, G. B. and SANDERS, H. D. (1963). A proposed common mechanism of action for general and local anesthetics in the central nervous system. Br. J. Pharmac. Chemother. 21, 1.

FRANK, G. B. and PINSKY, C. (1964). Evidence for a postsynaptic origin of the surface-negative response to direct stimulation of the cat's cerebral cortex. Nature, Lond. 202, 192.

FRANK, G. B. and PINSKY, C. (1966). Tetrodotoxin induced central nervous system depression. Br. J. Pharmac. Chemother. 26, 435.

FRANK, G. B. and JHAMANDAS, K. (1969). Interaction between the effects of phenobarbital and procaine in mice: the importance of timing in drug addition studies. Canad. J. Physiol. Pharmacol. In Press.

FERGUSON, J. (1939). The use of chemical potentials as indices of toxicity. Proc. R. Soc. Med. B. 127, 387.

FISHBEIN, W. N. and BESSMAN, S. P. (1964). Gammahydroxybutyrate in the mammalian brain. Reversible oxidation by lactic dehydrogenase. J. biol. Chem. 239, 357.

FRENCH, J. D., VERZEANE, M. and MAGOUN, H. W. (1953). A neural basis of the anesthetic state. Archs Neurol. Psychiat., Chicago, 69, 519.

FREY, H. H., HUSHAHN, E. W. and SOEHRING, K. (1956). 1st β,β - Aethyl-methyl-glutarimid ein spezifiseher barbiturate antagonist? Arzneim-Forsch 6, 583.

FUJITA, S., YASUHARA, M., YAMAMOTO, S. and OGIU, K. (1954). Studies on site of action of analgesics. Jap. J. Pharmacol. 4, 41.

FUHRMAN, F. A. and FIELD, J. (1948). Inhibition of brain respiration by ethyl alcohol at varied temperature levels. Proc. Soc. exp. Biol. Med. 69, 331.

GADDUM, J. H. (1957). Theories of antagonism. Pharmac. Rev. 9, 211.

GADDUM, J. H. (1937). The quantitative effects of antagonistic drugs. J. Physiol. 89, 7P.

GAGE, P. W. (1965). The effect of methyl, ethyl, n-propyl alcohol on neuromuscular transmission in the rat. J. Pharmac. exp. Ther. 150, 236.

GANGLOFF, H. and MONNIER, M. (1957a). Topic action of reserpine, serotonin and chlorpromazine on unanesthetised rabbit's brain. Helv. physiol. pharmac. Acta, 15, 83.

GANGLOFF, H. and MONNIER, M. (1957b). The topical action of morphine, levorphanal and morphine antagonist levallosphan on anesthetised rabbit's brain. J. Pharmac. exp. Ther. 121, 78.

GIARMAN, N. J. and PEPEU, G. (1962). Drug induced changes in brain acetylcholine. Br. J. Pharmac. 19, 226.

GOODMAN, L. and GILMAN, A. (1965). The pharmacological basis of therapeutics. London: Collier-McMillan.

GRAFSTEIN, B. (1963). Convulsant drug action on neuronally isolated cerebral cortex. Science, 142, 973.

GRUNDFEST, H. (1957). General problems of drug action on bioelectric phenomenon. Ann. N.Y. Acad. Sci. 66, 537.

GRUNDFEST, H. (1964). Effect of drugs on the central nervous system. A. Rev. Pharmacol. 4, 341.

HAAS, H. T. A. (1937). Über den antagonismus der narkotica gegen die analeptica coranin and picrotoxin. Arch. exp. Path. Pharmak. 184, 468.

HAHN, F. (1960). Analeptics. Pharmac. Rev. 12, 447.

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. Biol. Rev. 26, 339.

HODGKIN, A. L. (1958). Ionic movements and electrical activity in giant nerve fibres. Proc. R. Soc. B. 148, 1.

HODGKIN, A. L. and HUXLEY, A. F. (1939). Action potentials recorded from inside a nerve fibre. Nature, Lond. 144, 710.

HODGKIN, A. L. and HUXLEY, A. F. (1945). Resting and action potentials in single nerve fibres. J. Physiol. 104, 176.

HODGKIN, A. L. and KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. 108, 37.

HENDLEY, C. D., LYNES, T. E. and BERGER, F. M. (1957). Effect of meprobamate on electrical activity of thalamus and other subcortical aread. In Tranquilizing Drugs, ec. HIMWICH, H. Washington, D.C.: Am. Assoc. Advan. Sci.

HILLE, B. (1966). Common mode of action of three agents that decrease the transient change in sodium permeability in nerves. Nature, Lond. 210, 1220.

HIMWICH, H. E. and RINALDI, F. (1957). The effect of drugs on the reticular system. In Brain mechanisms of drug action, ed. FIELDS, W. S. Springfield, Illinois: Thomas, C. C.

INGERAR, D. H. (1955). Electrical activity of isolated cortex in the unanesthetised cat with intact brain stem. Acta physiol. scand. 33, 151.

INNES, I. R. and NICKERSON, M. (1965). Drugs inhibiting the action of acetylcholine on structures innervated by post-ganglionic parasympathetic nerves. In The pharmacological basis of therapeutics, ed. GOODMAN, L. and GILMAN, A. London: Collier-McMillan.

INOUE, F. and FRANK, G. B. (1962a). Actions of ether on skeletal muscle fibre membrane. Proc. Can. Fed. Biol. Soc. 5, 37.

INOUE, F. and FRANK, G. B. (1962b). Action of procaine on frog skeletal muscle. J. Pharmac. exp. Ther. 136, 190.

INOUE, F. and FRANK, G. B. (1965). Action of ether on frog skeletal muscle. Canad. J. Physiol. Pharmacol. 43, 751.

INOUE, F. and FRANK, G. B. (1967). Effects of ethyl alcohol on excitability and neuromuscular transmission in frog skeletal muscle. Br. J. Pharmac. 30, 186.

JAFFE, J. H. (1965). Narcotic analgesics. In The Pharmacological basis of therapeutics, ef. GOODMAN, L. and GILMAN, A. London: Collier-McMillan.

JOWETT, M. and QUASTEL, J. H. (1937). The effects of ether on brain oxidations. Biochem. J. 31, 1101.

KILLAM, E. K. (1962). Drug action on the brain stem reticular formation. Pharmac. Rev. 14, 175.

KIMURA, E. T. and RICHARDS, R. K. (1957). A comparative study of bemegride (β,β -methylethyl glutasimide, NP-13, megimide) as an analeptic in mice. Arch int. Pharmacodyn. 110, 29.

KLEIN, J. R. (1943). Inhibition of brain respiration by picrotoxin. J. biol. Chem. 151, 651.

KLETZKIN, M. and SWAN, K. (1959). The effects of meprobamate and pentobarbital upon cortical and subcortical responses to auditory stimulation. J. Pharmac. exp. Ther. 125, 35.

KOPERA, J. and ARMITAGE, A. K. (1954). Comparison of some properties of chlorpromazine, promethazine, and pethidine. Brit. J. Pharmac. 9, 392.

KRNJEVIC, K. (1964). Micro-iontophoretic studies on cortical neurons. Int. Rev. Neurobiol. 7, 41.

KRNJEVIC, K., RANDIC, M. and STRAUGHAN, D. W. (1964). Cortical inhibition. Nature, Lond. 201, 1294.

LARABEE, M. G. and POSTERNAK, J. M. (1952). Selective action of anesthesics on synapses and axons in the mammalian sympathetic ganglia. J. Neurophysiol. 15, 91.

LEWIN, J. and ESPLIN, D. H. (1961). Analysis of spinal excitatory action of pentylenetetrazole. J. Pharmac. exp. Ther. 132, 245.

LEWIS, J. J. (1964). An introduction to pharmacology. Edinburgh: Livingstone.

LILLIE, R. S. (1923). Protoplasmic action and nervous action. Univ. of Chicago, 1923.

LITCHFIELD, J. T. and WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. J. Pharmac. exp. Ther. 114, 185.

LOEW, E. R. (1950). Pharmacology of benadryl and specificity of antihistamine drugs. Ann. N.Y. Acad. Sci. 50, 1142.

LOEW, E. R. (1952). Pharmacological properties of antihistaminics in relation to allergic and nonallergic diseases. Boston med. Q. 3, 1.

LOEWE, S. (1955). Isobols of dose-effect relations in combination of rubethanide (cararine) and phenobarbital. J. Pharmac. exp. Ther. 114, 185.

LONGO, V. G. (1956). Effects of scopalamine and atropine on EEG and behavioural reactions due to hypothalamic stimulation. J. Pharmac. exp. Ther. 116, 198.

LONGO, V. G. (1966). Behavioral and electroencephalographic effects of atropine and related compounds. Pharmac. Rev. 18, 965.

LONGO, V. G. and SILVESTRINI, B. (1958). Contribution á l'étude des sapports entre le potentiel réticuloise evoque, l'état d'anesthésie et l'activité electrique cérébrale. Electroen. Neurophysiol. 10, 111.

LOYING, L., OSHIMA, T. and YOKOTA, T. (1964). The site of action of thiamylal sodium on monosynaptic reflex pathway in cats. J. Neurophysiol. 27, 408.

MAITI, A. and DOMINO, E. F. (1961). Effect of methylated xanthines on the neuronally isolated cerebral cortex. Expl. Neurol. 3, 18.

MARTIN, W. R. (1963). Analgesic and antipyritic drugs. Strong analgesics. In Physiological pharmacology, Vol. 1, ed. ROOT, W. S. and HOFFMAN, F. G. New York: Academic Press.

McLENNAN, H. (1963). Synaptic transmission. Philadelphia: Saunders.

MAYKUT, M. O. and KALOW, W. (1955). Experiments with animals on the combined action of procaine and barbiturates. Canad. Anaes. Soc. J. 2, 109.

MEYER, H. (1899). Zur theorie der alkohomarkose. Arch. exp. Path. Pharmak. 42, 109.

MILLER, S. L. (1961). A theory of gaseous anesthetics. Proc. natn. Acad. Sci. Wash. 47, 1515.

MIYAHARA, J. T., ESPLIN, D. W. and ZABLOCKA, B. (1966). Differential effects of depressant drugs on the pre-synaptic inhibition. J. Pharmac. exp. Ther. 154, 119.

MOORE, J. W., ULBRICHT, W. and TAKATA, M. (1964). Effects of ethanol on the sodium and potassium conductances of the squid axon membrane. J. gen. Physiol. 48, 279.

MORUZZI, G. and MAGOUN, H. W. (1949). Brain stem reticular formation and activation of the EEG. Electroen. Neurophysiol. 1, 455.

MULLINS, L. J. (1954). Some physical mechanisms in narcosis. Chem. Rev. 54, 289.

NGAI, S. H., TSENG, D. T. C. and WAONG, S. C. (1966). Effect of diazepam and other central nervous system depressants on spinal reflexes in cats. A study of site of action. J. Pharmac. exp. Ther. 153, 344.

NARAHASHI, T., MOORE, J. W. and SCOTT, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J. gen. Physiol. 47, 965.

OVERTON, E. (1901). Studien uber die narkose. Jena: Fischer.

OVERTON, E. (1902). Beitrage zur allgemeinen muskel-und nernenphysiologie. Pflug. Arch. ges. Physiol. 92, 346.

OSTERHOUT, W. J. V. (1913). Effect of anesthetics on permeability. Science, 37, 111.

PAULING, L. (1961). A molecular theory of general anesthesia. Science, 134, 15.

PITTINGER, C. B. and KEASLING, H. H. (1959). Theories of narcosis. Anesthesiology, 20, 204.

PHILLIS, J. W., TEBECIS, A. K. and YORK, D. H. (1968). Histamine and some antihistamines: their action on cerebral cortical neurones. Br. J. Pharmac. 33, 426.

PRESTON, J. B. (1955). Pentylenetetrazole and thioscomicarbazide: a study of convulsant activity in the isolated cortex preparation. J. Pharmac. exp. Ther. 115, 28.

PRESTON, J. B. (1956). Effects of chlorpromazine on the central nervous system of the cat: a possible neural basis of action. J. Pharmac. exp. Ther. 118, 100.

QUASTEL, J. H. (1939). Respiration in the central nervous system. Physiol. Rev. 19, 135.

QUASTEL, J. H. and WHEATLEY, A. H. M. (1932). Narcosis and oxidations of the brain. Proc. R. Soc. B. 112, 60.

QUASTEL, J. H. and WHEATLEY, A. H. M. (1933). The effects of amines on oxidations of the brain. Biochem. J. 27, 1609.

QUASTEL, J. H. and WHEATLEY, A. H. M. (1934). Narcotics and brain oxidations. Reversibility of narcotic action *in vitro*. Biochem. J. 28, 1521.

RANDALL, L. O., HEISE, G. A., SCHALLEK, W., BAGDON, R. E., BANZIGER, R. F., BEXIS, A., MOE, R. A. and ABRAMS, W. B. (1961). Pharmacological and clinical studies on valium. A new psychotherapeutic agent of the benzodiazepine class. Current Therap. Res. 3, 405.

REIFFENSTEIN, R. J. (1968). Effects of ion permeability changes on propagation of spreading cortical depression. Proc. int. Union Physiol. Sci. 7, 362.

RILEY, H. and SPINKS, A. J. (1958). Biological assessment of tranquilisers. J. Pharm. Pharmac. 10, 657.

RINALDI, F. and HIMWICH, H. E. (1955). Drugs affecting psychotic behavior and function of mesodiencephalic activating system. Diseases of Nervous System, 16, 133.

RITCHIE, J. M. and GREEGARD, P. (1961). On active structure of local anesthetics. J. Pharmac. exp. Ther. 133, 241.

SANDERS, H. D. (1963). Studies on a proposed common mechanism for the action of general and local anesthetics in the central nervous system. Ph.D. Thesis, University of Manitoba, Winnipeg.

SANDERS, H. D. (1967). A comparison of the convulsant activity of procaine and pentylenetetrazole. Arch. int. Pharmacodyn. 170, 165.

SCHALLEK, W. and ZABRANSKY, F. (1966). Effect of psychotropic drugs on pressor responses to central and peripheral stimulation in cat. Arch. int. Pharmacodyn. 161, 126.

SCHALLEK, W., KUEHN, A. and JEW, N. (1962). Effects of chlordiazepoxide (librium) and other psychotropic agents on the limbic system of the brain. Ann. N.Y. Acad. Sci. 96, 303.

SCHMIDT, R. F. (1964). The pharmacology of presynaptic inhibition. In Progress in Brain Research, ed. ECCLES, J. C. and SCHADE, J. P. pp. 119-134. Amsterdam: Elsvier Publishing Co.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in the excitable cells. Pharmac. Rev. 10, 59.

SHERRINGTON, C. S. (1906). In The integrative action of the nervous system. London: University Press.

SHIDEMAN, F. E. (1949). Effects of central nervous depressants and stimulants on intermediary metabolism of brain. Fedn. Proc. 8, 332.

SHULMAN, A. and LAYCOCK, G. M. (1957). The analeptic activity of bemegride to structurally unrelated hypnotics. Aust. J. exp. Biol. med. Sci. 35, 559.

SLATER, I. H. (1965). Analeptic and convulsive drugs. Strychnine, picrotoxin, pentylenetetrazole, and miscellaneous drugs. In Drill's Pharmacology in Medicine, ed. DiPALOMA, J. R. New York: McGraw-Hill.

SMITH, C. M. (1965). Relaxants of skeletal muscle. In Physiological Pharmacology, Vol. 2, ed. ROOT, W. S. and HOFFMAN, F. G. New York: Academic Press.

SOMJEN, G. G. and GILL, M. (1963). The mechanism of blockade of synaptic transmission in the mammalian spinal cord by diethyl ether and thiopental. J. Pharmac. exp. Ther. 140, 19.

TAYLOR, R. E. (1959). Effect of procaine on electrical properties of squid axon membrane. Am. J. Physiol. 196, 1071.

THESLEFF, S. (1958). The effect of anesthetic agents on skeletal muscle membrane. Acta Anesth. Scand. 2, 69.

TOMAN, J. E. P. and DAVIS, J. P. (1949). Effects of drugs upon electrical activity of the brain. Pharmac. Rev. 1, 425.

TRAUBE, J. (1904). Theorie du Osmose und Narkose. Pflugers Arch. ges. Physiol. 105, 541.

USUI, R. (1912). Uber messung von gewebsokydationen in vitro. (Leber, Zentralnervensystem). Pflugers Arch. ges. Physiol. 147, 100.

VAN HARREVELD, A. (1960). Physiological effects of gluatmate and some related compounds. In Inhibition in the Nervous System and Gamma aminobutyric acid, ed. ROBERTS, E. p.454. New York: Pergamon Press.

VERWORM, M. (1909). Ueber narkose. Deutsch med. Woschen. 35, 1593.

VIRTUE, R. W. and JONES, B. E. (1956). Influence of chlorpromazine on survival time of shocked rats. Anesthesiology, 17, 601.

WHITE, R. P., SEWELL, H. H. and RUDOLPH, A. S. (1965). Drug induced dissociation between evoked reticular potentials and EEG. Electroen. Neurophysiol. 19, 16.

WIKLER, A. (1950). Sites and mechanism of action of morphine and related drugs in the central nervous system. Pharmac. Rev. 2, 435.

WINTERS, W. D. and SPOONER, C. E. (1965). A neurophysiological comparison of gammahydroxybutyrate with pentobarbital in cats. Electroen. Neurophysiol. 18, 287.

WINTERSTEIN, H. (1915). Beitrage zur kenntnis der narkose III narkose und erstickung. Biochem. Z. 70, 130.

WERNER, H. W. (1939). Certain effects of benzedrine, caramine, metrazol, and picrotoxin in alcohol depression. J. Pharmac. exp. Ther. 66, 39.

YAMAGUCHI, T. (1961). Electrophysiological studies on the mechanism of anesthetics on isolated frog muscle fibres. J. Fac. Sci. Hokkaido U. Ser. IV Zool. 14, 552.

ZBINDEN, G. and RANDALL, L. O. (1967). Pharmacology of benzodiazepines. In Advances in Pharmacology, Vol. 5, ed. GARTINI New York: Academic Press.