1970

The Effects of Various Pharmacological Agents on the Electroconvulsive Seizure Pattern in Mice

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THE EFFECTS OF VARIOUS PHARMACOLOGICAL AGENTS ON THE ELECTROCONVULSIVE SEIZURE PATTERN IN MICE

BY

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A Thesis Submitted to the
Faculty of the Graduate School of
Loyola University in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

November, 1970

BIOGRAPHY

William Charles Hanigan was born in Chicago, Illinois on April 28, 1945. He graduated from St. Leo College Preparatory School at St. Leo, Florida in 1963 and received a Bachelor of Science degree, cum laude, from the University of Notre Dame in 1967.

In September of 1967, he entered the Loyola University, Stritch School of Medicine and in the same year enrolled as a graduate student in the Department of Pharmacology at the same institution.

Mr. Hanigan was married to the former Donna Gibson Holland in September of 1970.

ACKNOWLEDGEMENT

I would like to express my most sincere gratitude to Dr. C. L. Scudder and Donna, both of whom have influenced my thinking more than they realize and I care to admit.

"There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact."

Mark Twain

Table of Contents

Introduction

A. General Introduction

Experimental electroconvulsive seizure (ECS) has been used in the past twenty years for the analysis of the effects of drugs on brain excitability and function. Unfortunately, after initial work was performed in the early 1950's, the use of ECS was narrowed to the more restricted field of anti-epileptic pharmacology and its potential usefulness for the pharmacological analysis particularly of neurotransmitters of the central nervous system was disregarded. This thesis is intended to illustrate the value of ECS in analyzing centrally-acting drugs of all types.

This study touches upon two major categories of problems. The first involves the theories of mechanisms of ECS and the second the effects of drugs on the various parameters of ECS. In reference to the former problem, the literature and speculation is voluminous; there are as many theories of ECS as there are workers in the field. To paraphrase all of these would be beyond the scope of this thesis. Instead, a novel physiological mechanism will be proposed and enlarged upon as a foundation for a pharmacological analysis whereby certain parameters of ECS are defined and correlated with this physiological mechanism and with the effects of drugs on the parameters.

The physiological mechanism presented here is based on the hypothesis that the electroconvulsive shock is a maximal stimulus in the sense that most cells of the brain are affected and that the organism through its homeostatic construction maintains stability of certain variables within physiological limits. The centrally-acting drugs modify the various sub-systems in the central nervous system so that this maintenance of stability is affected to a greater or lesser degree. The two variables, latency and duration, which this study utilizes as measurements of this maintenance of stability are perhaps limited as to the type of information, which they yield, but they

are practical in that they are convenient to test and quantitate accurately.

B. The Unstable Neuron

1. Biochemistry

There has been evidence presented in the literature of the relationship between certain biochemical parameters and neuronal excitability. This section will deal with several of these parameters previously shown to result in an unstable neuron.

The administration of ammonium salts has been shown to induce convulsions in animals; similarly, during preconvulsive and convulsive phases of maximal seizures the ammonia concentration in brain tissue increases rapidly (Richter and Dawson, 1948; Benitez et al., 1954). However, Takahashi et al. (1961) demonstrated that rat brain ammonia level and cerebral excitability are not correlated but that ammonia increases are a result of the excitation of the neurons. Ammonia might be metabolized into an abnormal "convulsant", guanidinobutyric acid, as suggested by Waelsch (cited by Tower, 1960). There is known to be an accumulation of ammonium ions after the seizure (Torda, 1953) which Kreindler (1965) suggests may lead to the arrest of the convulsive episode. In any case, although ammonia is associated with cerebral excitability, its specific role has yet to be precisely defined.

The presence in the brain of gamma-aminobutyric acid (GABA) was initially demonstrated by Roberts and Franckel (1950). Bazemore et al. (1956) and Lovell et al. (1963) showed that the inhibition of seizure activity is closely related to the brain GABA level. Convulsant drugs have been shown to decrease to GABA levels which anti-convulsant drugs increase these levels (Killiam and Bain, 1957; Killiam, 1958; Lovell, 1963). Killiam (1957) demonstrated that the effect of hydrazines is due particularly to the blocking of glutamic acid decarboxylase which results in the increase of

GABA concentration. Similarly, it has been shown by Roberts et al. (1958) that the relative activities of glutamic acid decarboxylase and GABA transaminase are decreased with acidification; this, in turn, increases the concentration of GABA and the neuronal excitability decreases. The ancient treatment for epilepsy consisting of a ketogenic diet is assumed to have operated on this mechanism. Worum and Porszasy (1968) found that age is correlated with a decrease in cerebral excitability and an increase in GABA concentration in the central nervous system.

Closely associated with ammonia-GABA cycles and neuronal excitability is the finding by Gershenovisch et al. (1963) that both glutamine and glutamine synthesis increases during the preconvulsive stage of seizures induced by oxygen poisoning. Mison-Crighel et al. (1964) found a decrease in glutamic acid and glutamine and an increase in glutaminase within two minutes after a topical application of Mescaline. Vrba (1955) demonstrated that cerebral activity stimulated by prolonged muscle movements is associated with a fall in amino nitrogen of the brain proteins and concomitant increase in free glutamic acid and glutamine. He believes that the functional activity of the brain depends on a certain level of the glutamic-acid-glutamine cycle.

The level of oxygen has been shown to play an important part in the excitatory process of seizure activity. It has been shown that oxygen consumption increases during an epileptic seizure while a lack of oxygen induces the arrest of electrical activity in the cortex (Jasper and Erickson, 1941; Gibbs et al., 1947). In generalized convulsions induced by electrical stimulation, Lascar and Pintilie (1955) demonstrated that an inhibition of cellular oxidation takes place due to a decrease in cytochrome oxidase activity and an increase in succinic dehydrogenase activity in the rabbit motor cortex. The nerve tissue presumably consumes a larger amount of oxygen

after the seizure to compensate for and utilize the excess lactic acid and to restore the bound phosphorus high-energy compounds which have been shown to decrease during seizure (Gurdjan et al., 1947). Several other findings correlate somewhat with these results. Klein and Olsen (1947) found that in generalized convulsions induced by electrical stimulation the cerebral glucose, glycogen and cerebral glucose: blood glucose ratio were found to decrease. Elliott (1955) found that the increase in cerebral circulation seen during seizure activity was a result of vasodilitation from a build-up of lactic acid and a resultant acidification.

Changes in concentration of various ions have been associated with cerebral excitation. Mison-Crighel et al. (1955) demonstrated that magnesium levels increase considerably in the renal vein after a convulsive seizure. This may point to an excessive release of magnesium during a seizure. Since the synthesis of glutamine from glutamic acid and ammonia occurs in the presence of magnesium this may explain the increase in glutamic acid (Vrba, 1955) and decrease in glutamine (Mison-Crighel et al., 1964) seen after seizure activity. Gordon and Waelsch (1955) believe that in the passage of a nerve impulse across the membrane, calcium is liberated and that its restoration is necessary for a stable condition to return. If magnesium were to replace calcium at the membrane level, prolonged firing and seizure activity would result. On the other hand, Flink (1956) has shown that with a magnesium deficiency, generalized convulsions can occur and clinically, in epileptics, serum magnesium levels are low.

In conjunction with ionic changes, the theory of a change in membrane permeability has been brought forth in numerous papers. The permeability of the neuron would determine its excitability and stability. Woodbury et al. (1958b) described an increase in intracellular sodium which appears to precede the convulsive activity in convulsions induced by CO_2 inhalation. He believes the change in cation distribution causes the membrane excitability to

increase (partial depolarization) bringing about repeated discharges. An increase in intracellular sodium causes a decrease in the membrane responses to minimal stimuli. Colfer and Essex (1947) have found that during ECS, intracellular potassium decreases.

In summary it may be stated that evidence has been presented that ammonia levels, glutamine-glutamic acid-GABA cycles, metabolic byproducts, oxygen levels correlated with high-energy bound compounds and concentration of various ions all play a part in the activity of an excitable neuron, most probably through membrane permeability changes. The exact mechanism awaits explanation.

2. Electrophysiology

The cortical neuron according to Kreindler (1965) consists of three separate yet highly interrelated areas. The axon consists of a membrane responding to the all-or-none principle; it is electrically excitable and plays the part of a message transmitter. The perikaryon (cell body) membrane also responds to the all-or-none principle and it too, is electrically excitable. The dendritic membrane, however, is gradually responsive, electrically unexcitable and responds to chemical transmitter substances released by button-like formations covering the greater part of the dendritic surface. The dendritic membrane generates "standing post-synaptic potentials" which, according to Purpura (1959), may influence through electrotonic spread, the electrically excitable membrane of the perikaryon. These "standing post-synaptic potentials" evoked by the dendritic membrane do not spread except through electrotonic propagation. Bishop (1956) demonstrated the absolute refractory period of the perikaryon and axon, but the dendritic membrane was described as decremental and had no absolute refractory period.

Schmidt et al. (1959) in describing chronic epileptogenic foci in monkeys using extracellular electrodes postulated that the autonomous activity

of the epileptic neuron is due to a relatively prolonged dendritic depolarization with a resultant difference in potential between dendrites and cell body. The cell body's membrane would recover rapidly and acting as a current source produce a current flow to the depolarized dendrite acting as a sink. If the current flow reaches a threshold, a high frequency discharge occurs. During the earlier phase of a maximal tonic-clonic seizure, the frequency of a neuronal discharge is approximately 100 per second but may go as high as 800 per second (Morruzzi, 1950; 1953). As the dendritic tree is progressively involved, increasingly synchronized slow wave activity replaces the rapid neuronal discharge. In the later phases of a maximal tonic-clonic seizure, maximum "invasion" of the dendritic tree has taken place.

Brazier (1955) established a close relationship between dendritic depolarization and epileptic discharge holding that epileptic spikes may be generated by apical dendrites of the cerebral cortex. Other authors (Beritashvili et al., 1958; Rosenblueth et al., 1942; Morrell and Torres, 1958) either directly or indirectly point to the "superficial" i.e. the apical dendritic layer of the cortex as the main initiating point in the production of seizures. Rayport (1960) taking microelectrode records from the somatosensorial area I in the cat after repetitive stimulation found four different types of cortical units: (1) units that respond with a short latency; (2) units that fire together synchronously with the appearance of spindles from the electroencephalogram; (3) units that respond to isolated shocks applied to the surface of the cortes; and (4) units whose activity is not correlated with any of the above listed activities.

Moruzzi (1950, 1953) believes that the unstable neuron has abnormally great potentials in the cell body and dendrites and a high frequency potential spreading along the axon up to 1000 spikes per second. However there is a disequilibrium in the unstable neuron which amplifies the excitatory post-synaptic potential and inhibits the inhibitory post-synaptic

potential. Eccles (1957, 1959) points out that metrazoi-induced convulsions are examples of the former while strychnine-induced convulsions are examples of the latter.

Sawa et al. (1963) using extracellular electrodes, demonstrated that with repetitive electrical stimulus at high intensity and frequency, a progressive decrease of the hyperpolarizing wave and a temporal summation of the depolarizing wave occurred. After stimulation ceased, a state of sustained depolarization was maintained and large and long-lasting depolarization waves occurred periodically with concurrent waves in the corticogram. During the final phases of the seizure the falling phase of the depolarizing wave became more marked and finally the depolarization was reversed and hyperpolarization ensued. A long-lasting hyperpolarizing wave was observed ans subsequently a period of silence in the electrocorticogram. The author postulates that the decrease of the hyperpolarizing wave with the increase of the depolarizing wave results from a gradual loss of inhibitory substance while the excitatory transmitter remains active. He also suggests that an observed latency period prior to the polarizing changes may be due to an initial prolonged depolarization or to a stage of asynchronous activation.

Thus, there seems to be two lines of explanation for the self-sustained spontaneous activity of the unstable neuron. The first, postulated initially by Eccles (1951) and later by Bodian (1967) regards synaptic activity as the core of the excitability acting through excitatory or inhibitory transmitters or neurons. The second brought forth by Bishop (1958) regards the dendritic electrical activity as the major focus for the neuronal discharge in an unstable state. However, it is well to remember that any activity recorded by the electroencephalogram or corticogram represents the composite, more-or-less additive activity of myriads of elements in the central nervous system

during convulsive seizure. Any individual neuron may react differently from the responses of the neuronal aggregate. Several writers have gone so far as to suggest that self-sustaining activity exists only in the neuronal aggregate. This will be discussed later.

C. The Neuronal-Glial Interaction

1. Introduction

Within the past few years increasing attention was paid to the role of neuroglia in nerve activity. Once the hypothesis of glial interaction with nerve tissue is accepted as tenable, it appears that there is a possibility of an intrinsic relationship between the modulation of a maximal stimulus (e.g. ECS) by the glia and neuronal stability. This section of the thesis will be presented, not as a foundation for a theory of ECS mechanics, but as an effort to provide a meaningful over-all view of convulsive activity.

2. Anatomy

Earlier studies (Cajal, 1909; del Rio-Hortega, 1932; Penfield, 1932; and others) considered glia as the interstitial supportive tissue composed of a tangled mass of non-nerve tissue and fibers with numerous intracellular spaces, capillaries and homogenous intercellular substance. Some of these early authors feel that the term neuroglia connotes a "neural glue" that separates and holds together the neuron population. However, Palay (1967) admits that the cellular units of the nervous system, at whatever level considered, are organized by means of their connections and their intrinsic properties for integrative action.

Bodian (1967) divides the neuroglia into three major categories: astrocytes, oligodendrocytes and microglia. The microglia occur irregularly in the central nervous system and as yet have not been linked with any basic functional aspect of the neurons. They are found in both grey and white matter, have no foot plates, but are often closely opposed to neuron bodies as perineural satellites or to the walls of blood vessels as perivascular

satellites. In trauma or destructive lesions of the nervous system they are transformed into large, actively phagocytic scavenger cells which exhibit ameboid movement. They are, for the most part, considered part of the reticulo-endothelial system (Truex and Carpenter, 1964).

The astrocytes, present in both white and grey matter, have foot plates which are anchored to the outer walls of the blood vessels lying within the central nervous system. They form a continuous glial membrane around the blood vessels and the exterior surface of the brain. They play a major role in preserving the integrity of the nervous system. DeRobertis (1965) mentions that astrocytes are thought to be involved in the transport of water, electrolytes and metabolites in the brain and are the site of an active homeostatic mechanism that regulates in the brain the content of water and a pool of electrolytes containing a high sodium concentration.

The oligodendrocytes lack cytoplasmic foot plates, are present in both grey and white matter, but predominently in the latter, and contain numerous cytoplasmic bundles. They are intimately related to the axon and generally are believed to play the same role in the case of the central nervous system as the Schwann cell in peripheral nerves. They also have a characteristic slow pulsatile activity as in the Schwann cell (Pomerat, 1952). Hyden and Pigon (1960) and Hamberger and Hyden (1963) have shown that oligodendrocytes have a metabolic interaction with neurons and are linked in an active system with regards to the control of the neuronal membrane permeability.

The membrane connections of the neuroglia are under much study. Schmitt (1967) states that neuroglia are, in general, separated from neurons by a space of 100 to 200 Å. They never intervene between presynaptic and postsynaptic components of a synapse, but in certain regions may circumscribe a synaptic zone insulating the functional synaptic junction (Bodian, 1967). Work by Gonzalez and DeRobertis (1963) have demonstrated that the plasma membrane of the glial processes adhere to the membrane of the

synaptic ending. This sealing, either partial or total, of the extra-cellular space around the synaptic complexes, may physiologically act as a kind of a synaptic glial barrier either slowing down or preventing diffusion of transmitters. Palay (1967) ascribes synaptic inhibition to a gross alteration in the ionic environment surrounding the postsynaptic membrane rather than to a precise action of an inhibitory transmitter at a specific receptor of the membrane. In synapses in which there is a prolonged or residual action after a presynaptic stimulation, there has been physiological evidence for a prevention of diffusion of transmitters (Curtis and Eccles, 1959).

3. Biochemistry and Metabolism of Glial Tissue

It has been fairly well established that the glia do have intrinsically different metabolic properties from the neuron. The glial dry material has a lipid-to-protein ratio of around eighty-to-twenty whereas corresponding values for large neurons is twenty-to-eighty (Hyden, 1960). The enzyme systems for the hydrolysis of adenosine triphosphate differs between the two types of cells (Whittman 1969; Cummins and Hyden 1962). On the inner side of the neuronal membrane adenosine triphosphate activity activated by sodium and potassium with a maximal activity at a pH of 7.40 is carried out. The adenosine triphosphate activity of the glia is activated by potassium alone and exhibits a maximal activity at a pH of 8.00. Hyden and Lange (1966), Giacobini (1964), Friede (1965) and Lowry (1957) have demonstrated that oxygen consumption is five to ten times higher in neurons than in glia, while the capacity for anaerobic glycolysis is higher in glia which shows a predominence of glucose shunt mechanisms.

Giacobini (1961, 1962) has shown that the carbonic anhydrase activity is one hundred times higher in glia than in neurons; he believes that carbon dioxide built up in the neurons is released and converted to carbonic acid in the presence of this enzyme. Svaetichin et al. (1965) in their work on retinal cells in fish found a reciprocal electrical excitation-inhibition between

the glial and neuronal elements. The amplitude of the neuronal potential was inversely related to the height of the glial membrane potential. The membranes of the non-neural cells were electrically inexcitable and membrane activity depended on excitation spread from cell to cell. The authors reason that the position of carbon dioxide as the final metabolic product and regulation parameter of the respiratory rate makes it a critical parameter in this neuronal-glial interaction. Since carbon dioxide accelerates the Kreb's cycle as well as the respiratory rate due to a production of hydrogen ions in the presence of carbonic anhydrase, the glial cell membrane potential would be decreased, i.e. its membrane would be hyperpolarized as the neuron membrane potential would be increased, i.e. its membrane would be depolarized. The authors further suggest that the double membrane formed by the opposed glial and neuronal cells could be compared to a mitochondrial membrane. Through energy shifts along the electrically inexcitable membranes, involving the respiratory chain, the non-neuronal potential would affect the neuronal one. Svaetichin and his co-workers consider this mechanism to be an important one in controlling the excitability in the grey matter and basal ganglia where electrical insulation provided by the oligodendrocytes is largely absent.

There is further evidence for an energetic coupling between the neuron and its glia. Hyden (1967) demonstrated that the amount of RNA, protein and respiratory activities increase in the neurons as a function of stimulation. In the glia, the changes of amount of RNA and respiratory activity were inversely related to stimulation. However, the anaerobic glycolytic activity measured by production of carbon dioxide per nerve or glial sample, decreased in the neurons and increased in the glia after stimulation. Hamberger (1963), using a micrometric technique, found similar inversely related enzyme changes in neurons and glia after stimulation. Pevzner (1965) using electrical stimulation found that the content of RNA increased in the

neuron and decreased in the glia. Interestingly, Hamberger (1963) found that the capillary glial tissue did not decrease their enzyme activities as a response to stimulation.

Hyden (1967) postulates that the glia are metabolic stabilizers of the neurons. The rate of biosynthesis of the functioning unit can alternate between two states. Furthermore, Landauer (1964), Roberts (1964) and Schmitt (1964) have speculated on the phenomena of RNA transfer from glia to neurons especially with the production of "memory" responses. There has been reported a non-ribosomal bound RNA in the axoplasm of the Mauthner nerve cell. This RNA has the same base composition as the RNA in the myelin sheath and Schwann cell, but differs from that of the nerve cell body (Edstrom et al., 1962).

Altman (1967) has indicated that the packing density of the glia may show a metabolic relationship to the neurons. Brizzee and Jacobs (1959) and Brizzee et al. (1964) have found that the packing density of glia in rats and cats increases after birth while that of the neurons decreases. Friede (1954) reported that the glia/neuron ratio increases as one goe sup the phylogenetic scale reaching a maximum in man. He postulates this ratio as a possible evolutionary index of brain development.

4. Electrophysiology of the Neuronal-Glial Interaction

As stated previously, glia have been demonstrated to have a spontaneous pulsatory movement. Pomerat (1952), Canti et al. (1935), and Tasaki (1965) demonstrated that glia, in vitro, respond to a strong electrical stimulus with a slow contraction lasting one to three minutes in duration followed by a phase of relaxation lasting up to ten minutes. Tasaki (1965) using the method of Chang and Hild (1959) for electrical stimulation of single glia in vitro found that the total impedance of the brain slice increases as the phase of relaxation occurs. This author further states:

"A strong electrical shock applied to a

> glial cell with a large extracellular electrode is known to bring about a reversible, transient reduction in the resting potential of the cell. The degree of reduction depends upon the intensity of the applied shock. There is evidence indicating that this reduction in the resting potential is associated with a simultaneous reduction in the membrane impedance. The intensity of current needed to induce such a potential variation seems too large to link the phenomena with physiological events in living brain tissue."

It is safe to assume that with a stimulus large enough to induce a convulsive seizure, these mechanisms might come into play.

Adey et al. (1965) suggest that the glia play a role as impedance modulators to neurophysiological processes. They reason that, by subtle changes in shape, the glia are able to modify the impedance load on neurons. The glia performs this function by modifying the width and therefore the conductance of the intra-cellular conducting channel. Schmitt (1967) mentions that this intra-cellular channel of approximately 100-200 Å in width is the usual channel for the flow of sodium and potassium ions and currents. Spreading currents such as electro-tonic or self-regenerating ones are thought not to influence glial processes.

Li (1959) believes that the glia play a part in the synchronization mechanism of a convulsive seizure. He states that both the electrical phenomena and the mechanical contraction of the glia could influence the neuronal electrical activity to produce synchronization. In this regard, Ward (1961) has shown that dendrites show an unusual responsiveness to mechanical contraction of the glia.

On the other hand, glia may alter events through a functional action on the synapse. Koelle (1962) has demonstrated the presence of a non-specific cholinesterase in glia that surround the synapses. Through a mechanism of positive feedback he postulates that the secretion of large amounts of acetyl-

choline counteract the activity of this cholinesterase thereby modifying the electrical activity at the synapse. Moreover, it has been shown that synaptic vessels which may release acetylcholine are present in the Schwann cells of normal and denervated peripheral nerves (Birks et al., 1959; 1960). This would explain the mini-end plate potentials seen in skeletal muscle several hours after denervation (Thesleff, 1960).

D. Functional Patterning and Electroconvulsive Seizure

1. Introduction

It is impossible to review completely the mechanisms that have been postulated to explain experimental ECS. The preceding two sections in this thesis survey some of the factors which are fundamental to model-building and theorizing. These factors direct consideration of the ECS to the smallest possible physiological unit as a frame of reference, and the pertinent studies were interpreted in regards to isolated neurons and glia. However, the central nervous system consists of neuronal aggregates and in this case, convulsive behavior may be a property of the whole aggregation and not the sum of the properties of its parts. Karl Jaspers (1963) states: "Even the simplest phenomena (of behavior) are so complex . . . that in order to bring them about the whole brain is necessary."

The justification for the following theory of the ECS is based, to a certain degree, on a linguistic analysis of our conceptual difficulties in regard to seizure as behavior. There is a tendency to segment the whole behavior and correlated brain structures, not because the two are divided in any visible way, but because both our current techniques and language of the "scientific method" necessitates this approach. As a result, the overview is lost and the other possible patterns of the phenomena under study are not perceived. The "patterning" which is perceived is based on certain assumptions which may or may not be valid according to the level of organization with regard to which the "patterning" is perceived. For example, the data

may be incorporated into a mechanism which presumes certain rhythmic centers of the central nervous system which impose a pattern upon the maximal firing of neurons in any specific pattern. Another hypothesis might suggest that the organism is striving for stability when it receives an overpowering electrical stimulus, and the course of the seizure is a result of this regulation. This thesis employs this the latter "pattern" as the basis for the interpretation of the data presented.

The observations of this study are comprised of two principal events: a specific electrical stimulus of known intensity and duration and a specific series of muscular contractions. The following are the assumptions: (1) the stimulus, in some way, affects the motor components of the brain. (2) These motor components are effected through a final common neuronal pathway which when activated is invariable. (3) Whatever is variable in the muscular movement, changes as a result of activity within the dynamic framework of the central nervous system. (4) The basic mechanism explains observed changes by postulating spatial or temporal changes in circuits. (5) The electroencephalogram mirrors the activity of neurons involved in the ECS. These five assumptions and the basic "pattern" provide a meaningful frame of reference in which to study the phenomena of ECS.

2. Description of ECS

Woodbury and Davenport (1952) described various motor components in rats following ECS. When a mild shock was delivered, far below in current required for generalized convulsions, a brief tonic spasm occurred after which the animal quickly resumed its normal behavior. At a current of 1.0 to 1.5 miliamperes below seizure threshold, a response, labeled "furor" occurred which consisted of violent running or leaping with shrill squeaking. At a current of 0.5 miliamperes below seizure threshold the animal underwent a brief tonic spasm followed by a near catatonic state. At a current of 1.0 miliamperes above seizure threshold the animal exhibited clonic movements

of the head and forelimbs. And finally, at currents above 1.0 milliamperes above seizure threshold the animal exhibits maximal seizures consisting of: 1) hindleg flexion in tonus, 2) hindleg extension in tonus, 3) intermittent wholebody clonus and 4) muscular relaxation and post-ictal depression. For these authors, tonic extension of the hindlimbs signify that a maximal seizure is occurring. Generally, this maximal seizure movement pattern is not influenced by factors that increase or decrease the threshold.

Toman et al. (1946) described five motor components of maximal ECS in rabbits. The first they described as "latency" or a period of muscular inactivation. The second was described as an extreme flexion component of the tonus while the third was described as an extreme extension component of the tonic phase. The fourth was described as the clonic phase with one or more extensor thrusts and finally, the fifth was described as a post-seizure depression of muscular activity. The authors feel that tonic seizures are evidence of maximal seizure due to the inability of various factors to change the duration or severity of tonic seizures. The brain would appear to be maximally active during a tonic seizure and once the latter is initiated the seizure would be independent of the stimulus.

It is generally agreed that tonic-clonic convulsions indeed indicate maximal seizure activity (Rosenblueth and Cannon, 1942; Toman et al. 1946; and others). For purposes of this paper Toman's description of the seizure activity is adequate. It consists of four essential phases: a latency phase, a tonic phase consisting of first hindleg flexion, then extension, a clonic phase and lastly a post-ictal depressive phase. The latency phase was defined as that period of time from the initial shock to the hindleg extension component of the tonic phase (Toman et al., 1957). Regretably, this period which is defined as "latency" includes the flexion component of the tonic phase. However the definition is a convenient one and permits accuracy in observation. The period of time between the initial shock and the beginning

of the clonic phase was defined as the duration of the seizure (for further explanation see the Methods and Materials section).

3. The Pattern of Latency

Toman et al. (1946, 1948); Penfield and Jasper (1954); Bonnet and Bremer (1956); Gerin (1960); and Rosenblueth and Cannon (1942) have observed that following a maximal shock there is a brief period of what appears as a reduction of both electrical and motor activity as seen by the EEG and clinical observation. This inactivation represents inhibitory mechanisms acting in a temporal sequence, i.e. before the excitatory mechanisms, inactivating neuronal firing (Jeans and Toman, 1956). These authors have shown that this latency reaches a minimum which is inversely related to the shock intensity.

A phenomenum described by Leao and Morrison (1945) may be associated with latency data. This "spreading depression" was found in the EEG of rabbits, under dial anesthesia, following subthreshold moderate tetanic stimulation. The authors postulated a neuronal mechanism acted on by changes in the blood-brain barrier brought on by the electrical stimulation. Subcortical structures were not thought to play a part in this "spreading depression". However, the phenomenum was of the order of thirty seconds to four minutes; thus, it was not reducible to the latency of approximately two seconds described by Toman (1946).

McCulloch (1944) and Bailey et al. (1940) describe a suppressive area of the cortex which actively suppressed both the electrical and motor activity when stimulated. This "suppression" acted with a slow time course through cortico-subcortical circuits, by unknown pathways, to block thalamo-cortical circuits. Dusser de Barenne et al. (1942) and Sloan and Jasper (1950) subsequently proved that this suppression was actually a spreading depression elicited by electrotonic or chemical influences along cortico-cortical circuits.

Propagation of the seizure discharge may be explained by several mechanisms. For Green and Naquet (1967) propagation represents extra-synaptic irradiation from cell-to-cell following dendritic depolarization. French et al. (1956) suggests that it may be ephaptic and interdendritic. Kreindler (1965) compared propagation to an oil spot that comprises larger and larger groups of neighboring neurons by certain preferential circuitry. While one or several of these mechanisms may be active it is the contention of this thesis that the question of pathways involved and synaptic or extra-synaptic transmission of the seizure activity are not significant in this early period following maximal stimulation. The reasoning is thus: (1) we deal here with a maximal stimulus of high frequency and duration. It is logical to assume that all neurons capable of firing will fire; (2) the current will travel quickly throughout the brain as indicated by the fact that Russell (1949) and Spiegel (1942) have shown that as the intensity of the stimulus increases, the impedance of the brain tissue decreases. The time period fo· this current to pass through is in the order of 500 miliseconds or less. (3) Pathways are not significant in this early stage because of the total depolarization. This depolarization should result in an essentially un-patterned, random neuronal firing.

On an individual neuron level Sawa et al. (1963) did studies on the motor cortex, hippocampus and pyramidal tract areas during maximal electrical stimulus. It was demonstrated that there was a progressive decrease in hyperpolarizing waves and a gradual increase in depolarizing waves with a temporal summation. Following this phase, there was a sustained depolarization with no record of activity in the corticogram. Bullock (1967) states that one type of neuronal inhibition may be due to sustained depolarization. Sawa et al. (1963) further suggests that this sustained depolarization could be due to a blocking of inhibitory transmitters and a prolongation of the activity of the excitatory transmitters. In any case, this phenomena may

constitute a sufficient explanation for Toman's (1946) phenomena of latency.

However, the EEG notoriously records only the superficial level of neuronal activity. And, as stated before, the neuronal aggregate may act in a different manner from the individual neuron. The electrical stimulus applied to the whole brain is a maximal one and fires all neurons sometimes at rates approximating 1000 per second (Moruzzi, 1939). Moreover, it is certainly possible that ephaptic transmission as well as synaptic transmission occurs, as suggested by Bullock (1967), and that maximal stimulus alters glial-neuronal relationships, thereby altering membrane excitability. Therefore, all neurons fire during the latency in an essentially random manner; by so firing they may destroy whatever functional order that existed previous to the stimulus. Both inhibitory and excitatory synapses are active and consequently their cumulative effects tend to cancel one another in terms of their influences on circuitry patterns. Interneuronal patterns, with this acute stimulus, become unclear and disorganized. The EEG reflects the summed effects of inhibitory and excitatory influences. The motor activity reflects massive neuronal firing in a random manner with both peripheral flexors and extensors activated and consequently, an increase in muscle tone without an exhibition of predominently flexion or extension.

If several EEG tracings following maximal stimulus are observed, an initial period of absent spike activity lasting in each record for approximately one second to one second-and-a-half may be seen (See outlined patterns in Figures 1 and 2). Penfield and Jasper (1954) label this first period as a period of asynchronous activation. Rosenblueth and Cannon (1942) call it a latency phase.

Before further explanation is attempted it would be wise to suggest that there are certain correlations between EEG tracings and motor activity. Rosenblueth and Cannon (1942), Adrian and Moruzzi (1939), and Bremer (1938) report that potential waves in the cortex and discharge waves in the pyramidal

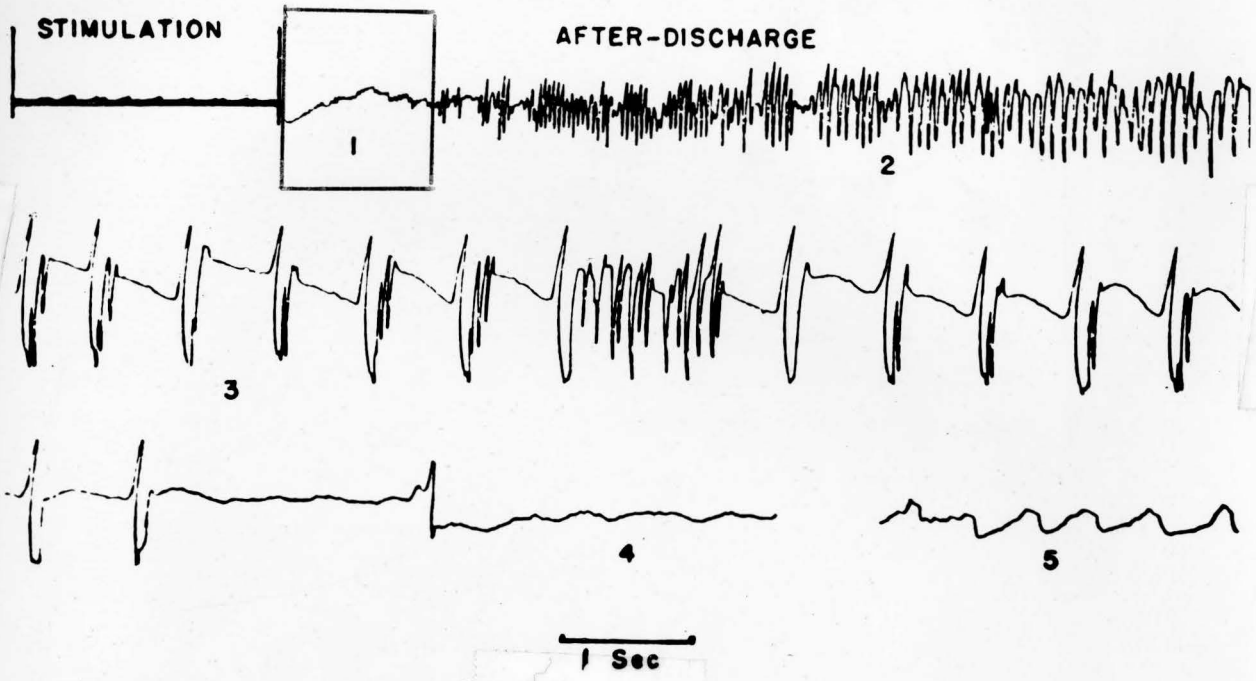

Figure 1. Electrocorticogram showing different stages of electrical after-discharge (From Penfield and Jasper, 1954, p. 201, cited by Kreindler, 1965, p. 3).

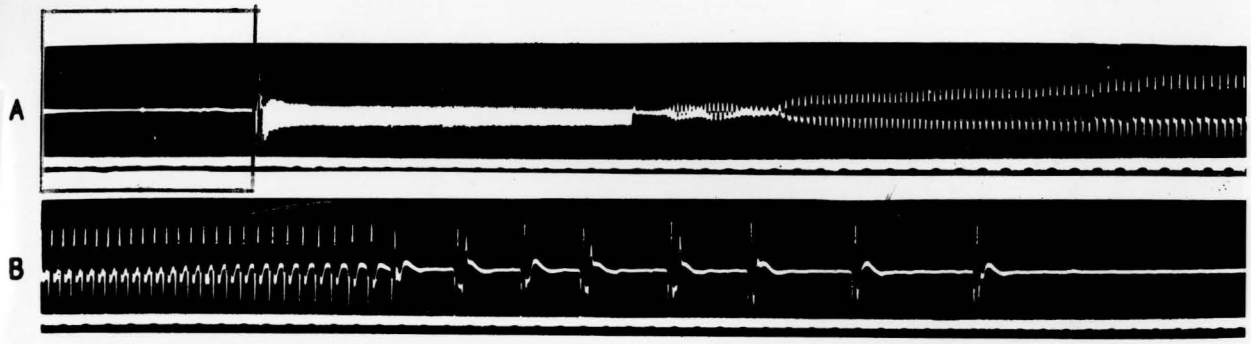

Figure 2. Oscillographic tracing of the cortical surface electrogram of area 4 following stimulation of this area. Stimulus was induced at point A. The time interval between A and B is one second (From Rosenblueth and Cannon, 1942 p. 700).

tract are closely related but not inseparable. Unfortunately, the latency phase has the poorest temporal correlation with the EEG and until the matter is investigated further. It may be stated only that there is an initial period after maximal ECS of approximately one to two seconds during which there is a flat EEG and an increase in muscle tone with no evidence of predominently flexor or extensor contraction.

4. The Pattern of Tonus

In decerebrate animals, rigidity of posture has been demonstrated by many investigators. Bazett and Penfield (1922) reported that with chronic decerebrate animals, flexor rigidity resulted while Ranson and Hinsey (1929) described the tensor rigidity resulting after decerebration.

As early as 1913, T. G. Brown noticed that stimulation of the reticular formation in decerebrate animals caused a decrease in rigidity. Ingram et al. (1932) demonstrated this in normal animals. It is well known however that the major portion of the reticular formation, when stimulated, facilitates or augments reflexes and cortically-induced movements (Truex and Carpenter, 1964).

According to Truex and Carpenter (1964) there are three general regions of the reticular formation. The first, a median region, contains the nuclei of the raphe and the paramedian nuclei. This region is apparently unimportant in generalized seizure activity. The second, a medial region, consisting of approximately the medial two-thirds of the total reticular substance, is regarded as the effector area. The third, a small lateral region, is reffered to as the sensory area because of the large amounts of collateral fibers projected to it from sensory pathways. The reticular formation can be further divided into rostral and caudal divisions, the rostral extending from the upper medulla to the hypothalamus and subthalamus, while the caudal extends through the medulla. The former, as stated previously, appears to be a facilitatory area while the latter is concerned with inhibitory functions.

Ward (1947) postulates that decerebrate lesions cause rigidity by interrupting all afferent impulses to the bulbar suppressor region; this deafferentation induces a paralysis of that region. The facilitatory region (in the lateral pontile tegmentum) continues to receive afferents from the cord and its influences continue to inbalance the behavioral manifestations.

Bergmann et al. (1963) induced generalized tonic-clonic convulsions accompanied by abolition of pupillary and corneal reflexes by stimulation of the mesencephalic reticular formation. The EEG showed cortical desynchronization. Gastaut (1958a) suggested that gross telencephalic discharges, induced by Metrazol, might liberate the tonic discharge of the bulbar reticular formation resulting in tonus. Kreindler et al. (1958) showed that in rats a tonic-clonic convulsion was induced by stimulation of the brain stem. The marked tonic component in the peripheral musculature constituted by the flexor system. These authors postulate that in the reticular substance of the brain stem there are neuronal structures which permit the development of a convulsive attack. The stimulus can give rise to neuronal reverberation and potentiation which preserve and intensify the excitation for a certain length of time. Decerebration, they point out, neither prevents the seizure nor alters its neuronal counterpart. Smith and Purpura (1960) found that a high frequency stimulation of the reticular formation inhibited low frequency afterdischarges of epileptogenic lesions which, in turn, facilitated high frequency afterdischarge. Gastaut and Fischer-Williams (1959) postulated that in grand mal seizures functional elimination of the thalamo-cortical system allows for loss of consciousness and liberation of the reticular system to product tonus. Gastaut (1958b) hypothesized that the tonic neuromuscular seizure depends on the caudal reticular formation while loss of consciousness would depend on the rostral reticular formation, the thalamus and the cortex. Thus, throughout the literature, the reticular formation has been heavily implicated in the causation or functional relation with the seizure discharge.

If the data point to the role of the reticular formation exerting control over tonic seizure activity, there is ample evidence that both peripheral and specific regions of the brain exert modulating influences on this control. Granit and Kaada (1952) demonstrated that as the facilitatory area of the reticular formation was stimulated, the efferent discharge of the gamma (γ) motor neurons was increased and the proprioceptive input from the muscle spindle was strengthened. Furthermore it is known that the gamma efferents are actively controlled by the reticular formation. Ward (1947) states that muscle proprioceptors play a role in the maintenance of rigidity in the decerebrate state. Sherrington and Frohlich (1902) and Rhines and Magoun (1946) demonstrated that stimulation of the direct spino-cerebellar tracts resulted in an increase in rigidity. Other peripheral sensory input involving the baroreceptors. (Kreindler, 1946), the sympathetic nervous system (Vardaptean, 1963), and the viscera (Kreindler, 1955) has been shown to influence the activity of the facilitatory mechanism involving maintenance of rigidity through decerebration.

The specific regions of the brain that appear to modify tonic seizure activity are, besides the reticular formation, the cerebellum, thalamus and hippocampus. Steriade (1960) demonstrated that penicillin-induced hypersynchrony of the derebral cortex occurred against a background of diffuse desynchrony brought about by cerebellar and thalamic stimulation. Suppression of the spikes by the cerebellum and diffuse thalamic nuclei would occur early in the seizure with facilitation of the spikes by these structures as the seizure proceeded. Generally, as the amplitude of the spikes increased, the facilitation increased. Inhibition of rigidity produced by decerebration has occurred by stimulation of the anterior lobe of the cerebellum. (Miller and Banting, 1922; Warren and Olmstead, 1923) Ablation of this region increased the decerebrate rigidity. Kreindler and Steriade (1964) reported that a high rate of stimulation of the dorsal amygdaloid structures resulted in a

reticular-like desynchronizing reaction while stimulation of the ventral amygdaloid structure resulted in synchronization of the EEG.

It is generally agreed that after the initial ECS there is a period of rapid low voltage waves in the EEG tracing. Following this, there is a gradual decrease in the wave frequency and an increase in the wave voltage with a characteristic spike-wave complex appearing. The spike-wave complex is an intermittent one alternating with periods of no apparent electrical activity. There is some correlation between the spike-wave complex and the clonic phase of the seizure (Rosenblueth and Cannon, 1942); this will be discussed later in the thesis. However, if a comparison is made between EEG recordings of the early phase of the seizure with recordings of stimulation of the cortex through the reticular formation a similarity will be seen. (Outlined in Figures 3 and 4).

The evidence presented above points to an important function of the reticular formation in the tonic phase of the ECS. It is the belief of many of the authors mentioned above that tonic-clonic convulsions produced by maximal stimulation are not essentially cortical seizures, but subcortical ones induced primarily as a reaction to the supra-maximal stimulus. It is certainly possible that the EEG tracings and neuromuscular activities are correlated if only in a general manner. As Rosenblueth and Cannon (1942) and others imply, the EEG represents a complex of brain structures while the neuromuscular activity represents only the manifestation of activation of the final common pathway.

During the latency period of the ECS there is a phase of maximal firing of all neurons and maximal glial interaction. In the single neuron there is a gradual decrease in the membrane hyperpolarization and an increase in the depolarization. While the latency consists of maximal unpatterned, essentially random firing, during the tonic phase of the ECS the reticular formation fires to pattern the random activity of the cerebrum.

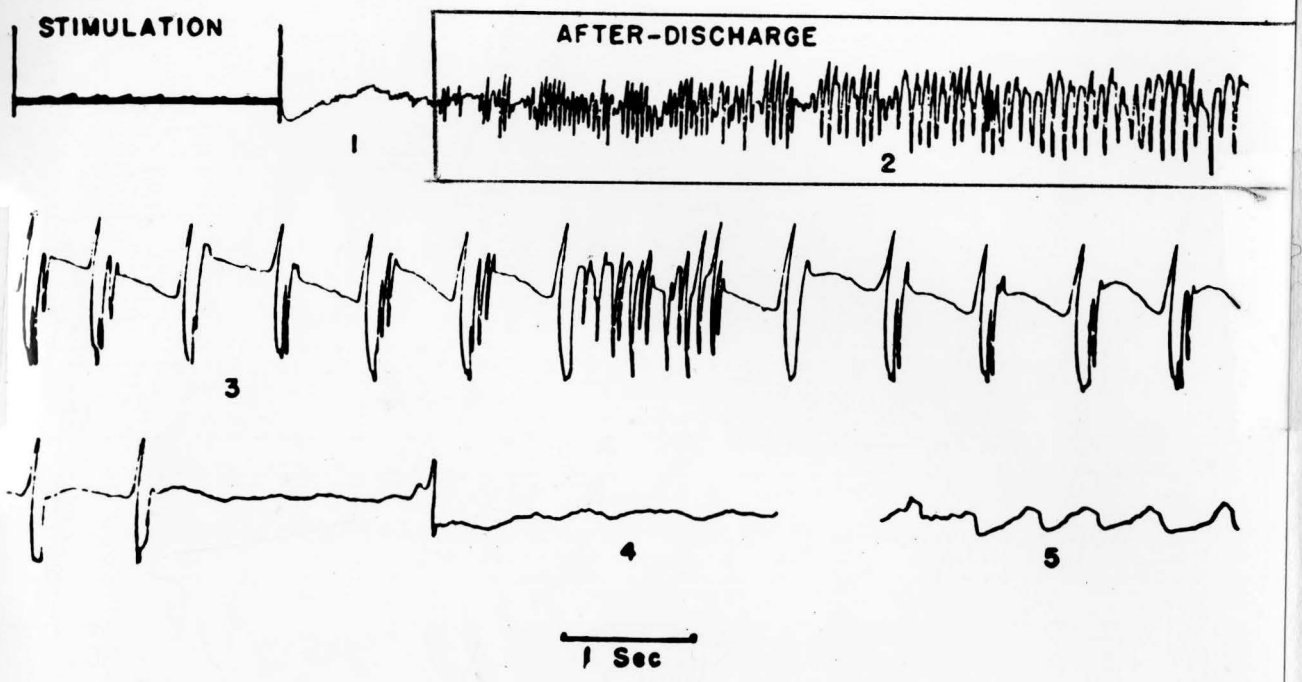

Figure 3. Electrocorticogram showing different stages of electrical after-discharge (From Penfield and Jasper, 1954, p. 201, cited by Kreindler, 1965, p. 3).

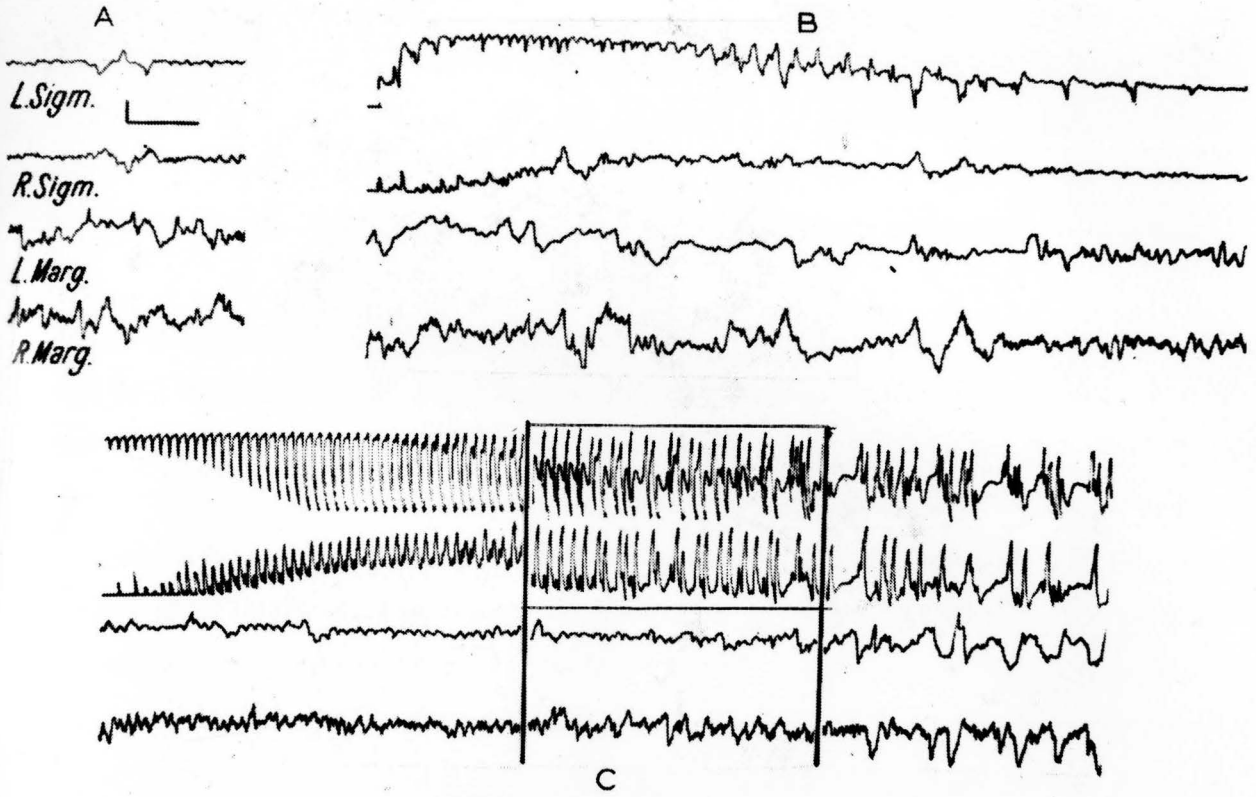

Figure 4. Facilitatory effect of reticular stimulation on neocortical discharge. A = background activity; B = afterdischarge induced by stimulating the left sigmoid gyrus; C = marked increase in sigmoidal after-discharges associating the neocortical stimulation to a fast repetitive reticular stimulation. Vertical bars indicate a lapse of 5 seconds. (Kreindler, 1965, p. 147).

This results in two activities: the descending activation of the reticular formation influences the flexor rigidity of tonus (due, in part, to the inhibition by the anterior cerebellum)$_1$ and the ascending activation influences the desynchrony pattern of the EEG. It is important to realize that the animal is functionally decerebrate at this time, i.e. it exhibits no patterned cortical activity.

Following this phase there is no longer a clear correlation between the EEG and neuromuscular activity, although certain speculations seem permissible. Flexor proprioceptive input, particularly from the gamma-efferent, and facilitation by the cerebellum results in an abrupt change from flexor to extensor rigidity as seen in decerebrate animals. The EEG shows a gradual increase in voltage and decrease in frequency of spike activity. This may be explained as a competitive phenomenon among the reticular formation, cerebellum, hippocampal and reverberating thalamic circuits. Another possibility is the neuronal-glial interaction with a decrease in excitability due to a change in individual membrane permeability.

It is known that after a convulsive attack the ascending reticular activating system is one of the first functional systems to resume pre-ictal activity (Kreindler, 1965), thus illustrating the biological priority of this system. For purposes of this thesis the tonic phase will represent total activation of the reticular formation as a result of an overpowering stimulus. The muscular activity seen in the tonic phase represents modulation of the reticular activity by cerebellar and hippocampal influences. The EEG tracings demonstrates a gradual summation of these modulatory influences after a period of initial reticular desynchrony.

5. The Pattern of Clonus

Rosenblueth and Cannon (1942) reported that the clonic neuromuscular contractions correlated in rate and amplitude with the irregular, low frequency and high voltage spike-wave complexes in the EEG tracings. On the

EEG tracings (See non-outlined patterns of Figures 1 and 3) it is seen that a development of the spike-wave complex does indeed occur which according to Kreindler et al. (1956) is originated from cortical and thalamic areas in common with the phenomena of paroxysmal hypersynchronism. Penfield and Jasper (1954) propose a thalamic origin for this activity. Petsche (1963) reported that this activity is of cortical origin due to a process of synchronization while the thalamus plays a small role in its appearance. As stated before, it has been shown that cerebellar influences (Kreindler and Steriade, 1960b; Steriade, 1960) cause a facilitation of hypersynchrony and that these influences can act on a cortical level.

Gastaut (1958) believes that the thalamo-caudate system produces the slow wave activity and actively inhibits the reticular desynchrony. He also states that convulsions involving the telencephalon and diencephalon have only a clonic phase. Kreindler (1955) suggests that the cortex disassociates the tonic attack and is significantly involved in the arrest of the epileptic seizure. Von Euler (1958) reported that the hippocampus, non-specific thalamic systems and the caudate nucleus are inhibitory structures acting on the high frequency, low voltage EEG waves. Fernandez-Guardiola (1962) states that in Metrazol seizures the cerebellum functions during clonus by an increase in frequency of unit discharges with each spike-wave complex. Other authors believe that clonus represents an active inhibitory process by the caudate nucleus (Jung, 1949), ventral thalamic nuclei (Voinescu et al., 1956) or fatigue and decrease of metabolic reserves (Gastaut, 1954). Kreindler (1965) postulates that separate neuronal chains distinct from the ones involved in the seizure activity of tonus as they exhibit longer refractory periods, are involved in the clonic neuromuscular movements.

It is here maintained that clonus represents the first signs of cortical influences on neuronmuscular activity. As the EEG tracing demonstrates a gradual change, the clonic movements represent abrupt activity on the part of

the cortex and reverberant activity on the part of subcortical structures. The gradual build-up of the spike-wave complex in the EEG represents these reverberant circuits overriding the decreasing tonic desynchrony of the reticular formation. The demonstrated flexor movements are adjustments from the severe tonic extensor stretch. Statistically, most neurons are becoming more hyperpolarized (Sawa et al., 1963) and inhibitory mechanisms are coming into effect to offset the maximal bursts produced during latency and tonus.

6. The End of Seizure Activity

It is probable that the hyperexcitable neurons become anoxic rapidly during the ECS. Lactic acid and other toxic metabolites are formed to a greater extent than in the course of normal activity. Undoubtably, this neuronal exhaustion plays a significant part in the termination of seizure activity. As for active inhibitory mechanisms, they are many, quite varied and subject to much discussion (Kreindler and Zuckermann, 1956; Fernandez-Guardiola et al., 1962; Tersuolo, 1954; Jasper, 1955 and others). In this thesis, the fatality rates of ECS will be discussed in this context, and further references will be presented.

7. Summary

Correlation between the EEG tracing, neuromuscular manifestations, functional patterning and stages of the ECS is given in Table I.

E. The Pharmacology of the Electroconvulsive Seizure

1. Introduction

The drugs utilized in this study will be presented in four major catagories: cholinergic, serotonergic, adrenergic and miscellaneous compounds. Earlier data on the former three categories of drugs and their influence on the ECS, behavior and the EEG is most controversial. A general review of the literature is presented in this section as a background to the data presented in this thesis.

Stage of the ECS	Skeletal Musculature Manifestations	Functional Patterning	EEG Tracing
Latency-	generalized increase in muscle tone	all neurons and circuitry firing in an unpatterned fashion-essentially a decerebrate animal	inactivation
	↓ gradual shift to:	↓ abrupt shift	↓ gradual shift to:
Tonus-	extreme increase in flexor muscle tone	firing of reticular formation with modulation by the anterior cerebellum, hippocampus and other regions (?)	desynchrony with gradual increase in amplitude and decrease in frequency
Flexion:	↓ abrupt shift	↓ abrupt shift to:	↓ abrupt shift to:
Extension:	extreme increase in extensor muscle tone	cortico-thalamic reverberation and modulatory affects of peripheral input	high amplitude low frequency spikes correlated with flexor movements
	↓ abrupt shift		
Clonus-	irregular, jerky flexion movements		

Table I. Correlation of the stages of the ECS with skeletal musculature manifestations, functional patterning and EEG tracing in the generalized tonic-clonic seizure.

2. Cholinergic Drugs

Richter and Crossland (1949) demonstrated that within a few tenths of a second after the application of an electrical stimulus the level of acetylcholine (ACh) in the protein-bound form in the brain dropped rapidly. They suggested that convulsive activity is associated with a release of free ACh so that the concentration of bound ACh decreases and the rate of the formation of free ACh increases as a compensation. McIlwain (1955) and Takahashi et al. (1961) suggested that an increase in ACh is necessary for convulsive activity. Pope et al. (1947) and Tower and Elliott (1952) demonstrated an increase in activity of cholinesterase in epileptic foci which could be interpreted as a compensation for the increase in free ACh due to its increased synthesis. Bose et al. (1958a, 1958b) showed that anticonvulsant drugs usually reduce the amount of ACh in the brain. (Elliott (1955) measuring ACh levels during seizure, reported that when the concentration of ACh decreases to forty per cent of normal in the brain, convulsive activity ceases). Voiculescu et al. (1957) showed that at the end of seizure activity the free ACh concentration falls to normal values and that a compensatory increase in bound ACh, sometimes up to three hundred per cent, occurs. It seems reasonable to assert, therefore, from this evidence that ACh in the free form generally increases neuronal excitability.

However, behaviorally, there is data suggestive of a central cholinergic system inhibiting general central nervous system responsiveness (Carlton, 1963; Warburton, 1969). Grossman and Grossman (1966) have found that stimulation with carbamylcholine in the mid-brain reticular structure decreased avoidance behavior but increased apetative behavior. They postulate that their data points to a decrease in the level of general reactivity which in turn decreases irrelevant response tendencies. Warburton and Russell (1969) confirmed these results involving the hippocampus as one site of the inhibition of the general reactivity. Anderson (1966) found fibers from the septo-

hippocampal area terminating in excitatory synapses on the pyramidal dendrites. Shute and Lewis (1966), in turn, traced a "cholinergic" pathway from the reticular formation to the septal area. In any case, there does appear to be some type of cholinergic mechanism acting on the reticular formation in the brain stem to inhibit or modulate certain behaviors.

3. Serotonergic Drugs

In single doses, serotonin has not been reported to affect seizure activity. In multiple doses, given by a general route, serotonin causes an antagonism of Metrazol-induced convulsions (Cahn, 1957). However, Wada (1961) found that in cats and monkeys, five-hydroxytryptophan (5-HTP) favors epileptic activity.

After ECS treatment, an increase in 5-HTP has been reported without an increase in monoamine oxidase activity (Spilman, 1960). Electroshock was observed to elevate the brain serotonin levels even after the administration of anticonvulsant drugs (Garattini et al., 1960). These results have been questioned by other investigators. Several studies (DeSchaepdryver et al., 1962; P'An et al., 1961) have indicated that there is no relationship between sensitivity to convulsions and brain serotonin levels.

Serotonin has been described as a depressant of the electrical activity of the brain (Curtis, 1959; Pineda and Sunder, 1963). Monnier (1960) described a depressant action with low doses of 5-HT or 5-HTP and an excitatory effect with high doses on brain electrical activity. It has been shown that serotonin depresses the monosynaptic reflex in the isolated spinal cord of the frog (Carels, 1962) Koelle et al. (1960) ascribe a modulating role to serotonin.

Behaviorally, Brodie et al. (1960) believe that the sedative effect of reserpine is due to its depletion of bound centrally-acting serotonin. Smith (1960) and Himwich and Costa (1960) contradicted this principle in their studies of α-methyldopa and 5-HTP. Giarman and Schanberg (1961) believe

that the sedative effect of 5-HT is due to an increase in the ratio of free 5-HT to bound 5-HT in the cell. Lysergic acid as an excitant is a powerful and specific antagonist of 5-HT on peripheral tissues but not in the brain where these two drugs stimulate some centers and inhibit others (Gaddum, 1962). Udenfriend (1957) found that low levels of free 5-HT caused sedation while high levels caused excitement.

4. Adrenergic Drugs

Norepinephrine and epinephrine are found in various areas of the central nervous system especially in areas concerned with central autonomic regulation including the reticular formation (Vogt, 1954). Kety (1967) implies that norepinephrine and dopamine are ubiquitous regulators but are located predominantly in the higher brain centers including the hypothalamus and mid-brain. Dahlstrom and Fuxe (1964) found catecholaminergic neurons in the lateral regions of the pons, medulla and mesencephalon while serotonergic neurons were located primarily in the Raphe nuclei of the brain stem.

Bonvallet (1954) and Dell and Bonvallet (1956) postulate an adrenergic mechanism in the reticular formation having ascending and descending influences. Both epinephrine and norepinephrine cause EEG arousal when given intravenously but not by close arterial injection. Gaddum (1962) and Rothballer (1956) confirmed these studies. DeMaar and Martin (1956) demonstrated that in unanesthesized cats epinephrine caused EEG arousal and spindle bursts while norepinephrine did not. Repeated administration of norepinephrine caused EEG slowing. Reserpine causes depletion of catecholamines with resultant depression, but Spector et al. (1960) believe that there is better evidence relating increased levels of the catecholamines with arousal.

Bulbring et al. (1948) found that epinephrine facilitated the extensor and depressed the flexor movements evoked by stimulating the de-

scending motor tracts. Skoglund (1952) found that epinephrine directly increases the monosynaptic extensor responses.

Behaviorally, Toman (1957) found that monoamines generally increased seizure latency and relate this to a "diffuse inhibition" following maximal ECS. Carlton (1963) on the basis of the studies of the sympathomimetic drug, amphetamine, postulated an adrenergic activating system antagonized by a cholinergic system. Brodie and Shore (1957) working with amphetamine and lysergic acid postulated a sympathetic center activated by norepinephrine and antagonized by serotonin. It has been shown that 5-HTP and dopamine. Precursors for serotonin and norepinephrine respectively, compete with each other for the same transport mechanisms in the brain (Schanberg, 1963).

In summary, there is a plethora of conflicting data concerning the neurohumors; but cholinergic and serotonergic inhibitory functions with adrenergic activating functions predominate in terms of both EEG and behavioral data. Further correlation is discussed later in this thesis.

Materials and Methods

A. Laboratory Animals

The mice used in this study were adult, male albino Mus musculus SC-1 obtained from Abrams Breeders in Chicago, Illinois. This strain originates from the inbred strains of CF-1 from Carworth Farms. All mice weighed between twenty-five and forty-five grams and were approximately fifty-nine to ninety days old. During a two week post-reception period the mice were housed in a room maintained at a constant temperature of 75° F. ± 5° F. Food and water were available ad libitum. All electroshock experiments were carried out between 9:00 A.M. and 3:00 P.M.

B. Apparatus

The stimulator for inducing the maximal electroshock seizure was similar to that described by Woodbury and Davenport (Woodbury and Davenport, 1952). The shock duration was 0.2 seconds in duration and the current intensity was a constant 21 milliamperes at 60 cps measured directly. This current was approximately three times the minimum current used to produce tonic-clonic convulsions in the strain CF-1 (Scudder et al., 1966) and below the current value inducing irreversible damage to nerve tissue and blood vessels (Ferraro et al., 1946). This current was also at the level of the ED100 value measured previously in this laboratory for this strain of mice. Since the impedance was quite high despite the use of both Spiegel corneal electrodes (see below) and a primary continuous variable autotransformer which varied the voltage up to 2000 volts, the current was assumed to be independent of the small changes in resistance of the tissue (Russell, 1949; Spiegel and Henry, 1942). The resistance (electrical) of the animals was assumed to be 500 ohms. In this study, tonic-clonic convulsions were considered as evidence of the induction of maximal seizures and of the corresponding electrical activity of the brain (Toman et al., 1946).

C. Procedure

Electric shocks for producing tonic-clonic convulsions were delivered through corneal electrodes (Spiegel, 1937). This technique lowers the high tissue impedance when current is applied through the intact skull of the animal. Several drops of 2.0% sodium chloride solution were put on each electrode before the shock. The animals were held firmly in place by hand, the shock was administered and simultaneously two electrical elapsed-time meters were started. Immediately after receiving the shock the animals were placed in a clear plastic cage.

The first timer was stopped at the beginning of the extensor thrust of tonus and this measurement was labeled latency. The second timer was stopped at the first signs of clonic movements, i.c. short, jerky flexor movements of the hind limbs which follow the tonus: this measurement expressed the ECS duration (Toman et al., 1946; Toman et al., 1957). The changes in latency and duration were measured for control animals and animals in various drug-treated groups.

D. Drugs and Dosages

All drugs and drug combinations were given intraperitoneally. Dilutions of drugs were such that all animals received a constant volume of solution per unit of body weight (o.ol ml/g.).

The total duration of the study covered a period of approximately three months. During this time period, four separate groups of mice used as controls were shocked at various intervals to obtain any change in latency or duration which might be age or situation related. These groups were staggered throughout these three months so that a control check was carried out during the entire study.

Tables II through VI illustrate the drugs, dosages and regimens utilized in this study.

E. Statistical Analysis

Table II. Adrenergic and serotonergic drugs utilized in this study with methods of preparation, dosages and administration.

Drug	Preparation	Dose	Time Elapsed Prior to ECS
Reserpine	Dissolved in 0.9% sodium chloride solution	10 mgm/Kgm of body weight	4 hours
D, L-Dopa	Warmed and dissolved in 0.9% sodium chloride solution	50 mgm/Kgm of body weight	15 minutes
Alpha-methyl-para-tyrosine (-MPT)	Mixed and dissolved in 0.9% sodium chloride solution and 2 mgm of tragacanth per cc of solution; suspension is spinned until homogenous	80 mgm/Kgm of body weight	One injection every four hours until a total of three injections; 4th injection one hour prior to ECS, 11 hours later
Pargyline (Abbott Labs, MO 911)	Dissolved in 0.9% sodium chloride solution	100 mgm/Kgm of body weight	24 hours
five-hydroxy-D, L-tryptophan (5-HTP)	Warmed and dissolved in 0.9% sodium chloride solution	100 mgm/Kgm of body weight	15 minutes
p-chlrophenyl-alanine (CP-10)	Dissolved in 0.9% sodium chloride solution and 2 mgm of tragacanth per cc of solution; suspension is spinned until homogenous	316 mgm/Kgm of body weight	One injection per day for three days; on fourth day, one hour prior to ECS

Table III. Cholinergic drugs utilized in this study with methods of preparation, dosages and administration.

Drug	Preparation	Dose	Time Elapsed Prior to ECS
Scopolamine Hydrobromide	Dissolved in 0.9% sodium chloride solution	0.03 mgm/Kgm of body weight	15 minutes
		0.03 " " " " "	60 minutes
		1.00 " " " " "	15 minutes
		1.00 " " " " "	60 minutes
		2.00 " " " " "	15 minutes
		2.00 " " " " "	60 minutes
Physostigmine Salicylate, U.S.P.	Dissolved in 0.9% sodium chloride solution	0.001 mgm/Kgm of body weight	15 minutes
		0.001 " " " " "	60 minutes
		0.005 " " " " "	15 minutes
		0.005 " " " " "	60 minutes
		0.010 " " " " "	15 minutes
		0.010 " " " " "	60 minutes
		0.050 " " " " "	15 minutes
		0.050 " " " " "	60 minutes
Diisopropyl-fluorophosphate (DFP)	Dissolved in peanut oil	0.05 mgm/Kgm of body weight	15 minutes
		0.05 " " " " "	60 minutes
		0.10 " " " " "	15 minutes
		0.10 " " " " "	60 minutes
		1.00 " " " " "	15 minutes
		1.00 " " " " "	60 minutes

Table IV. Miscellaneous drugs and regimens utilized in this study with methods of preparation, dosages and administration.

Drug or Regimen	Preparation and/or dosage	Time Elapsed
Ethanol, U.S.P.	15% by volume; 0.01 mgm/Kgm of body weight	5 minutes
" "	" " " ; 0.01 mgm/Kgm of body weight	60 minutes
Enovid, 10	Dissolved in 0.9% sodium chloride solution 0.10 mgm/Kgm of body weight	60 minutes
Isolated Mice	Mice were isolated for fourteen days	ECS was induced on fifteenth day of isolation
Fighting Mice	Mice were isolated for fourteen days and on fifteenth day pairs of mice were placed in cages together	ECS was induced during fighting
Mice shocked after frustration	Mice received 5 second foot shock with 65 second rest period between shocks; repeat for fifty times	ECS was induced immediately after frustration
L-methionine-D, L-sulfoxamine	Dissolved in 0.9% sodium chloride solution 25 mgm/Kgm of body wt.	15 minutes
	25 mgm/Kgm of body wt.	60 minutes
	25 " " " " "	240 minutes
	50 " " " " "	15 minutes
	50 " " " " "	60 minutes
	50 " " " " "	240 minutes
	75 " " " " "	15 minutes
	75 " " " " "	60 minutes
	75 " " " " "	240 minutes
	100 " " " " "	15 minutes
	100 " " " " "	60 minutes
	100 " " " " "	240 minutes
Parahexyl	Boiled and dissolved in 0.9% sodium chloride solution 3.0 mgm/Kgm of body weight	15 minutes
	3.0 mgm/Kgm of body weight	60 minutes
	7.0 " " " " "	15 minutes
	7.0 " " " " "	60 minutes
	15.0 " " " " "	15 minutes
	15.0 " " " " "	60 minutes

Table V. The preparation, dosage and method of administration of methamphetamine utilized in this study.

Preparation	Method of Administration	Dose	Time Elapsed Prior to ECS
Dissolved in 0.9% sodium chloride solution	Acute	3.0 mgm/Kgm of body weight	60 minutes
	"	6.0 " " " " "	60 minutes
	"	12.0 " " " " "	60 minutes
	"	24.0 " " " " "	60 minutes
	"	48.0 " " " " "	60 minutes
	"	96.0 " " " " "	60 minutes
Dissolved in 0.9% sodium chloride solution	Drug given twice a day at increasing doses, ECS induced after morning injection	3.0 mgm/Kgm of body weight	Day#1 60 minutes
		6.0 " " " " "	Day#2 60 minutes
		12.0 " " " " "	Day#3 60 minutes
		24.0 " " " " "	Day#4 60 minutes
		48.0 " " " " "	Day#5 60 minutes
Dissolved in 0.9% sodium chloride solution	Constant dose; drug given twice a day, ECS induced after morning injection	3.0 mgm/Kgm of body weight	Day#1 60 minutes
		3.0 " " " " "	Day#2 60 minutes
		3.0 " " " " "	Day#3 60 minutes
		3.0 " " " " "	Day#4 60 minutes
		3.0 " " " " "	Day#5 60 minutes
Dissolved in 0.9% sodium chloride solution	Constant dose; drug given twice a day, ECS induced after morning injection	7.0 mgm/Kgm of body weight	Day#1 60 minutes
		7.0 " " " " "	Day#2 60 minutes
		7.0 " " " " "	Day#3 60 minutes
		7.0 " " " " "	Day#4 60 minutes
		7.0 " " " " "	Day#5 60 minutes

Most groups of mice contained ten to twenty animals for a given control run or an experimental drug run. The means and standard errors for the latency and duration were determined for each run from the values obtained from these ten to twenty animals. The various values obtained for the four control groups during the period of experimentation were placed into four separate control blocks respectively and each block was analyzed for significant variation between each other. The four separate blocks were then pooled for a mean control value for latency and duration. Each experimental drug run was analyzed with Fischer's unpaired "T" distribution versus the major control value (Dunn, 1964). The degree of freedom were assumed to be infinite. All data was analyzed at the Data Processing Center of Loyola University.

Results

A. Control Groups

The data for the four series of control groups is presented in Figures 5 through 7. Figure 7 illustrates the percentage of fatalities occurring on each shock trial of the control animals to the age of the animals. There was initially a relatively high rate of fatalities (up to 30 per cent) which gradually decreased to zero and remains at this level for the remainder of the experimental period. Figure 5 illustrates the mean control latencies of the four control groups to the age of the animals. There is no significant change in latency in any of the four groups ($p \geq .3$) when analyzed for variability against the lowest and highest values of each group. Figure 6 demonstrates the changes in duration of the four control groups with the age of the animals. In control group II there was a gradual decrease in variability of the duration value as the age of the animal increased. In control groups I and III there is a gradual decrease in duration as the age of the animal decreases. However, these changes were not significant ($p \geq .3$) when analyzed for variation against the lowest and highest values of each group.

The four control group values were pooled to yield a single control value. (mean ± SE, seconds) The latency and duration values of this control were: 1.947 - .048 seconds (259 animals) for latency and 16.638 - .309 seconds (253 animals) for duration.

B. Adrenergic and Serotonergic Drugs

The data for these drugs are presented in Table 7 and Figure 8. In the figure (as with the remainder of the figures in this study) the data is presented as percentage above or below mean control levels, the probability of each value included with the data. When a large number of fatality rates prevented a legitimate statistical analysis with any particular drug regimen the term SEE TEXT is given in the figure. These large fatality rates will be discussed in a subsequent section in this study. In Figure 8 it is seen that

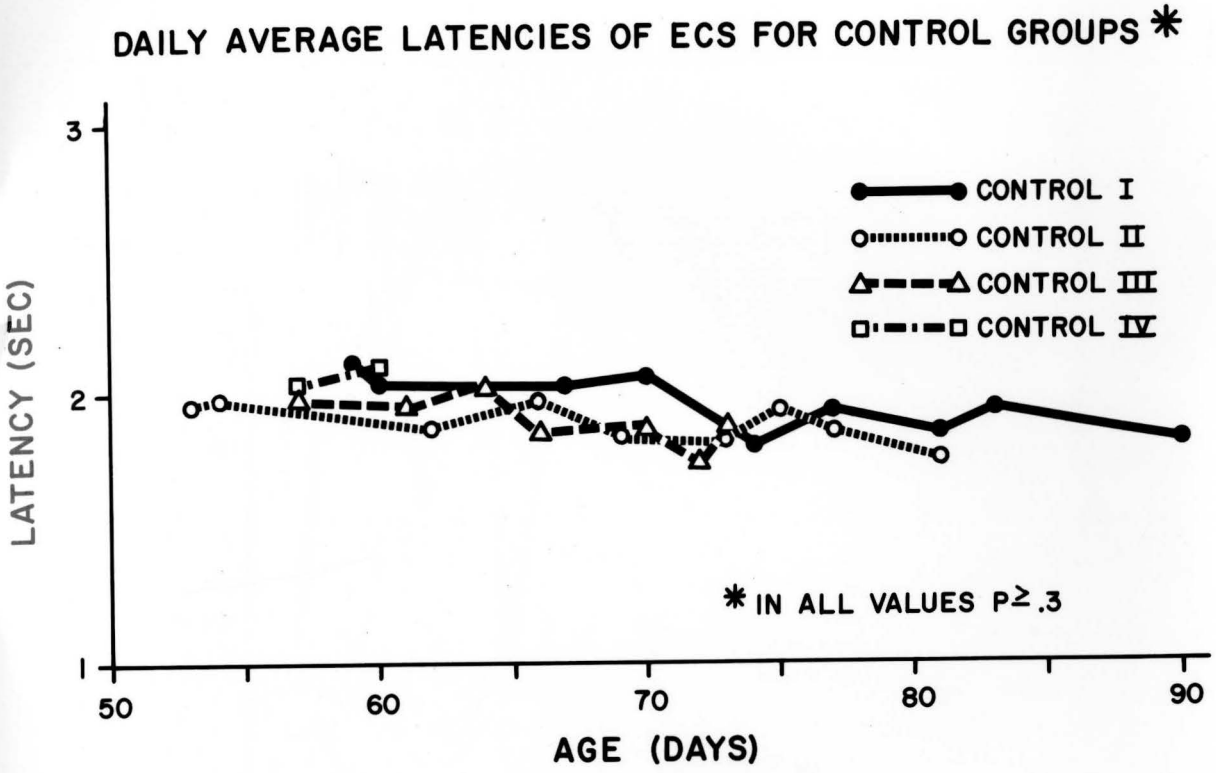

Figure 5. Average, daily latencies for the four control groups during the experimental period.

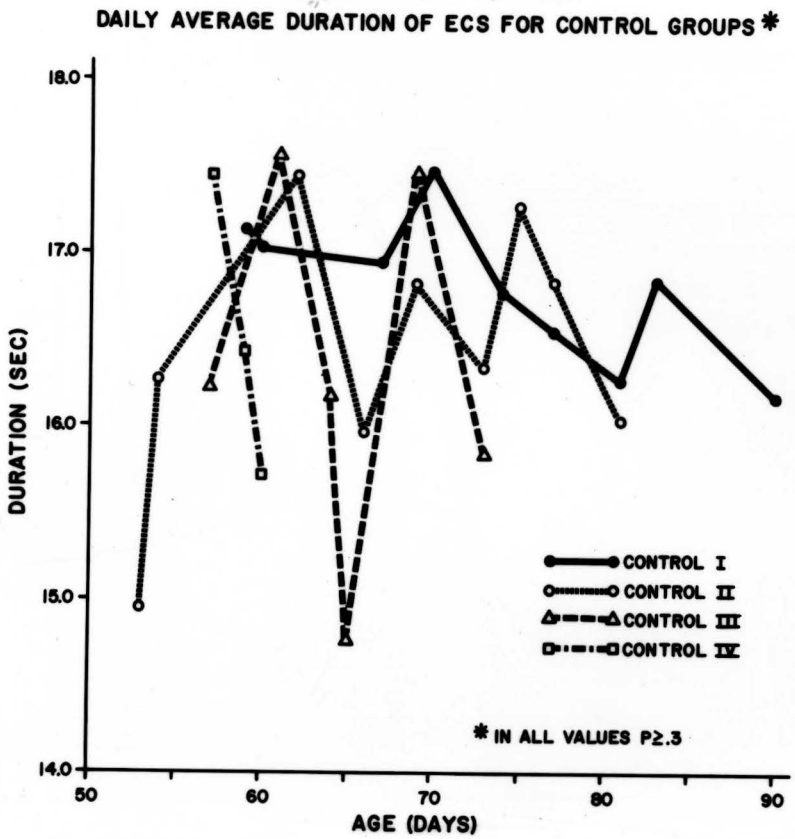

Figure 6. Average, daily duration values for the four control groups during the experimental period.

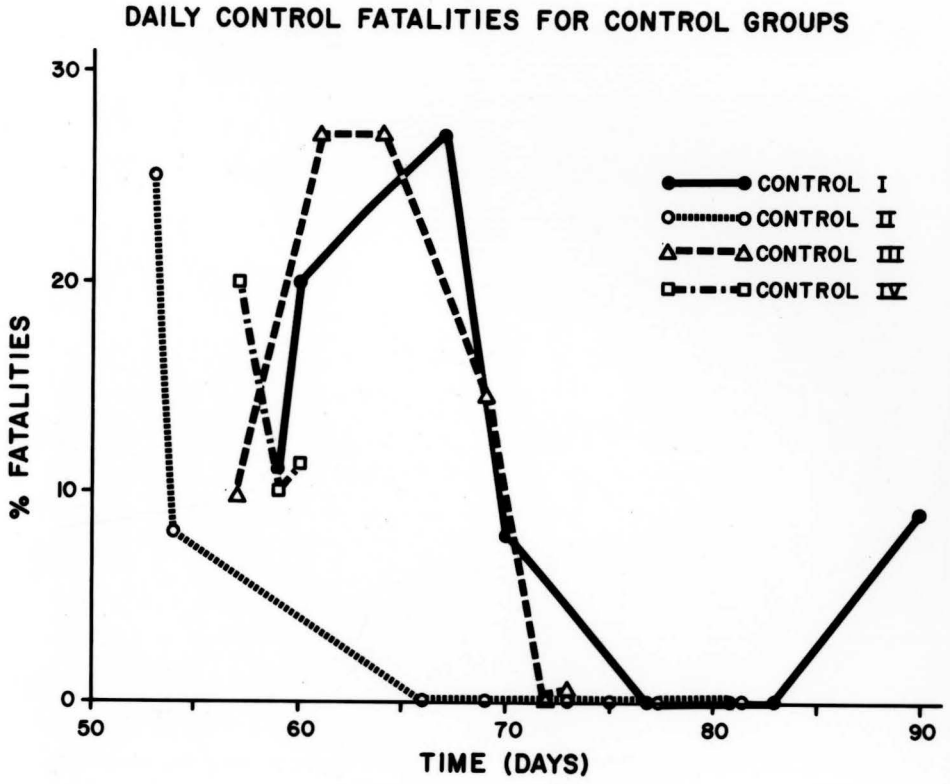

Figure 7. Daily control fatalities for the four control groups during the experimental period.

Table 7. The effect of adrenergic and serotonergic drugs on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug	Number of Determinations	Latency	Number of	Duration
Control	259	1.947±.048	253	16.639±.309
Reserpine	10	1.745±.069	10	17.810±.683
D, L-Dopa	14	2.279±.166	14	15.604±.341
∝-MPT	13	1.850±.073	14	18.204±.650
5-HTP	16	2.128±.083	15	18.477±.991
CP-10	16	2.063±.090	16	16.841±.336

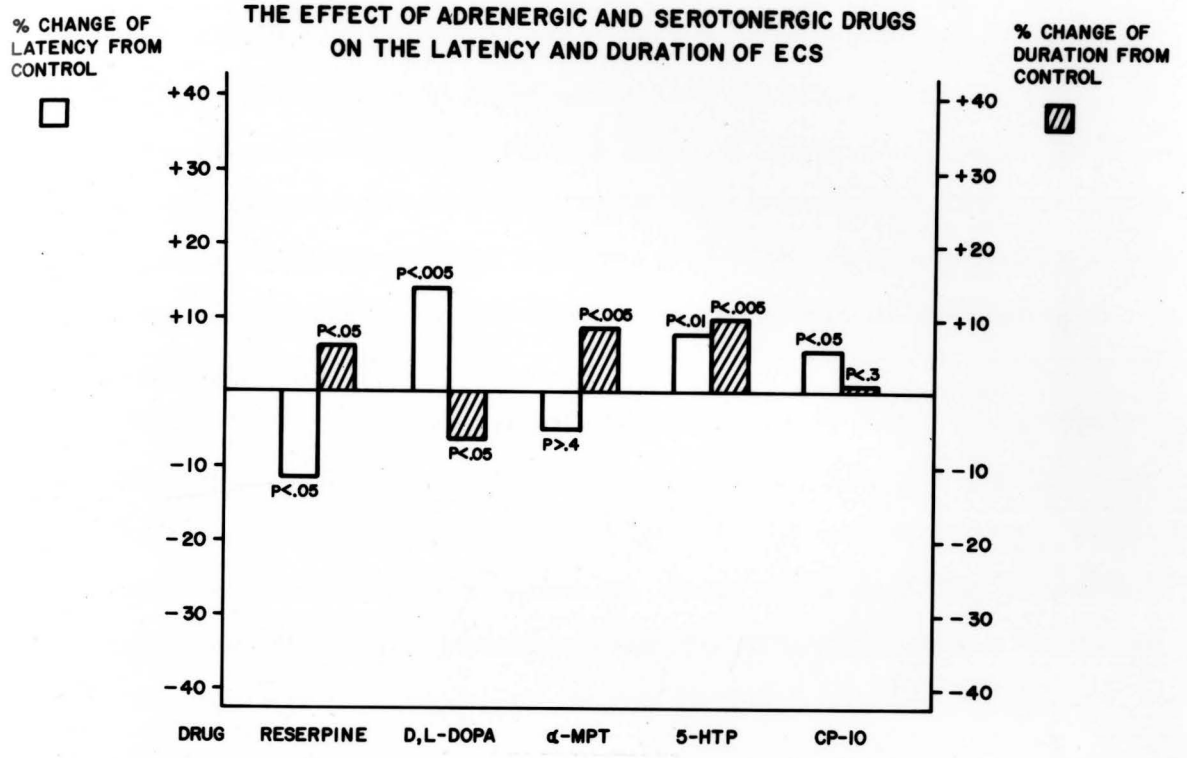

Figure 8. Percent change from control latency and duration values for the values for the various adrenergic and serotonergic drugs utilized in this study. P values are given for % changes for each drug in the experimental group.

D, L-Dopa. A drug that increase the levels of catecholamines in the central nervous system induced an increase in latency values and a decrease in duration values. 5-HTP which may selectively increase central serotonin levels caused an increase in duration value with no significant change in the latency value. Para-chlorophenylalanine. (CP-10, a drug which induces a differential lowering of serotonin levels in the central nervous system is correlated with an increase in both latency and duration in the seizure.

The administration of reserpine induced changes opposite to that seen with the administration of D, L-Dopa, i.e. a significant decrease in latency and increase in duration. The addition of α-MPT resulted in a slight, non-significant decrease in latency and a significant increase in duration.

C. Cholinergic Drugs

The data for the scopolamine are presented in Table 8 and Figure 15. This drug was administered to the mice in various dosages and they were schocked at various times after injection. At the lowest dosage, 0.03 mgm/Kgm, at fifteen minutes after injection, there was a decrease in duration and no significant change in latency. When ECS was induced one hour after injection, there is no significant change in either variable. With the two higher dosages, fatalities prevented a meaningful statistical analysis when the drugs were given fifteen minutes prior to ECS. When ECS was induced one hour after injection there was an increase in latency and duration values for the highest dosage.

The data for the drug physostigmine are presented in Table 8 and Figure 14. At the lowest dose, 0.001 mgm/Kgm, there was a significant decrease in duration at the fifteen minute period, but no change in latency or duration for the one hour period. At the dose level of 0.005 mgm/Kgm there was a decrease in duration after fifteen minutes and one hour but no significant change in latency at either time period. With 0.01 mgm/Kgm there was a decrease in duration after fifteen minutes while after one hour there is an

Table 8. The effect of scopolamine, physostigmine and diisopropyl phosphoroflouridate on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug and Method of Administration	Number of Determinations	Latency	Number of Determinations	Duration
Control	259	1.947±.048	253	16.638±.309
Scopolamine				
0.03mgm/Kgm				
15minutes prior to ECS	16	2.047+.087	14	15.132±.325
60 minutes prior to ECS	12	1.996±.089	10	16.275±.914
1.00 mgm/Kgm				
15 minutes prior to ECS		94.0% Fatalities		
60 " " " "	12	2.221±.095	9	16.224±.570
2.00 mgm/Kgm				
15 minutes prior to ECS		100% Fatalities		
60 " " " "	14	2.214±.079	10	19.140±1.963
Physostigmine				
0.000 mgm/Kgm				
15 minutes prior to ECS	13	1.812±.080	14	15.318±.643
60 " " " "	14	1.868±.073	12	15.829±.392
0.005 mgm/Kgm				
15 minutes prior to ECS	10	1.990±.141	12	15.027±.392
60 " " " "	14	1.957±.105	13	15.185±.312
0.01 mgm/Kgm				
15 minutes prior to ECS	10	1.775±.056	10	14.875±.598
60 " " " "	14	2.150±.109	14	15.900±.567
0.05 mgm/Kgm				
15 minutes prior to ECS	14	1.771±.066	15	15.567±.747
60 " " " "	15	2.023±.087	15	15.647±.242
Diisopropyl phosphoroflouridate				
0.05 mgm/Kgm				
15 minutes prior to ECS	12	1.800±.064	11	14.995±.515
60 " " " "	18	1.989±.091	18	15.478±.362
0.10 mgm/Kgm				
15 minutes prior to ECS	12	2.046±.115	11	14.649±.517
60 " " " "	12	1.938±.090	12	16.479±.651
1.00 mgm/Kgm				
15 minutes prior to ECS	12	1.854±.074	14	15.250±.677
60 " " " "	14	1.982±.147	14	17.382±.372

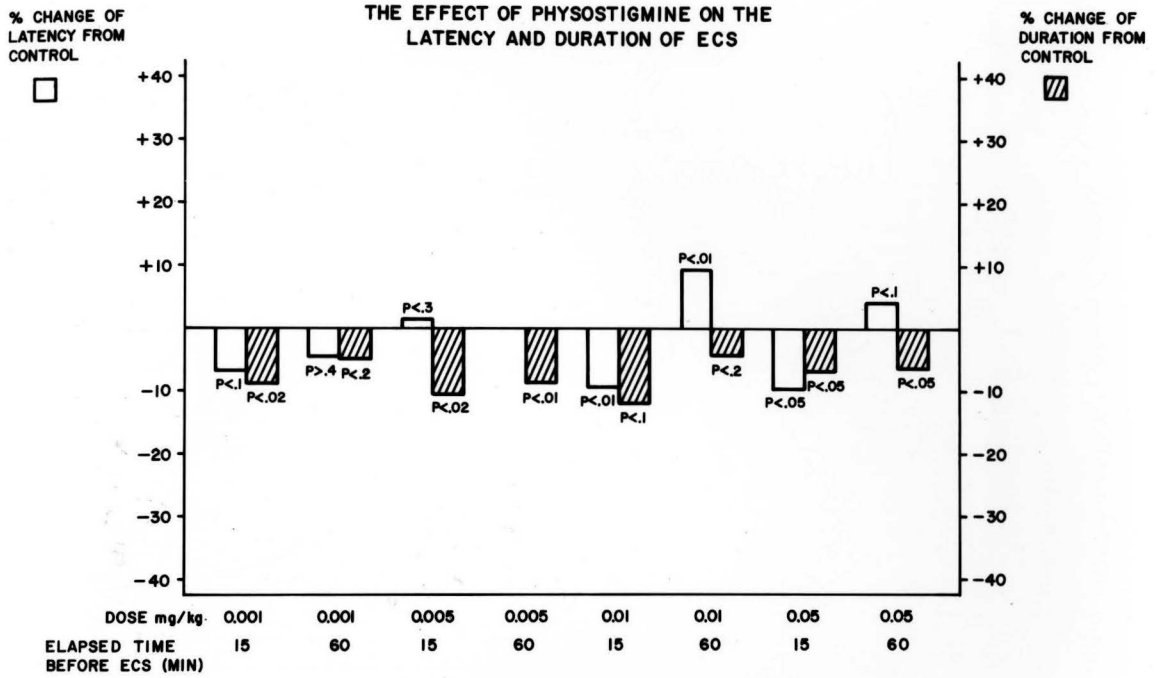

Figure 14. Percent change from control latency and duration values for physostigmine given in various doses and time periods prior to ECS. P values are given for % changes for each regimen in the experimental group.

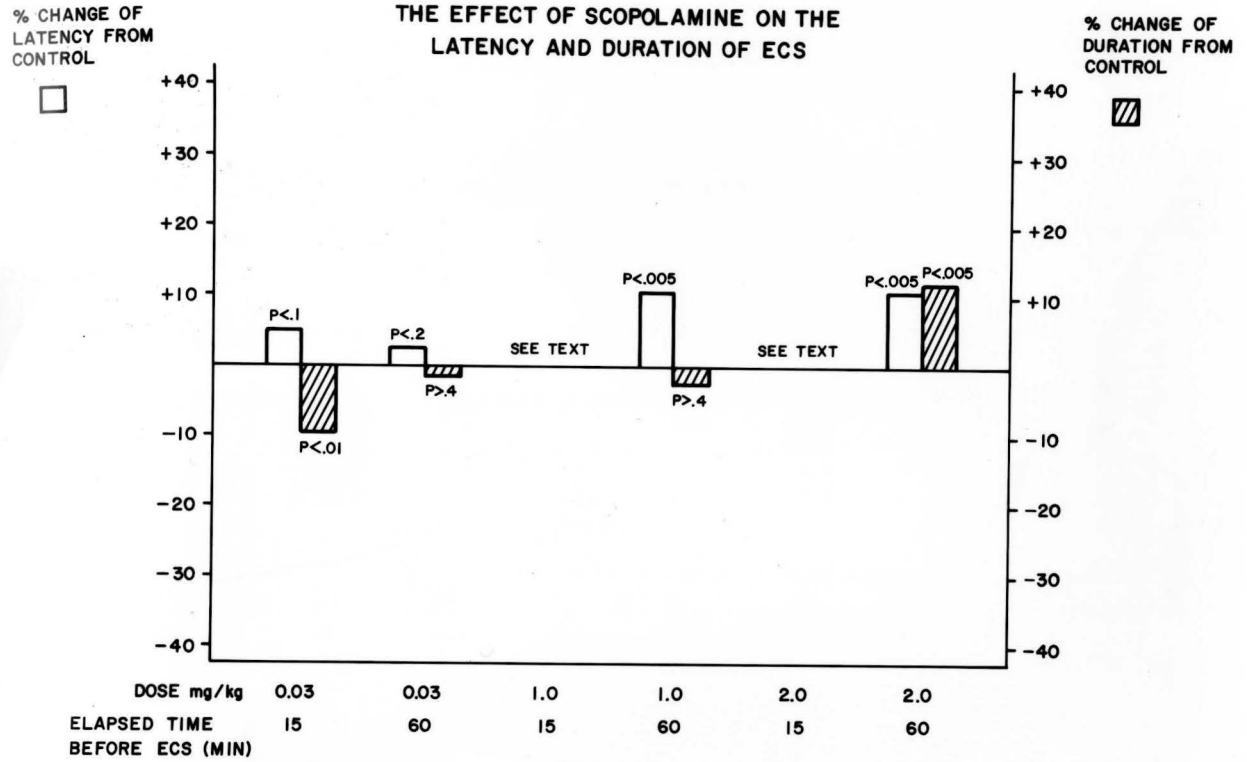

Figure 15. Percent change from control latency and duration values for scopolamine given in various dosages and time periods prior to ECS. P values are given for % changes for each regimen of the experimental group.

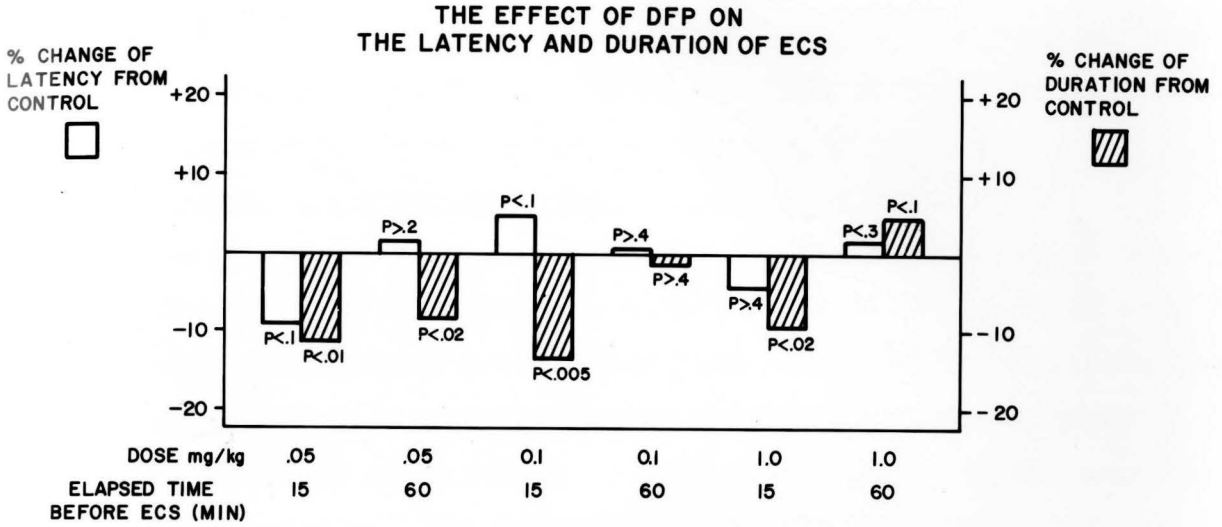

Figure 16. Percent change from control latency and duration values for diisopropyl phosphorofluoridate (DFP) given in various dosages and time periods prior to ECS. P values are given for % changes for each regimen of the experimental group.

increase in latency with no significant change in duration. At the highest dose of 0.05 mgm/Kgm of body weight there was a decrease in latency and duration after fifteen minutes and a decrease in duration after one hour.

The data, diisopropyl phosphoroflouridate (DFP) is presented in Table 8 and Figure 16. There was no significant change in latency at any dose or time period with this drug. At all doses there is a decrease in duration, but at the highest dose, 1.0 mgm/Kgm of body weight, after one hour the increase is statistically non-significant. There was a greater change with all doses in duration after the fifteen minute period than after one hour.

D. Adrenergic and Serotonergic Drug Combinations

The data for these drug combinations are presented in Tables 11 and 11b and in Figures 9 and 9b. Reserpine given with CP-10 is associated with a large decrease in latency and a marked increase in duration while reserpine given with 5-HTP increased duration but caused no significant change in latency. Reserpine given with D, L-Dopa resulted in no significant change in latency or duration. Reserpine given with α-MPT is associated with a decrease in latency and an increase in duration. Reserpine given with α-MPT and CP-10 resulted in an increase in duration and a decrease in latency. Reserpine given with α-MPT, CP-10, 5-HTP and pargyline is associated with an increase in latency and duration. Pargyline given with 5-HTP resulted in an increase in latency and decrease in duration. Pargyline with D, L-Dopa markedly increased in latency and decrease in duration while pargyline with α-MPT increased both the latency and duration. Pargyline given with CP-10 and 5-HTP showed an increase in latency and decrease in duration. Pargyline given with D, L-Dopa and α-MPT was associated with an increase in latency and no significant change in duration. Pargyline given with 5-HTP and α-MPT increased in the latency and decreased duration while pargyline given with α-MPT, CP-10, and 5-HTP increased the duration, but caused no significant change in latency.

Table 11. The effect of various adrenergic and seretonergic drug combinations on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug Combinations	Number of determinations	Latency	Number of determinations	Duration
Control	259	1.947±0.048	253	16.638±0.309
Reserpine CP-10	13	1.438±0.031	8	28.738±0.759
Reserpine 5-HTP	13	1.973±0.090	12	18.775±0.693
Reserpine D, L-Dopa	12	2.046±0.061	11	16.255±0.491
Reserpine ∝-MPT	11	1.623±0.034	9	26.378±0956
Reserpine ∝-MPT CP-10	11	1.668±0.046	6	28.608±2.513
Reserpine -MPT CP-10 Pargyline 5-HTP	9	2.156±0.138	8	20.888±1.422
Pargyline 5-HTP	10	2.755±0.166	10	14.655±0.701
Pargyline D, L-Dopa	13	2.527±0.153	13	14.342±0.305
Pargyline ∝-MPT	18	2.239±0.110	18	18.125±0.960
Pargyline CP-10 5-HTP	9	2.467±0.253	11	14.514±0.894
Pargyline D, L-Dopa ∝-MPT	15	2.450±0.157	15	16.443±0.747
Parygyline D, L-Dopa 5-HTP		81.8% Fatalities		

Table 11b. The effect of various adrenergic and serotonergic drug combinations on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug Combination	Number of Determinations	Latency	Number of determinations	Duration
Control	259 259	1.947±.048	253	16.638±.309
Pargyline 5-HTP α-MPT	5	3.260±.474	5	13.340±.423
Pargyline CP-10 5-HTP α-MPT	12	1.888±.070	12	20.717±1.650
CP-10 α-MPT	16	1.766±.067	11	19.946±1.832
5-HTP α-MPT	14	1.761±.049	11	25.936±1.597

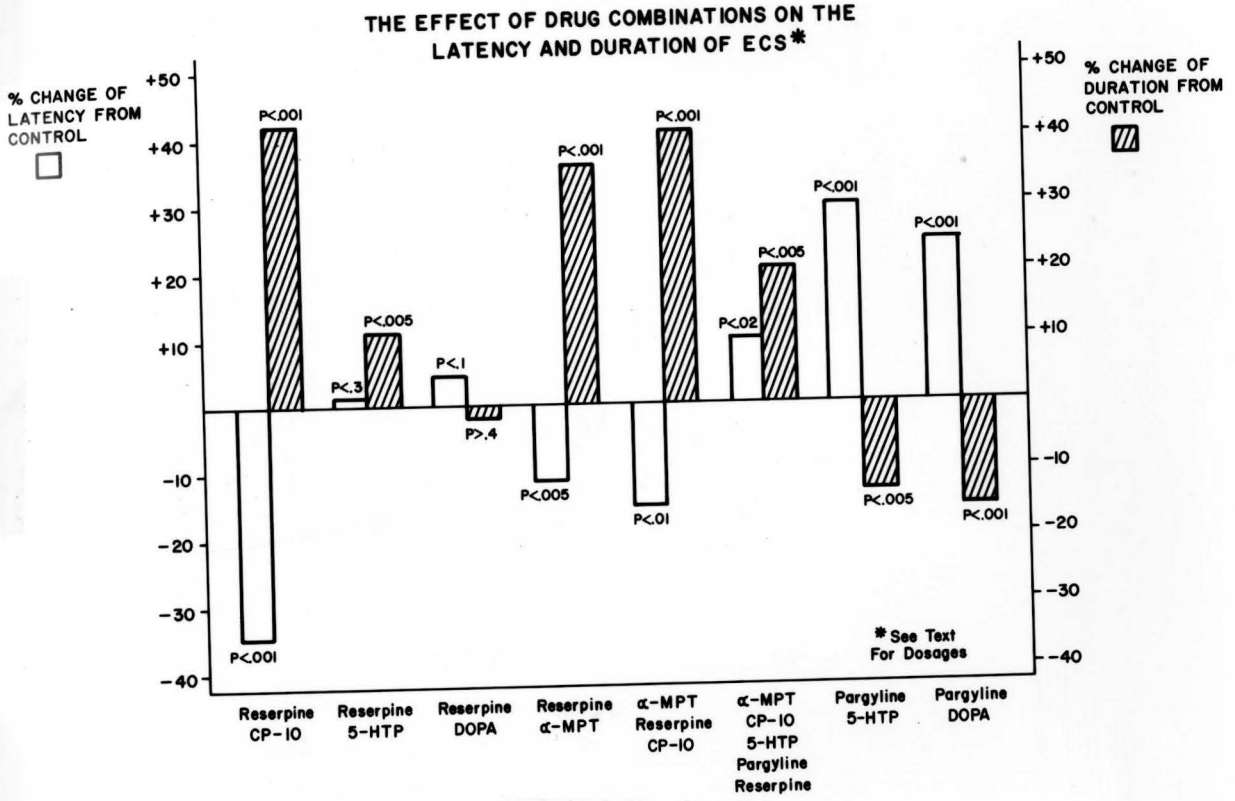

Figure 9a. Percent change from control latency and duration values for the various adrenergic and serotonergic drug combinations utilized in this study. P values are given for the % changes for each drug combination in the experimental group.

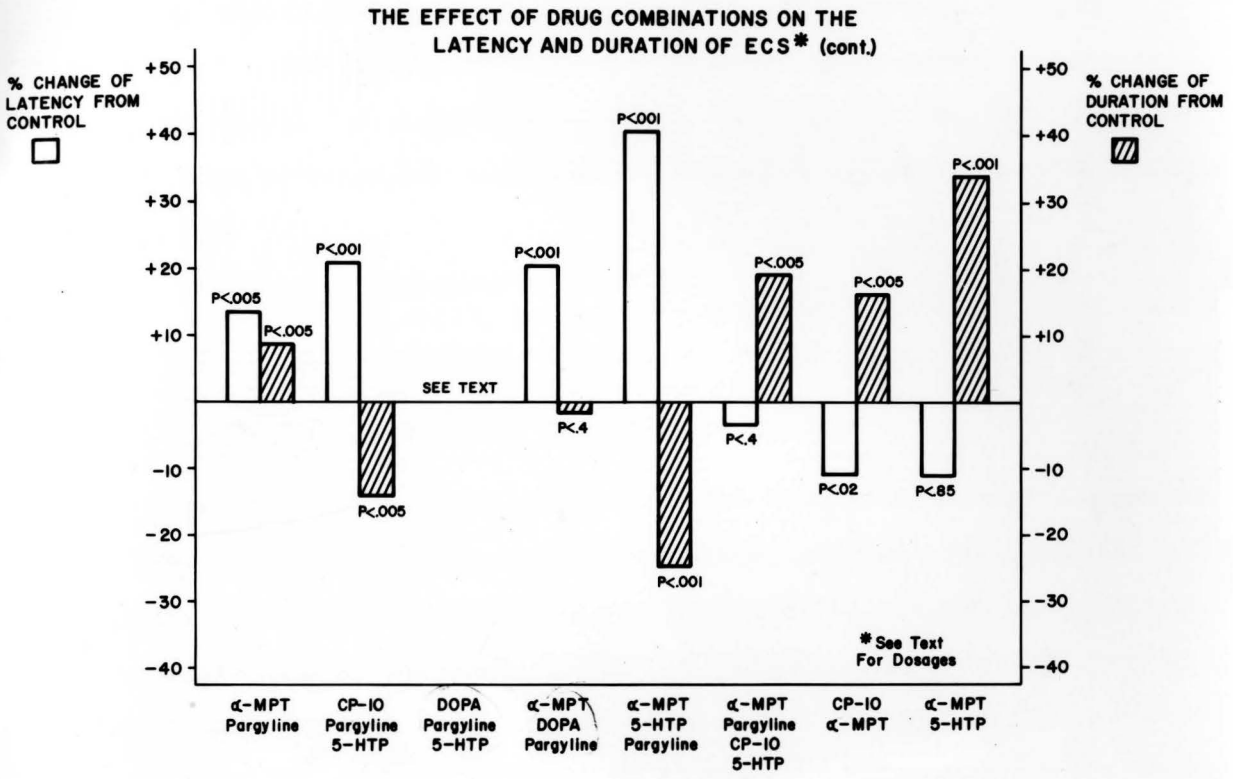

Figure 9b. Percent change from control latency and duration values for the various adrenergic and serotonergic drug combinations utilized in this study. P values are given for % change for each drug combination in the experimental group.

The administration of α-MPT and CP-10 together resulted in a decrease in latency and an increase in duration. α-MPT given with 5-HTP caused a marked increase in duration, but no significant change in latency.

The administration of α-MPT and CP-10 together resulted in a decrease in latency and an increase in duration, while that of α-MPT and of 5-HTP resulted in an increase in duration and a decrease in latency.

The fatality rates are interesting in this series in the fact that five combinations of drugs had somewhat increased fatality rates. These combinations are:

1.	pargyline and 5-HTP	36.5%
2.	α-MPT, reserpine and CP-10	37.4%
3.	pargyline, 5-HTP and -MPT	61.6%
4.	pargyline, 5-HTP and CP-10	40.0%
5.	pargyline, D, L-Dopa, 5-HTP	81.6%

In the case of the last combination the fatality rates were increased to diminish, statistically, any appropriate statement that could be made as to the latency or duration values (Figure 9b) However, as can be seen from these combinations, the drugs 5-HTP and pargyline are given together in four of these combinations and in three of these combinations (1, 3 and 5) the serotonin levels were raised to 400% or more above mean levels (Richardson, unpublished observations) with varying levels of catecholamines from 35% below mean to 200% above mean levels.

E. Methamphetamine

1. Acute Regimen

The data for methamphetamine given at various doses one hour prior to ECS are given in Table 9 and Figure 10. At all dose levels and one hour past injection, there was an increase in latency while there is a gradual, dose response, decrease in duration. The two higher dosages were associated with a 100.0% mortality following the seizure.

2. Chronic, constant, 3.0 mgm/Kgm of body weight dose

Table 9. The effect of acutely-given methamphetamine and chronic, increasing doses given over a five day period on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug and Regimen	Number of determinations	Latency	Number of determinations	Duration
Methamphetamine Acute 60 minutes prior to ECS				
3.0 mgm/Kgm	13	2.262±.088	13	16.554±.763
6.0 mgm/Kgm	14	2.471±.103	14	13.679±.579
12.0 mgm/Kgm	11	2.391±.092	8	12.506±.728
24.0 mgm/Kgm	8	2.200±.187	7	11.206±.764
48.0 mgm/Kgm		100.0% Fatalities		
96.0 mgm/Kgm		100.0% Fatalities		
Methamphetamine Chronic, Increasing dose				
Day #1 3.0 mgm/Kgm	23	1.776±.018	22	15.934±.280
Day #2 6.0 mgm/Kgm	23	1.872±.035	21	17.076±.484
Day #3 12.0 mgm/Kgm	19	2.313±.114	16	17.631±.565
Day #4 24.0 mgm/Kgm	3	2.250±.155	4	18.800±.1.463
Day #5 48.0 mgm/Kgm		100.0% Fatalities		
Control	259	1.947±.048	253	16.638±.309

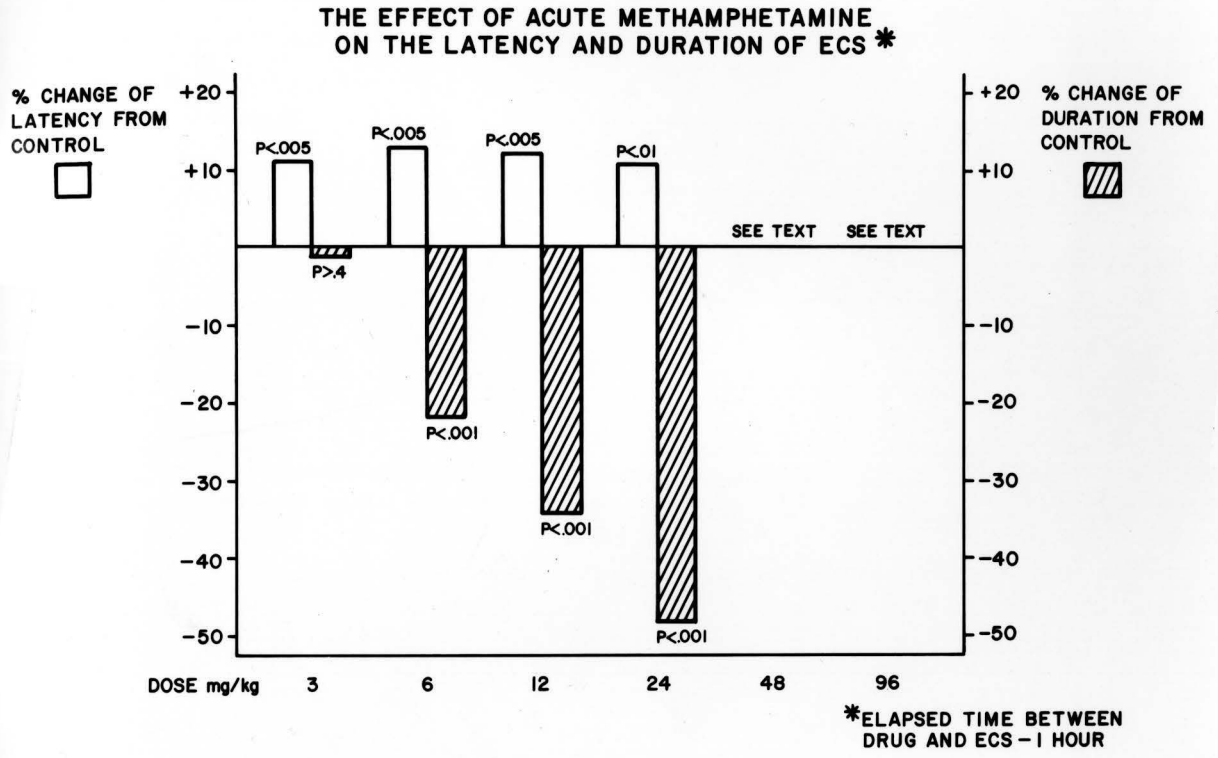

Figure 10. Percent change from control latency and duration values for methamphetamine given at various dosages one hour prior to ECS. P values are given for % changes for each dose in each experimental group.

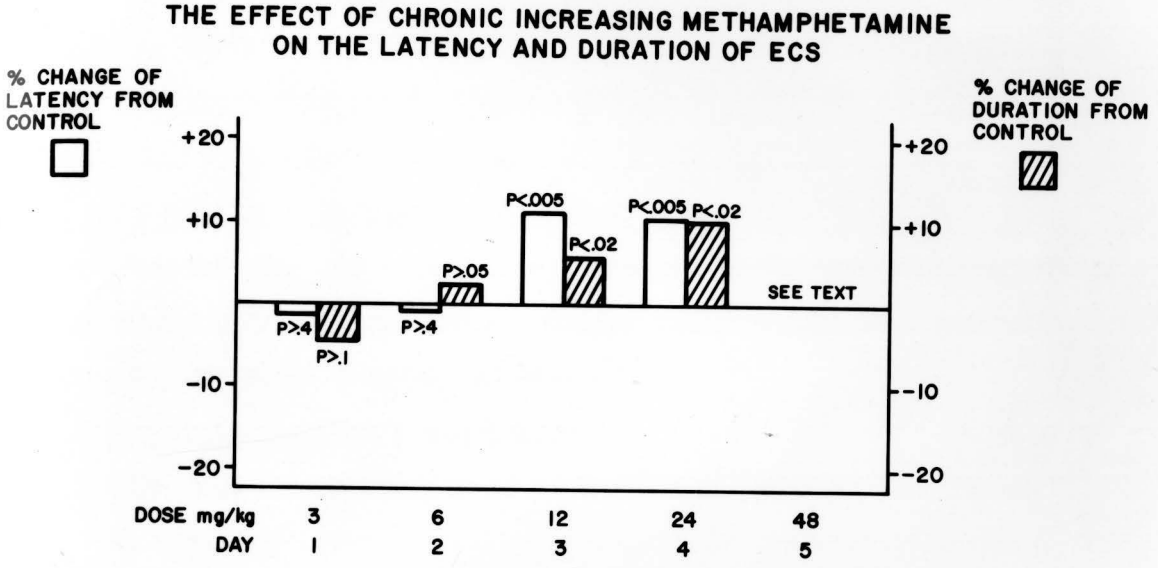

Figure 13. Percent change from control latency and duration values for methamphetamine given in a chronic increasing dosage with ECS induced on the morning of each day over a five day period. P values are given for % changes for each day of the experimental trial period.

The data for this particular regimen are given in Table 10 and Figure 11. In all cases, there is an increase in latency and decrease in duration which is apparently not dose-related.

3. Chronic, constant 7.0 mgm/Kgm of body weight dose

The data for this particular regimen are given in Table 10 and Figure 12. In all cases, there is an increase in latency and duration. However, there is evidence for habituation to the effects produced by methamphetamine on the durational changes. There is no evidence for habituation to changes in latency.

4. Chronic, increasing regimen

The data for this regimen is presented in Table 9 and Figure 13. There was a gradual increase in latency and duration from non-significance to significant statistical levels.

F. Miscellaneous Drugs and Regimens

The data for this series is presented in Table 12 and Figure 17.

1. Ethanol

Ethanol given at 15.0% by volume after both five minutes and one hour prior to ECS very markedly increased the latency and decreased the duration of the seizure. Interestingly, it was difficult to distinguish the tonic phase from the clonic phase, i.e. the tonic phase was not abrupt and tended to run into the clonic phase.

2. Enovid 10

Enovid 10 given at 1.0 mgm/Kgm of body weight and one hour prior to ECS failed to change the latency or duration. Female mice were analyzed against a female control after the female control failed to show any significant change in latency or duration against the male control group.

3. Isolated Mice

Mice isolated for a period of fourteen days with ECS induced on the morning of the fifteenth day failed to show a significant change in

Table 10. The effect of chronic, steady doses of methamphetamine given over a five day period on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug and Regimen	Number of determinations	Latency	Number of determinations	Duration
Control	259	1.947±0.048	253	16.638±0.309
Methamphetamine Chronic, steady				
Day #1 3.0mgm/Kgm	22	2.014±0.037	23	13.193±0.551
Day #2 3.0 mgm/Kgm	17	2.118±0.057	15	12.643±0.461
Day #3 3.0 mgm/Kgm	17	2.147±0.065	16	12.809±0.419
Day #4 3.0 mgm/Kgm	18	2.100±0.057	21	13.431±0.480
Day #5 3.0 mgm/Kgm	16	2.094±0.061	17	13.253±0.554
Methamphetamine Chronic, Steady				
Day #1 7.0 mgm/Kgm	21	2.119±0.036	22	23.789±1.112
Day #2 7.0 mgm/Kgm	16	2.131±0.104	17	19.412±0.946
Day #3 7.0 mgm/Kgm	14	2.089±0.064	14	19.311±0.768
Day #4 7.0 mgm/Kgm	12	2.196±0.144	13	18.504±1.143
Day #5 7.0 mgm/Kgm	9	2.128±0.049	8	19.288±0.808

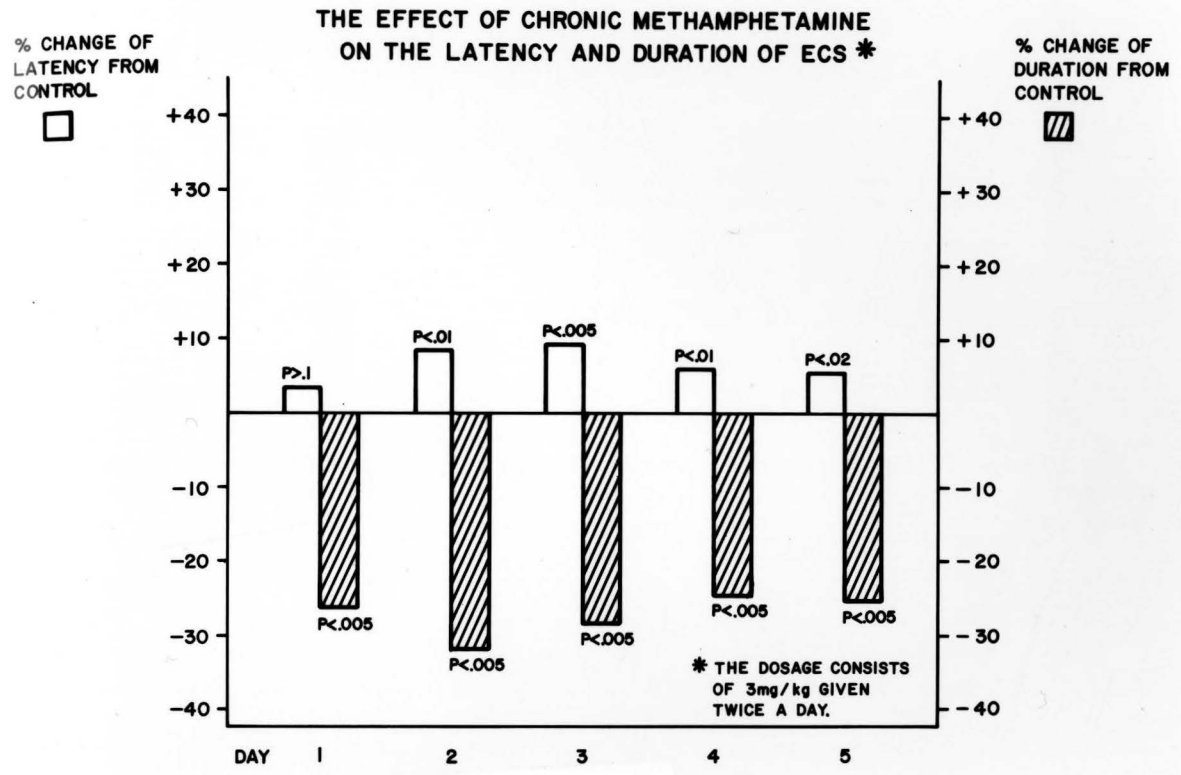

Figure 11. Percent change from control latency and duration values for methamphetamine given in a chronic steady dosage each day. P values are given for % changes for each day of the experimental trial period.

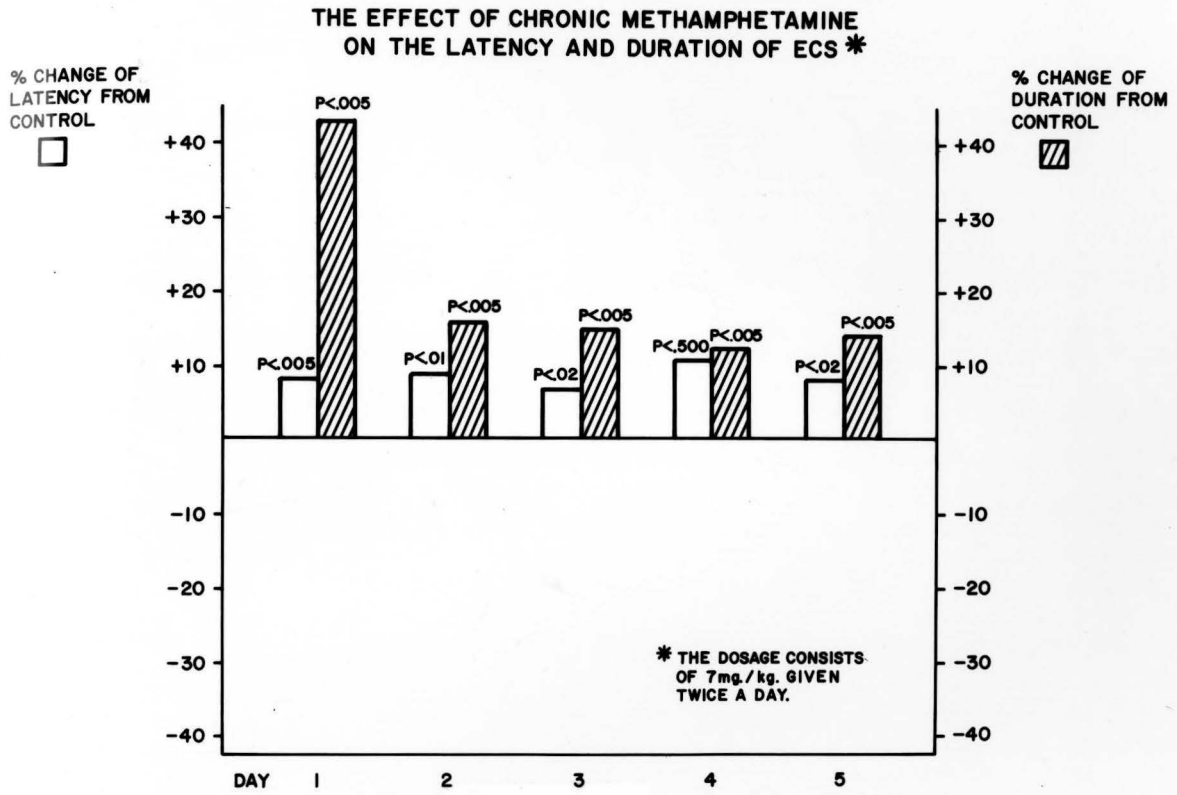

Figure 12. Percent change from control latency and duration values for methamphetamine given in a chronic steady dosage over a five day period with ECS induced on the morning of each day. P values are given for % changes for each day of the experimental trial period.

Table 12. The effect of miscellaneous drugs and regimens on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drugs and/or Regimen	Number of determinations	Latency	Number of determinations	Duration
Control	259	1.947±.048	253	16.638±.309
Ethanol-15%				
5 minutes prior to ECS	10	3.155±.196	13	11.277±.583
60 " " " "	16	2.784±.229	16	13.625±.741
Enovid 10 1.0 mgm/Kgm	31	1.871±.046	29	15.605±.339
female control	11	1.882±.046	11	15.973±.331
Isolated	14	2.043±.123	14	16.354±.582
Mice shocked after stress	11	2.127±.107	10	14.815±1.017
Fighting	5	2.230±.134	6	13.642±.428

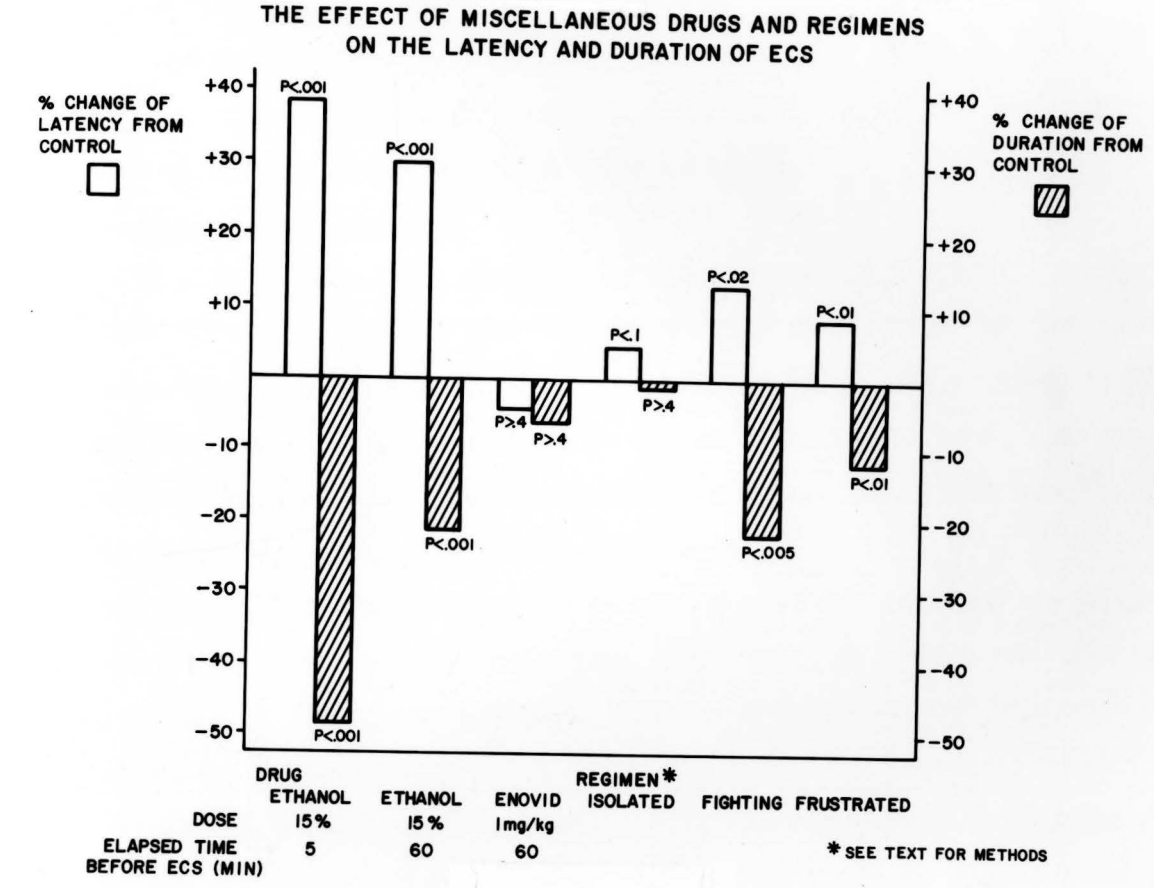

Figure 17. Percent change from control latency and duration values for the miscellaneous drugs and regimens utilized in this study. P values are given for % changes for each drug and/or regimen in the experimental group.

latency or duration.

4. Fighting Mice

Mice were isolated for fourteen days and were allowed to fight after being placed in pairs in cages. The latency was significantly increased and the duration was significantly decreased during this activity.

5. Stress

Mice, after undergoing an unavoidable shock situation, showed an increase in latency and a decrease in duration.

C. Methionine Sulfoxamine

The data for this drug given in various dosages and time periods prior to ECS is shown on Table 13 and Figures 19a and 19b. At the lowest dose, 25.0 mgm/Kgm after fifteen minutes and duration of the seizure was decreased but there was no significant change in latency. After one hour, the latency is increased with no significant change in duration. After four hours, there was no significant change in latency or duration.

At the 50.0 mgm/Kgm there is an increase in latency and a decrease in duration fifteen minutes after the drug. After one hour, there was no significant change in latency or duration. After four hours, there is an increase in both the latency and duration.

At the 75.0 mgm/Kgm there was no significant change in latency at any time period following the drug. After fifteen minutes there is a decrease in duration which disappears and then increases after one and four hours respectively.

The administration of 100.0 mgm/Kgm of body weight dosage, after fifteen minutes there is a decrease in duration and no change in latency. After one hour, there was a significant increase both of latency and duration. After four hours, the latency increased and the duration of the seizure decreased.

H. Parahexyl.

Table 13. The effect of methionine sulfoxamine and parahexyl on the latency and duration values (mean ± SE, seconds) of ECS in mice.

Drug	Number of determinations	Latency	Number of determinations	Duration
Control	259	1.947±.048	253	16.638±.309
Methionine Sulfoxamine				
25.0 mgm/Kgm				
15 minutes prior to ECS	16	1.988±.076	16	14.913±.483
60 " " " "	17	2.241±.121	16	16.297±.347
240 " " " "	12	2.017±.093	12	16.598±.523
50.0 mgm/Kgm				
15 minutes prior to ECS	14	2.204±.084	12	14.188±.365
60 " " " "	12	2.004±.071	11	15.686±.719
240 " " " "	14	2.161±.112		17.732±.450
75.0 mgm/Kgm				
15 minutes prior to ECS	19	1.963±.058	16	14.797±.390
60 " " " "	12	2.021±.516	14	15.761±.516
240 " " " "	13	1.988±.083	15	17.320±.515
100.0 mgm/Kgm				
15 minutes prior to ECS	13	2.023±.095	14	14.664±.797
60 " " " "	17	2.076±.069	17	18.032±.503
240 " " " "	16	2.281±.174	15	15.587±.460
Parahexyl				
3.0 mgm/Kgm	13	1.892±.059	13	15.812±.720
15 minutes prior to ECS	11	2.018±.099	11	16.100±.515
60 " " " "				
7.0 mgm/Kgm				
15 minutes prior to ECS	10	1.850±.072	11	15.364±.475
60 " " " "	8	2.144±.144	6	14.817±.603
15.0 mgm/Kgm				
15 minutes prior to ECS	16	1.978±.077	16	17.244±.563
60 " " " "	16	2.147±.106	15	17.380±.767

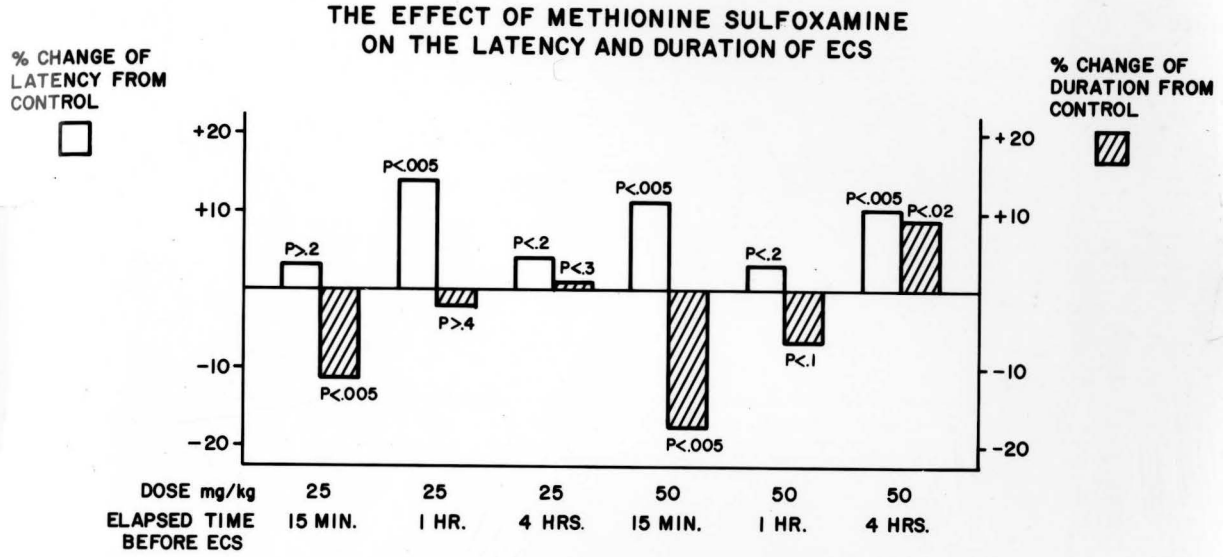

Figure 19a. Percent change from control latency and duration values for methionine sulfoxamine given at various dosages and time periods prior to ECS. P values are given for % changes for each regimen of methionine sulfoxamine in the experimental group.

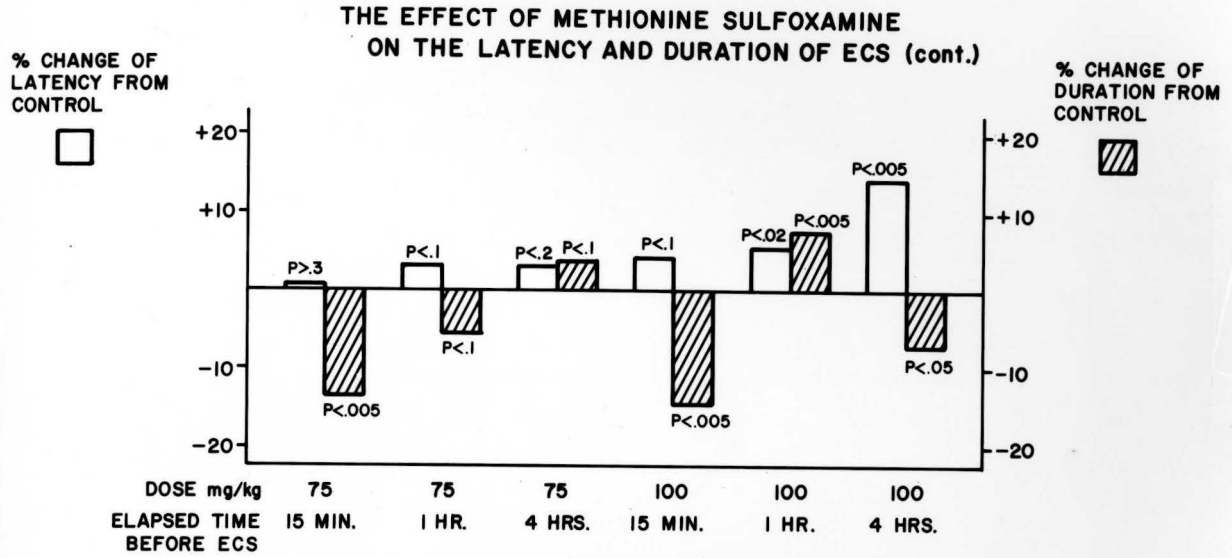

Figure 19b. Percent change from control latency and duration values for methionine sulfoxamine given at various dosages and time periods prior to ECS. P values are given for % changes of each regimen of methionine sulfoxamine in the experimental group.

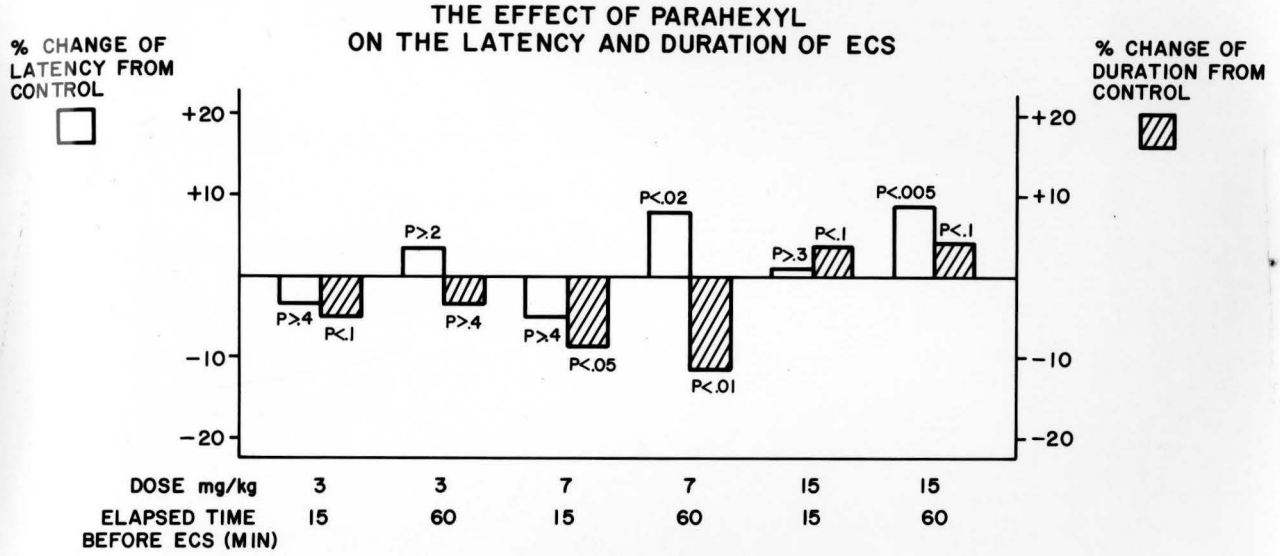

Figure 18. Percent change from control latency and duration values for parahexyl given in various dosages and time periods prior to ECS. P values are given for % changes for each regimen of parahexyl in the experimental group.

The data for this drug is given on Table 13 and Figure 18. At the lowest dose of this drug there was no significant change in latency or duration at any time period. At 7.0 mgm/Kgm after fifteen minutes there was a decrease in duration while after one hour there was an increase in latency and decrease in duration. At 15.0 mgm/Kgm after fifteen minutes there was no significant change in latency or duration. After one hour there was an increase in latency with no significant change in duration.

Discussion

A. Introduction

This discussion is divided into two sections. The first, dealing with a pharmacological analysis of the data, attempts to present the results in the light of recent pharmacological information on some of the mechanisms and action of these drugs. The second reviews the data in reference to a general systems analysis. It represents an effort to synthesize the patterns, shown by ECS latency and duration, of the organism's reaction to the overwhelming stimulus and bring them into a coherent whole. These sections are not clearly separable; the second section builds and enlarges on the concepts developed and clarified in the first. Therefore, the latter section assumes mechanisms that are postulated and discussed in the preceding parts of the thesis. In part this analysis demonstrates that there are many ways of perceiving or interpreting the data, all of them interconnected in a suitable frame of reference without which they may appear to be independent.

B. Pharmacological Analysis

1. Adrenergic and Serotonergic Drugs

From previous studies it has been shown that reserpine depletes the brain of its dopamine, norepinephrine and serotonin contents (cf for instance Carlsson et al., 1958). The mechanism underlying this phenomena is open to debate at the present time, but generally it is thought to be one concerned with a long-lasting block of the storage function of the amines (Dahlstrom et al., 1965). In a different manner, D, L-dihydroxyphenylalanine employed in the dose range utilized in this study, has been shown to increase dopamine and to a lesser extent, norepinephrine levels in the central nervous system. However, this drug has minimal effects on the levels of serotonin in the brain (Hillarp et al., 1966). Alpha-methyl-paratyrosine (α-MPT) an inhibitor of tyrosine hydroxylase, has been shown to inhibit catecholamine synthesis

(Spector et al., 1965), while para-chlorophenyalanine (CP-10) inhibits tryptophan hydroxylase resulting in a depletion of serotonin in the central nervous system with little or no effect on catecholamine levels (Koe and Weissman, 1966) Five-hydroxytryptophan (5-HTP) is the immediate precursor of serotonin (Udenfriend, 1959) resulting in a specific increase of serotonin levels in the brain. Finally, pargyline is a monoamine oxidase inhibitor (MAOI) which acts to raise levels of serotonin and dopamine in the central nervous system (Richardson, unpublished data).

The data on the ECS effect of the adrenergic drugs are relatively consistent, permitting convincing correlations. As shown before (Toman et al., 1946) increasing levels of amines centrally leads to an increase in latency; the decrease of the levels of central amines, leads to an increase in latency. However, Toman (personal communication, 1969) was never able to demonstrate a change in duration of the seizure correlated with catecholamine levels. In Figures 8, 9a and 9b we would show that with increases in amine levels there is a decrease in seizure duration and with decreases in central catecholamine levels there is an increase in seizure duration. In Figure 8 the seizure duration values for reserpine and D, L-Dopa were directly opposed which was correlated with their opposing effects on catecholamine concentration in the brain while from Figure 9a it is seen that D, L-Dopa given with reserpine will directly antagonize its action resulting in no significant change in either latency or duration. This antireserpine effect of D, L-Dopa has been demonstrated many times both pharmacologically and behaviorally (Carlsson et al., 1957; Blaschko and Chrusciel, 1960). Additionally a MAOI potentiates the effect of D, L-Dopa on the seizure pattern (Figure 9a), as would be expected if the effects of D, L-Dopa on the seizure were mediated through catecholamine levels.

Similarly, α-MPT as a specific depletor of central catecholamines increased seizure and possibly decreased latency (Figure 8). However, when

given in combination with reserpine the results are indicative of a depletion of catecholamines (Figure 9a). Furthermore the changes in duration are potentiated suggesting that the catecholamines may be more closely associated with durational changes than latency changes. Although this point is debated and others have shown an initial rise in catecholamines with α-MPT given in combination with reserpine (Spector, 1966), data from this laboratory (Richardson, unpublished observations) have demonstrated a synergistic depression of catecholamines with reserpine and α-MPT given in combination. CP-10 given in combination with reserpine and α-MPT (Figure 9a) results in a potentiation of the former combination with extremely high durational values. When α-MPT was given in combination with a MAOI (Figure 9b) an increase in latency and duration resulted. Data from this laboratory indicates that with this particular combination there is a decrease in brain catecholamines of approximately 25%-30% thus accounting for the increase in duration, but also a 380% increase in serotonin levels which could possibly account for the increase in latency.

The data for the drugs which affect the central serotonin levels are less clear. 5-HTP alone (Figure 8) increased latency and duration while CP-10 alone resulted in an increase in latency and caused no significant change in duration. Both these drugs appear to act specifically on serotonin levels (Richardson, unpublished observations) to raise or lower the levels of serotonin respectively. Moreover, CP-10 potentiates reserpine's effect resulting in the largest decrease in latency and increase in duration in this series (Figure 9a). Data from this lab confirms this fact with levels of brain amines and serotonin. 5-HTP given with reserpine results in an increase in duration corresponding with a decrease in catecholamines while the latency values return to mean levels (Figure 9a) Again, data from this laboratory confirm this result with serotonin returning to mean levels while catecholamine levels remain decreased. This data may indicate that serotonin's role may be that of a potentiator of the catecholamines as far as the durational values are

concerned, but exert its own distinct effect on the latency of the seizure. Pargyline, as an MAOI, results in an increase in central serotonin and norepinephrine levels (Shore, 1962; Richardson, unpublished observations). Given in combination with 5-HTP (Figure 9a) it induces a potentiation of the effects seen with increased levels of catecholamines such as D, L-Dopa given alone (Figure 8). However, the effect of an increased duration when 5-HTP is given alone is completely antagonized by pargyline. This may suggest that the increase in catecholamines play a major role with regards to the durational values. This may be borne out by the results due to the combination of CP-10, pargyline and 5-HTP (Figure 9b). Here the increase in serotonin and amine levels is not as great as in the case of 5-HTP-pargyline combination (Richardson, unpublished observations), and consequently the effect of the increased amines is diminished somewhat.

CP-10 given with α-MPT and 5-HTP given with α-MPT (Figure 9b) both potentiates α-MPT's effects on the duration of the seizure. As the serotonin levels in the brain rise the durational changes are potentiated. Yet with both combinations the changes in catecholamines, were decreased in a similar manner as did the latency values. When α-MPT, pargyline, CP-10 and 5-HTP were given in combination (Figure 9b) the amine, serotonin and durational levels remain approximately the same as with the α-MPT, and 5-HTP combination yet the latency does not significantly change. This may point to a specific effect of pargyline or of CP-10 on the latency values. If a comparison is made with α-MPT and 5-HTP versus α-MPT, 5-HTP and pargyline (Figure 9b) it is seen that the addition of pargyline reverses the latency and duration values to a great degree. Recent experimental evidence (Aghajanian et al., 1970) demonstrated that the MAOI in the same dosage used in this study exerts a specific response in depressing the firing of serotonin neurons in the Raphe nuclei of the brainstem, while 5-HTP does not exert this effect.

In summary, it is seen that with an increase in catecholamines result in an increase in seizure latency and a decrease in seizure duration; the opposite effects were found to hold true. D, L-Dopa will antagonize reserpine's effects on the seizure parameters while 5-HTP antagonized only the D, L-Dopa induced increase in latency. In general, drugs that act on the serotonin levels will potentiate the effects of the changes in catecholamines in so far as durational calues are concerned. The effect of serotonergic drugs on the latency of ECS remains unclear and further work must be done to clarify its mechanism.

Bertler and Rosengren (1959) as well as Carlsson (1959) reported that the levels of dopamine in the brain were highest in the caudate nucleus and putamen while levels of norepinephrine were highest in the hypothalamus. Hillarp et al. (1966) state that cells that stain for five-hydroxytryptamine (5-HT) are in the lower brain stem localized in the Raphe complex while catecholaminergic nerve cells were located laterally in the same area. Hornykiewicz (1966) states that dopamine cells are found predominently in the cephalic portion of the mesencephalon while norepinephrine and 5-HT-containing cells are found in the caudal portion of the mesencephalic reticular formation. In the spinal cord, Hillarp et al. (1966) reported that 5-HT and norepinephrine-containing cells occupy approximately similar distributions, i.e. highly concentrated in the sympathetic lateral columns and the lateral motor cell area. There are no dopaminergic neurons yet found in the spinal cord.

Physiologically a case may be stated for catecholamines and serotonin causing behavioral excitement and electrophysiological alerting (Marley, 1966) and depression respectively. Dispute has arisen over the effects of dopamine versus that of norepinephrine as mediators of catecholamine activity. Some authors feel (Krnjevic and Phillis, 1963) that L-Dopa may have an effect of its own rather than merely as a precursor for dopamine or nor-

epinephrine. The data presented in this study indicates that catecholamines, acting through the reticular formation, affect a behavioral and electrophysiological excitatory activity. It might be postulated that the latency is increased because there is increased random firing from the disorganized neurons. The duration, however, is decreased because there is activation of the reticular formation resulting in less time for the tonic phase to be completed. Serotonin's role, as potentiator of the adrenergic system is shown only in the durational values and therefore the reticular formation activation. This effect of serotonin is a late one in terms of pregression of the seizure; the data on its initial effects on latency are confusing and need further clarifying. Koella et al. (1960) believe that serotonin's primary role lies in its modulating behavioral activities.

Data from this laboratory on brain amine levels in association with the drugs used in this study do not point to a specific effect of either dopamine or norepinephrine as a primary mediator of the catecholamine effect. Rather the data point to a centrally located, catecholamine action.

On the other hand, there is good evidence that the role of catecholamines in seizure may be peripherally located. Certainly the location of norepinephrine-containing neurons in the lateral aspect of the reticular formation may point to a role in sensitizing this structure and raising its level of activity. Baust et al. (1963) believe that arousal after injection (I.V.) of catecholamine in cat encephale isole preparations was peripheral in origin and mediated through the reticular activating system. Livingston (1957) has shown that the reticular activating system does, in fact, mediate sensory cortical input. Similarly, the lethal effect of the combination of 5-HTP and pargyline may be a peripheral one stemming from either highly increased peripheral levels of serotonin or increased activity, beyond tolerance levels, of the reticular formation. Further studies in this area are certainly needed to clarify these mechanisms.

2. Methamphetamine

Amphetamines are generally believed to act through a mechanism involved with catecholamines (Dingell et al., 1967; Hanson, 1965) although the specific action is disputed. Smith (1963) and others have found that reserpine and the reserpine-induced norepinephrine depletion does not exert an anti-amphetamine effect, yet Weissman et al. (1966) found that Alpha-methyl-tyrosine (-MT) which blockades the synthesis of norepinephrine antagonizes amphetamine effect with regard to certain behavioral parameters. In Figure 10 it is shown that a various doses of methamphetamine, the latency is increased while the duration is decreased. The latter value shows a dose-response relationship.

In this study it is assumed that methamphetamine is closely related pharmacologically to the amphetamines although exhibiting a larger than amphetamine central to peripheral effect ratio (Goodman and Gilman, 1970). At 12 mgm/Kgm of body weight the fatality rate was 38.0% while at 24 mgm/Kgm of body weight the fatality rate was 60.0%. At 48 mgm/Kgm of body weight the fatality rate was 100.0% which shows an acceptable lethal dose-response curve.

Methamphetamine is seen to act on the seizure variables in a manner similar to the catecholamines, i.e. induces increase in latency and decrease in duration. It is interesting to note that while the effect on latency was relatively fixed, the duration shows a dose-response curve. The latency values follow those shown by Toman et al. (1957) using amphetamines. Data from this laboratory (Richardson, unpublished observations) shows that with measurements taken one hour after injection of methamphetamine at these dosages the neurotransmitters concentration rises, although the dose-response relationship is seen only with the rise exhibited in serotonin levels. This will be discussed at length later in this section.

Figure 11 shows the changes in latency and duration associated with

methamphetamine at 3.0 mgm/Kgm given twice a day for five days. The amine levels measured at this laboratory and by others (McLean and McCartney, 1961) shows that this response induces a gradual decrease in the catecholamine levels in the central nervous system. Yet, the ECS pattern does not indicate this concentration change in any way. However, McLean and McCartney (1961) measured a significant increase in the serotonin levels in brain. This may be a possible explanation for the response observed.

A dose of 7.0 mgm/Kgm of body weight, given twice a day for five consecutive days, induced a more or less fixed increase of latency while the duration showed first an increase and then significant tolerance with regard to the durational changes. Two possible explanations arise: first, the durational values may be associated with the rise in serotonin levels previously shown by McLean and McCartney (1961). Confirmation of this is given in Figure 8 for 5-HTP given alone. Therefore at this dosage methamphetamine affects serotonergic receptors in the caudal mesencephalon. Secondly, the effects observed may be specific for this dosage of methamphetamine acting to combine with norepinephrine receptors made available by depletion of this neurotransmitter by the administration of methamphetamine as shown previously by Handon (1965). The latter explanation appears to be more tenable since the amphetamines have been shown to activate the reticular formation (Goodman and Gilman, 1970) resulting in both electroencephalographic and behavioral evidence of alerting. (Bradley and Elkes, 1953; Bradley, 1957)

Bradley and Key (1958) demonstrated that amphetamines lower the threshold of the reticular formation to electrical stimulation. However, this was not dose dependent. As shown from Figure 11 and 12 strikingly dissimilar changes are produced in durational values dependent on the dosage of methamphetamine that was used.

In the case of methamphetamine given in gradually increasing doses

over a five day period, no tolerance is shown to the fatality rates with this regimen due to the fact that at 24.0 mgm/Kgm the fatality rate was 60.0% while at 48.0 mgm/Kgm the rate was 100.0%. As can be seen in the figure there is an abrupt increase in latency on the third day while the duration values show a gradual change from a non-significant decrease on the first day to a significant increase on the fifth day (Figure 13). A threshold phenomena would not be expected in regards to latency particularly when previous data (Figures 11 and 12) show at lower dosages, slight a large increase in latency. Data from this laboratory (Richardson, unpublished observations) do not show any abrupt changes in catecholamine at this time; however, there is an abrupt rise in serotonin levels on the fourth and fifth days. This may lend further support that serotonin may augment catecholamine effects on duration values through serotonergic pathways in the lower reticular formation. On the other hand, as the dose in increased to above 6.0 mgm/Kgm of body weight changes appear to shift in the seizure pattern so that the duration is increased. Thus the change in duration from chronically injected methamphetamine at 3.0 mgm/Kgm of body weight (Figure 11) to 7.0 mgm/Kgm of body weight (Figure 12) reflects an increase in duration while with chronically increasing doses above 6.0 mgm/Kgm of body weight the duration increase significantly. (Figure 13) This may reflect a specific effect of methamphetamine related to low or high dosages when given in a chronic regimen. In acute regimens, the duration shows a dose dependent decrease (Figure 10).

In summary then it is seen that in the case of acute administration of methamphetamine the latency is increased while the duration decreases in a dose dependent manner. This may reflect methamphetamine's effect as a sympathomimetic agent associated with rises in catecholamine and serotonin levels in the central nervous system. At low chronic doses, methamphetamine acts again as a sympathomimetic agent. No tolerance is shown at the lower dosage. At higher doses methamphetamine may exert a specific effect

or attach to serotonin receptors rather than catecholamine receptors. The dose of 6.0 mgm/Kgm of body weight may represent a threshold limit in a gradual shift from sympathomimetic activity to serotonergic activity.

3. Controls and Fatality rates

Figures 5 and 6 show the findings for the control groups (each control group consisting of twenty or more animals) and the changes of latency and duration values of the ECS with the age of the animals. There was no significant change in latency or duration for a period of thirty to forty days during which the animals were randomly shocked approximately twice a week. This period of time represents approximately 3.5% of the total life span for this species of mouse (Abrams Breeders, personal communication). This non-significant change in latency is in conflict with a earlier report (Toman et al., 1957) which states that latency increases with age. However, the study cited does not give any variables associated with that statement, nor is there any reported data concerning the duration values. Woodbury and Davenport (1952) state that the threshold for ECS in male Sprague-Dawley rats increases 0.022 mA with each gram increase of body weight. They also associate the increase in threshold to age of the animal. Worum and Proszasy (1968) report that an increase in GABA occurs in the central nervous system with aging and relate this to an over-all inhibition of activity. However, these differences were measured over a six month period for Sprague-Dawley rats, rather than to mice.

To view the data differently perhaps repeated ECS may result in a change in the concentration of neurotransmitters resulting in the reported increase in latency. Huesley et al. (1968) found that twenty minutes and 24 hours, respectively after single or multiple shocks, the 5-HT levels in the pons and medulla rose significantly while twenty-four hours after this treatment the norepinephrine levels rose significantly. However, in rats treated with pseudoshock similar results occurred and the authors state that over-all

significance of the data is lacking. Kety et al. (1967) found an increase in norepinephrine turnover in the central nervous system with rats treated with ECS once a day for eleven days. Pryor and Ctis (1969) reported an increase in acetylcholinesterase activity in rat brains after two weeks while an increase in MAO occurred after one week. However, the rats were treated either daily or twice daily with maximal ECS. The results presented in this study are not inconsistent with these findings primarily because the mice were shocked approximately twice a week for only a thirty day period. These other studies include far greater frequencies of ECS and duration of study thus allowing for changes to appear that may be compensated for in this particular regimen.

Figure 7 shows the fatality rates for the control groups. After reaching a peak of 27.0% rate the fatality percentages dropped after the control groups were subjected to repeated ECS. There are several alternative explanations for these data. It is possible that the animals with lower thresholds for ECS were diminished after repeated shocks; the lower thresholds to ECS resulting in a higher state of excitement within the brain stem and a consequent destruction of centers vital to life. It is also conceivable that this excitability is related to neurotransmitter levels and consequently the control groups after the first deaths had changed transmitter values and were no longer randomized. This possibility does not hold when the changes in time for latency and duration are seen. Since there were no significant changes, one must conclude that if the levels of neurotransmitters had changed they were at a level that if the levels of neurotransmitters had changed they were at a level that did not affect the latency or duration values. Conceivably, some animals expired and were not included in the mean values; thereafter, the latency and duration did not change because these animals were not included. This is a possibility, but intuitively one might feel that the means would not be as consistent as the data shows.

It is possible that peripheral effects may have played a part in the fatality rates. That the ECS is an overwhelming stimulus which requires a totally efficient organism is an important idea. One may say that a selective process was taking place with each trial of seizures for it is obvious that the animals which could adapt to the stimuli without destroying vital brain centers, survived. However, any infections, any pathological process in the lung tissue resulting in poor tissue perfusion and a period of hypoxia exceeding that tolerated by the organism would result in death of the animal. Any myocardial damage previous to the stimuli would certainly be aggravated by the tremendous demands put on this system by the convulsive process. Any structural abnormalities resulting in weakness of arterial walls would lead to massive hemorrhage and death. These and a host of other peripheral effects are sufficient to account for the fatality rates observed in the study. It is maintained here that the initial deaths represent a removal of these randomly aberrant animals.

As 27.0% was the highest value for the fatalities, this figure was arbitrarily selected as a cut-off point and any value in the experimental groups that exceeded it was reported as a significant increase in fatality rates. These "abnormal" rates have already been mentioned and will be so stated in the entire discussion of the thesis.

4. Cholinergic Drugs

Although there has been no direct evidence of acetylcholine's (ACh) function as a neurotransmitter in the central nervous system there is solid indirect evidence of its activities (Karczmar, 1970). Eccles (1964) and others have given the criteria for the prerequisites for a substance to be considered as a chemical synaptic transmitter, and Sobotka (1969) has reviewed the evidence for ACh's role and found it entirely favorable. In any case, the presumption of a central cholinergic mechanism does not, at the present time, seem unwarranted.

Hano, et al. (1964), Karczmar et al. (1968) and others have found ACh to be present in brain of mice including Mus musculus. Furthermore, ACh does seem to be specific for grey matter of the brain (MacIntosh, 1941) although fibrous areas such as the corpus collosum and the internal capsule have significant amounts thus arguing for a specific function. Fink and Urban (1966) found that ACh levels were highest in the basal ganglia. Others have found that the midbrain-diencephalon area (comprising the hypothalamus, thalamus and micbrain) contain the largest amounts with the pons-medulla, telencephalon and cerebellum following in that order in decreasing concentration (Aprison et al., 1968; Bowers et al., 1966; MacIntosh, 1941).

On the other hand, Krnjevic (1969) using acetylcholinesterase determinations (AChE) found a general agreement with the distribution of ACh, although he states that the cholinergic pathways, if present, must belong to a series of pathways distinct from the fast conducting tracts best known to the neurophysiologists.

Despite the classical work reviewed by Eccles (1969) and Curtis and Eccles (1958a, 1958b) and others on the activity of Renshaw cells, there is still much dispute concerning the cholinergic mechanisms elsewhere (for review cf. Karczmar, 1969). The Renshaw cell with its nicotinic character, high sensitivity to ACh and rapid excitatory effect seems to be opposed to the general cholinergic response in the central nervous system which is slow in its excitatory effects, may be blocked by atropine and is muscarinic in character. There have been numerous studies concerning the muscarinic excitatory effects of forebrain cells innervated by an ascending system originating in the striatum and tegmentum (Krnjevic and Silver, 1965; Krnjevic and Phillis, 1963). On the other hand the nicotinic excitatory effects in the thalamus (Anderson and Curtis, 1964) and medulla (Bradley et al., 1966) and the muscarinic depression of neuronal firing in various areas. (Bradley et al., 1966; Curtis et al., 1966).

The significance of these findings vary with the investigator's opinion. Krnjevic (1969) believes that the data points to ACh mediating the durational response of the central nervous system to stimuli. ACh promotes repetitive discharges in response to single stimuli resulting in a "general facilitatory" action on the cortex. Its muscarinic, slow-acting effect may mediate tonic arousal of the brain. As a caution to this type of interpretation Karczmar (1969) warns that in many areas, the cortical cells' response depends on the state of arousal of the central nervous system and not on how the cell reacts to applied ACh. Cholinergic synapses may mediate noncholinergic, inhibitory phenomena or they may be coupled with inhibitory interneurons. To make the issue even more intractable, the Burn-Rand hypothesis (Burn and Rand, 1962) states that the cholinergic synapse may operate through the release of another non-cholinergic transmitter or mediator. Functional activity may result in decreases of ACh levels in the brain (Giarman and Pepeu, 1962) or increases in ACh levels in the brain; the latter is suggested indirectly by the increases in AChE activity (Rosenzweig et al., 1968) induced by certain behavioral paradigms. As Karczmar (1969) states:

> "The functioning of the cholinergic synapse has to be carefully evaluated in terms of the state of its membrane, of simultaneous activity of other synapses, and of the interplay of ACh with other transmitters or modulatory substances. Indeed, studies of the cholinergic system demonstrated admirably the cybernetic character of the central nervous system."

Scopolamine, a potent central nervous system cholinolytic (Goodman and Gilman, 1970) decreased the duration significantly at 0.03 mg/Kgm given fifteen minutes prior to ECS while the latency was increased very slightly.

This pattern is similar as that seen with an increase in brain catecholamines (Figure 8). However, after one hour the latency and duration values remained unchanged. This pattern may represent several different effects. Scopolamine has been shown to lower the ACh levels in both the cerebral and mesencephalic areas of the brain (Fink and Urban, 1966; Sobotka, 1969) with brain levels approximating those of the control after one hour. Although dissimilar results have been found (Beani et al., 1964) it is postulated that scopolamine does indeed alter ACh levels allowing several possible alternatives. The first may be a lessened antagonism to a generalized adrenergic-serotonergic system allowing this system to assume dominance over the ECS patterning (Carlton, 1963; White and Rudolph, 1968). This is possibly the most likely explanation for the changes seen after one hour. However it is conceivable that scopolamine due to its EEG depressing action (Bradley, 1968) may depress the cholinergic arousal system (Rinaldi and Himwich, 1955; Krnjevic, 1969) altering the ascending reticular formation. Another theory put forth by Meyers and Domino (1964) states that the depression of the cholinergic system interferes with the organization of sensory stimuli. This would explain the increase in latency as a function of disorganization, but wouldnnot explain the decrease in duration which represents significantly greater organization of the stimuli and a resultant shorter seizure period.

At the dose of 1.0 and 2.0 mgm/Kgm of scopolamine administered fifteen minutes prior to the ECS the fatality rate was 94.0% and 100% respectively ending the collection of significant data as to the ECS parameters. These fatality rates may represent a lowering of ACh levels to the point of a minimal reaction to the stimuli by the organism resulting in death. Sobotka (1969) found that with 2.0 mgm/Kgm of body weight of scopolamine given in the same regimen, the levels of ACh dropped to 51.0% of control value in the telencephalon and 37.0% of control value in the midbrain-diencephalon area respectively. Behavioral excitant effectssof scopolamine

could also explain the increased fatality rates at these dosages and regimens. Following Meyers and Domino (1964) theory, a paralysis of the sensory system might result in complete disorientation during the convulsion and subsequent anoxia of the medullary respiratory centers. In contrast to these theories, it has been suggested (Takahashi et al., 1961; Kurokowa et al., 1963) that seizure intensity is directly related to ACh levels of the central nervous system. Therefore one would not expect a higher seizure intensity resulting in greater fatalities with lower ACh levels as demonstrated by Sobotka (1969).

At 1.0 and 2.0 mgm/Kgm of scopolamine given one hour prior to the ECS significantly increased the seizure latency paralleling the effects seen at the lower dosage, while the duration showed significantly increased only with the larger dose. This data are in keeping with the disorganization of sensory stimuli theory of Meyers and Domino (1964): a general lowering of ACh level would lead to a disorganization of sensory stimuli, a consequent loss of habituation to sensory stimuli (Carlton, 1968). This would explain prolonged latency and duration. Sobotka (1969) found that one hour after the administration of 2.0 mgm/Kgm of scopolamine the levels of ACh in the midbrain-diencephalon had returned to normal control levels while the levels of ACh in the telencephalon had remained significantly below control levels. This may suggest a cortical control of the temporal activity of the seizure with lower ACh levels resulting in lower cortical excitability and therefore less feedback into the reticular formation resulting in a prolonged tonic phase. In this regard, Longo (1966) postulated that a lowering of ACh in the cortex retards integration of an aggression-generating stimulus. If this were so, the initial integration of the low amplitude, high frequency arousal input of the ascending reticular formation seen in the tonic phase of the seizure (Figure 1 and 3) could be retarded and the duration thus prolonged.

Multiphasic effects were obtained with physostigmine given at various doses and times prior to the ECS. At 0.001 mgm/Kgm of body weight given fifteen minutes prior to the ECS there is a significant decrease in duration with no change in the latency value from control levels. This dose given one hour prior to the ECS shows no significant change in either latency or duration. This effect of a shortened duration at the lowest level of physostigmine dosage is a minimal one and certainly open to question. Although physostigmine is a potent anticholinesterase (anti-ChE) resulting in increased levels of ACh (Goodman and Gilman, 1970) in the central nervous system, this data parallels that of an increase in catecholamine pattern. (Graph 8) However, antiChE's are known for their propensity in inducing the "divorce" phenomena (Karczmar, 1969) in which EEG alerting is produced without concomitant behavioral arousal. Similarly, the propensity of these compounds to produce "fast" sleep suggests that they may act through a thalamic-reticular activating pathway thus not affecting the latency yet shortening the duration of the seizure. Koelle's hypothesis (Koelle, 1966) of the anti-ChE's acting through a percussive mechanism liberating modulatory substances particularly catecholamines, may explain the data. As can be seen at the dose of 0.005 mgm/Kgm of body weight given at both fifteen minutes and one hour prior to the ECS this same relationship holds i.e. a decrease in duration with no significant change in latency with a general tendency for the changes in duration to return to control levels after one hour.

However, at the two higher dosages, i.e. 0.01 mgm/Kgm and 0.05 mgm/Kgm changes in latency are seen after fifteen minutes with a curious "rebound" phenomena after one hour. The changes in duration parallel the changes seen at the two lower dosages. Thus the pattern seen at the two higher doses are closely related to the pattern associated with increased levels of catecholamines (Figure 8) and the lower dosage of scopolamine,

(Figure 15) and may be a result of an increase in the activity of the adrenergic-serotonergic system or a depression of the cholinergic system allowing for a dominance of the former system. (Carlton, 1963; Goodman and Gilman, 1970) The data in this dose range may point to Koelle's percussive mechanism acting on generalized cholinergic pathways releasing amines. The tendency for antiChE's to produce long-acting repetitive responses and through a basically adrenergic activity may illustrate the "cholinergic-link" phenomena described by Burn and Rand (1962).

At 0.005 mgm/Kgm of body weight given fifteen minutes prior to ECS there was a significantly higher fatality rate of 36.5%. This isolated finding is not given any significance for the drug in this particular regimen.

Figure 16 illustrates the effect of diisopropyl phosphoroflouridate (DFP) on the seizure pattern. DFP is unique in the fact that it results in a so-called "irreversible" inactivation of AChE through a mechanism of alkyl-phosphorylation of the esterase. However, Karczmar et al. (1970) warn that the term "irreversible" may be a misnomer for the chemical reactions are similar for all inhibitors of AChE. In addition, compounds known as reactivators activate AChE whether the enzyme is inhibited by reversible or irreversible inhibitors. In any case, it is generally agreed that the duration of inhibition of DFP is certainly longer than one hour which is the longer of the two time periods utilized in this study.

From the figure it is seen that there are no significant latency changes at any given dosage or time period. Although this general lack of effect on latency does parallel that seen with physostigmine given at lower doses, there is no "rebound" phenomena on the latency values as seen with the higher doses, of physostigmine, i.e. an abrupt reversal of values at two different time periods prior to ECS. While durational changes there does seem to be a recovery period after one hour. In all dosages after fifteen minutes the duration is significantly prolonged while at the two higher dosages after one

hour the duration is not significantly prolonged. At the dose of 0.05 mgm/Kgm of body weight the duration remains significantly shortened after one hour. Data from this laboratory (Richardson, unpublished observations) concerning the amine changes with this regimen of DFP shows that after fifteen minutes in all the dosages there is a significant increase in serotonin levels while after one hour only the lower dose (0.05 mgm/Kgm of body weight) returns to control levels. The catecholamines at this lower dosage show a significant increase with a return to control level after one hour. At the two higher doses the catecholamines show a gradual decrease (not statistically significant) with return to control level after one hour. There may be a slight case made for the adrenergic-serotonergic system reflecting the changes in duration, but the patterns do not correlate well and it is assumed that DFP acts predominently on the cholinoceptive neurons in a manner generally parallel to physostigmine.

In summary with regards to the cholinergic drugs it is seen that scopolamine exhibits a biphasic effect which is dose dependent. At lower doses, it is seen to act in a manner parallel to the previously described adrenergic-serotonergic system while at higher doses it acts in a manner consistent with a cholinolytic effect. This biphasic effect of atropinics has been previously reported (Karczmar and Scudder, 1969) with reference to aggressive behavior and cholinergic mechanisms. Whether the effects of the adrenergic-serotonergic system is equivalent to a cholinolytic effect can not be decided, pharmacologically, from this data. The antiChE's act in a manner that is quite dissimilar from the adrenergic-serotonergic system, although there is no direct polarity. In general their effects are in direct polarity from the higher doses of scopolamine. Physostigmine, at higher doses, shows an abrupt reversal in latency values ("rebound") at fifteen minutes and one hour prior to the ECS while DFP shows a recovery of the latency to control levels during the same two time periods. These antiChE's

may act through a variety of mechanisms which have been discussed. In any case, a complete pharmacological investigations is warranted using various combinations of cholinolytics and cholinomimetics in an effort to clarify further reciprocal relationships.

5. Miscellaneous Drugs

a. Methionine Sulfoxamine

Methionine Sulfoxamine (MSI) has been shown to mimic human epilepsy more closely than any other known drug (Tower, 1955; Tower and Elliott, 1953) The seizure induced by the administration of MSI includes running fits, dilated pupils, piloerection and a "fearful expression" (Wada et al., 1967). These seizures, closely related to the so-called "psychomotor" seizures in man are frequently accompanied by typical tonic-clonic seizures. However, there is a latency period of one to several hours, depending on the dosage and type of animal used, from the time of administration to the seizure proper. Harris (1964) conducted EEG studies during this latent period and found a polarity of effects. Initially, immediately after administration of MSI, the cortical potentials were increased; prior to the onset of convulsions after a specific latent period, the cortical potentials decreased signalling the onset of convulsions.

There have been conflicting theories concerning the biochemical effects of MSI with resultant convulsions. DeRobertis et al. (1967) and Lamar (1968) found that in vivo MSI irreversibly inhibits glutamine synthetase some time before seizure activity. However, since MSI depresses the activity of several other enzymes in the glutamic acid-glutamine-GABA cycle, e.g. glutamic acid decarboxylase, aspartate-amino-transferase, alanine-amino-transferase (DeRobertis et al., 1967) and glutamine transferase (Lamar and Sellinger, 1965), Lamar (1968) did not postulate that the development of seizures due to the inhibition of glutamine synthetase alone. Tower (1955) found that with administration of MSI there was a concomitant decrease of glutamic acid,

glutamine and GABA. Since methionine or asparagine could restore glutamic acid and/or GABA to control levels and prevent seizure activity, Peters and Tower (1959) postulated that a deficiency of glutamine synthetase resulting in a deficiency of glutamine normally derived from glutamic acid and ammonia results in the seizure activity.

Naruse et al. (1960) found that in convulsive strains of mice there was lower levels of GABA, glutamine, glutamic acid and ammonia as well as higher levels of ACh than in the non-convulsive strains. Demonstration of the function of increased levels of ACh in the development of seizures has been amply shown by previous investigators (Tower and Elliott, 1953; Takahashi et al., 1961; Fink, 1966; Reeves, 1966) but the mechanism is disputed. Tower and Elliott (1953) believe that a deficit of glutamine results in an interference of ACh-binding without changes in free or bound ACh levels. Wada and Ikeda (1966) found that scopolamine reduced the frequency of MSI-induced seizures while eserine facilitated such activity. Sobotka (1969) found with various dosages of MSI a significant change in ACh levels post-injection and prior to convulsions. Lamar and Sellinger (1965) found a direct time-response relationship with the inhibition of glutamine synthetase, glutamine transferase and seizure activity. However in conflict with these findings, Gershoff and Elvehjen (1951) found that there was no change in whole brain levels of ACh in the MSI-convulsed animals compared to normals.

DeRobertis et al. (1967) because of Gershoff and Elvehjen's findings and because they found decreased levels of norepinephrine and 5-HT with treatments by MSI dismissed the cholinergic mechanism of MSI convulsions. Associated with these changes in amine levels, Scudder et al. (1966), Schlesinger et al. (1968) and Toman and Everett (1958) found that an increase in amine levels could be related to a decrease in seizure susceptibility. The opposite effects also held true.

A third theory of MSI induced seizures was postulated by Heath (1966). He attributed an anticholinergic effect to the drug and a promotion of a schizophrenic-like behavior in control patients. He reasoned that MSI due to its antimetabolic effects of methionine (Gershenovich et al., 1963) interfers with the formation of ACh by blocking the synthesis of choline in which methionine participates. Similarly, it has been found in this laboratory that MSI depresses aggression and increases exploration of mice at lower dosages which is similar to the effect of anticholinergic agents (Richardson, unpublished observations; Bradley, 1968). In addition, Sobotka (1969) found that lower dosages of MSI do potentiate hexobarbital sleeping time as is characteristic of atropine and scopolamine (Giarman and Pepeu, 1962).

Our own data on the effects of MSI on ECS showed in the case of all lower subconvulsive dosages (25.0 mgm/Kgm of body weight through 75.0 mgm/Kgm of body weight) there is a striking similarity of pattern with regards to seizure duration (Figures 19a-19b). Beginning at fifteen minutes after injection and through one hour and four hours past injection, the duration changes from a significant decrease to no significant change to a significant increase. The latency values, if significantly changed, were all increased, although there was no apparent pattern associated with dose or time. This pattern of an increase in latency and decrease in duration seen at the lower subconvulsive dosages after fifteen minutes was also seen at the convulsive dosage of 100.0 mgm/Kgm after fifteen minutes. It is interesting to note that scopolamine with increasing dosages and time after injection produce the same result as outlined above (Figure 15). Correlated with this is Sobotka's finding (1969) that after fifteen minutes at 100.0 mgm/Kgm dosage, the ACh levels are decreased in the telencephalon and midbrain-diencephalon but increased in the pons-medulla area. After four hours he found that ACh levels were increased in all three areas. This indicates that the durational changes as a result of MSI administration are

directly correlated with ACh levels in the telencephalon and midbrain-diencephalon areas of brain and in general, MSI has an anticholinergic effect on the pattern of subconvulsant dosages. Although this result has been arrived at in the previous literature (Sobotka, 1969; Heath, 1966) there is a discrepancy in the fact that at four hours post-injection the pattern with subconvulsive dosages showed a pattern similar to higher dosages of scopolamine yet the ACh levels were increased in the total brain tissue (Sobotka, 1969). Hexobarbitol sleeping times were prolonged (Sobotka, 1969) with this regimen and the animals appeared to be behaviorally depressed which indicates a high degree of biological inactivity with a subsequent depression of the central cholinergic system. These increased ACh levels could be the result of a variety of mechanisms involved in MSI action. Ammonia and glutamic acid may be building up disrupting the mechanism of ACh-binding capacity and further resulting in increased ACh levels (Peters and Tower, 1959). However this ACh does not have to be in the active form, but could be inactivated in the bound form as this disruption can also explain a decrease in ACh levels. Since the ACh levels had been raised during the four hour period in the telencephalon and midbrain-diencephalon areas it is conceivable that these areas control the seizure duration; due to a build-up of ammonia and glutamic acid and a subsequent disruption of the ACh-binding mechanism, there may be an inhibition of the cholinergic mechanism in these areas. Aprinson et al. (1968) believe that a telencephalic cholinergic system is active in states of behavioral excitation, thus confirming in part, these speculations.

The latency changes due to MSI were those seen with the adrenergic-serotonergic drugs with methamphetamine and higher doses of scopolamine. They are consistent with the postulate of an anticholinergic effect inducing a dominance of catecholamine activity (Carlton, 1963). However, there is no true pattern observed and the significant increases in latency appear

randomized in terms of the regimen of the drug; moreover the majority of latency changes with MSI were not significant.

The convulsant dose of MSI (100.0 mgm/Kgm) shows a pattern of the ECS quite distinct from the nonconvulsant dosages. Fifteen minutes and one hour after MSI, the duration dose change from a significant decrease to significant increase but after four hours the durational pattern changes to that seen at fifteen minutes. Behaviorally, there is no evidence of any radical change at this time aside from a more severe form of motor depression (Richardson, unpublished observations). It is interesting to note that at this time the threshold to seizure is lowered to a great extent, yet the ECS pattern takes the same form, i.e. increased latency and decreased duration, as that seen with an increase of catecholamines. Previous data relate that an increase in amine levels lowers the susceptibility to seizure (Toman and Everett, 1958; Schlesinger et al., 1965; Scudder et al., 1966). Data from this laboratory indicates that with this dose the total catecholamine levels are significantly increased (Richardson, unpublished observations).

Various authors have suggested that the increasing ammonia levels disrupt the ACh-binding mechanism (Quastel, 1962) allowing a greater conversion to free ACh. Sobotka (1969) postulated that a triggering mechanism in the form of strong sensory stimuli could complete this disruption and release ACh. If this were the case the convulsive doses lower the level of ACh due to this disruption of the binding mechanism. This would account for the catecholamine-like pattern of the ECS if a dominance of the adrenergic-serotonergic system occurred. However, Sobotka's (1969) own data on the increased levels of ACh immediately prior to the seizure in the total brain tissue offers evidence against this theory to some degree. Certainly, further research into the exact mechanism of release of ACh by a disruption of the ACh-binding system is warranted and may clarify the conflicting data.

That MSI can result in a convulsion given by itself has been well documented (Harris, 1964; Lamar and Sellinger, 1965). Some authors feel that anoxia resulting from administration of MSI results in the convulsive activity through a release of ACh (Sobotka, 1969). However it has been well documented that anoxia can induce catecholamine release (Goodman and Gilman, 1970) peripherally and in this way may influence the seizure pattern.

There was one case of high fatalities at the dosage of 25.0 mgm/Kgm of body weight four hours after injection. Here the rate increased to 27.4%. This finding is not, perhaps, of any significance in the overall pattern. It is well to note that Sobotka (1969) found that scopolamine enhanced the lethal effect of MSI. With the high fatality rates noted with scopolamine (Figure 15) it is an indication of the efficacy of anticholinergic effects in producing respiratory death during the ECS.

Altogether, it is seen that at subconvulsant doses, MSI produced an ECS pattern strikingly similar to that of higher doses of scopolamine, i.e. an initial decrease in duration followed in time by a return to normal levels or an increase in duration. The latency changes are consistent with an anticholinergic/adrenergic effect. At convulsant doses, MSI produced, initially, a pattern simulating subconvulsant doses, but this pattern is of shorter duration than that seen with the subconvulsant dosages. Prior to convulsions induced by MSI the pattern of the ECS changes to one that is consistent with an increase in catecholamines in the central nervous system.

b. Parahexyl

Parahexyl (Synhexyl) is a derivative of tetrahydrocannabinol (THC), first synthesized by Adams et al. (1940). Soon after its synthesis it was subject to several clinical trials and it was found to have several similar actions and a remarkably high LD_{50}. (Adams et al., 1945; Lowe, 1945) In the late 1940's the drug was clinically tested for use in alcohol and opiate withdrawal with conflicting results. (Himmelsbach, 1944; Thompson and

Proctor, 1953). Stockings (1947) reported that synhexyl was effective in alleviating neurotic depressions, but a later report failed to validate this opinion (Parker and Wrigley, 1950). Because of these discouraging early results very little was done with this drug; the majority of research has been limited to the natural or semi-synthetic analogs of THC.

In 1964, 1-Δ^{1}-3, 4-trans-THC was identified as a naturally occurring constituent of marihuana resin and the year following, its synthesis was achieved (Gaoni and Mechoulam, 1964). Since that time, this compound has been recognized as the major psychoactive component of marihuana (Pillard, 1970). Synhexyl differs from this compound in that it contains an n-amyl group instead of an n-hexyl radical at the 4' position (monoterpene numbering). The levorotatory form is several times less active than its dextrorotatory form (Stockings, 1947).

The potency, pharmacological and psychological properties of Synhexyl has been compared to THC by Hollister et al. (1968). It is three times as potent as THC, has a latency of one to two hours and a duration of affectiveness of upwards of twenty-four hours, which has significantly larger than that of THC. In comparable doses, the physiological and psychological effects were considered to be similar. Thus, a brief review of the literature on the effects of THC (marihuana) may be pertinent.

It is useless to describe the difficulties investigators have experienced in working with Cannabis sativa or its extracts. Both federal law and ignorance of active components have hindered investigators up until several years ago. (Pillard, 1970) Pharmacologically, marihuana is in a class by itself. Both THC and Cannabis extract have been shown to potentiate hexobarbitol sleeping time (Gershon, 1970) and amphetamine stimulation (Garriott et al., 1967) in a variety of animals. In man, transient physiological changes consisting of a slight increase in pulse rate and conjungtival injection have been reported (Weil et al., 1968). However

there was no change in pupil size or respiration rate and minimal changes in blood pressure and body temperature. There has also been noted a distinct lack of other sympathomimetic effects such as increase in serum free fatty acids (FFA) or hyperglycemia (Pillard, 1970; Hollister et al., 1968; Weil et al., 1968).

Garattini (1965) and Carlini (1969) found in laboratory animals that Cannabis extract decreases fighting behavior in doses below that required to reduce total activity. However, Carlini and Masur (1969) found that in starved rats with similar doses of Cannabis extract, aggressiveness was markedly increased. In man, the behavioral results do not differ from those often found in the study of effects of many other drugs on complex behaviors (Clark and Nakashima, 1968). Digit symbolization, complex reaction time, serial addition have all been reported as being impaired with a great deal of variability among chronic or acute users (Mayor's Committee, 1944; Weil et al., 1968). With increasing doses of THC feelings of euphoria and relaxation gave way to hallucinations, depersonalization and loss of reality testing. However, marihuana can not be classed as a true hallucinogenic as it shows no cross-tolerance to lysergic acid diethylamide (LSD, dimethyltryptamine (DMT), mescaline or peyote all of which are hallucinogenic and show partial cross-tolerance to each other (Silva et al., 1968; Isbell and Jasinski, 1969).

Perhaps, at the present time, one of the biggest questions in Cannabis research is the dose standardization with regards to the extract and THC. Generally speaking, the extract is used in the dose range of 20 mgm/Kgm while the pure THC is used in doses of 5-10 mgm/Kgm (Carlini, 1968). Synhexyl, although it has been used in doses of 15 mgm/Kgm of body weight (Himmelsbach, 1944) in opiate addicts without any untoward effects, is generally given in doses of 2-3 mgm/Kgm of body weight (Hollister et al.,

1969).

With regard to its action on ECS, parahexyl caused in small doses no significant change in latency or duration values when it was given at several time periods preceding the ECS (Figure 18). At the two higher doses (7.0 and 15.0 mg/Kgm) which are considered pharmacologically high doses, although well below the LD_{50}, there was no demonstrable pattern of change. At 7.0 mgm/Kgm the duration was significantly decreasedaat fifteen minutes, and decreased further when parahexyl was given sixty minutes prior to the ECS. At the end of sixty minutes which is also the minimum latent period observed for the pharmacological effects of parahexyl to appear (Hollister et al., 1968) the latency was increased producing a pattern similar to that consistent with an increase in central catecholamines. However, at the higher dose, i.e. 15.0 mgm/Kgm the duration did not significantly change at any time period while the latency was increased at the end of one hour as in the case of lower dosage. Data from this laboratory (Richardson, unpublished observations) indicates that parahexyl given at 7.0 mgm/Kgm increases central serotonin levels while not affecting the catecholamine levels. Partially confirming this are the studies of Holtzman et al. (1969) which demonstrate that the administration of THC results in an elevation of serotonin with a minimum of depression of catecholamines in the brain tissue. The data presented in this study are not consistent with those findings. The response at 7.0 mgm/Kgm of parahexyl approximates that shown by physostigmine (Figure 14) at higher dosages with an initial cholinergic dominance and "rebound" phenomena. However, this response is not seen at the higher dose of 15.0 mgm/Kgm. The increases in latency at 7.0 mgm/Kgm and 15.0 mgm/Kgm after sixty minutes can be explained at corticolthalamic sympathomimetic effects (Stokings, 1947) which is correlated with the potentiation of amphetamine toxicity as shown in THC-treated mice (Garriott, et al., 1967). This might explain the cholinolytic-like effect of potentiation of the hexobarbitol sleeping time (Gershon, 1970) as due

to an imbalance of neurotransmitters with dominance of the adrenergic-serotonergic system. The slight sympathomimetic peripheral effects would also correlate with this explanation.

The only statistically significant increase in fatalities was seen at 7.0 mgm/Kgm of body weight with a rate of 50.0%. No pharmacological interpretation can be given to this isolated finding.

In summary, the data indicates that parahexyl at pharmacologically active does not affect the ECS pattern in a manner comparable to other drugs used in this study. At higher dose levels, increased latencies are seen one hour after the administration of the drug. At an intermediate dose the duration is significantly decreased at fifteen and sixty minutes post-injection. At high doses, the duration is not significantly changed. These erratic patterns may point to activation of a higher level adrenergic-serotonertic system by extremely high doses of this synthetic Cannabis derivative or to a direct effect of the drug independent of the various neurotransmitter systems. Altogether, the pharmacological data do not fit readily into any set pattern. Certainly with this drug, the difficult method of preparation (See Materials and Methods section) may account for inexplicable effects at various dose levels. Direct effects of the drug on neuronal populations independent of neurotransmitter levels also have to be taken into account for a more complete explanation.

c. Isolated and Fighting Mice

It is well known that isolation in certain strains of Mus for a period of approximately three weeks will produce aggressive activity when these mice are placed in an aggregate situation (Karczmar and Scudder, 1966; Yen et al., 1959). It is also known that this behavior will only occur in the male animal and then only if there is an intact pituitary-gonadal axis (Valzelli and Garattini, 1967; Sigg et al., 1966). Scott and Fredricson (1951) theorized that an

endocrine center located in the pituitary body with nerve connections to the hypothalamus was responsible for the aggressive behavior during isolation.

Several biochemical changes have been reported for isolated animals. Norepinephrine (NE) brain levels have been found to either increased (Geller et al., 1965; Welch and Welch, 1964; Richardson, unpublished observations) or remain unchanged (DeVanzo et al., 1963). Serotonin levels have been reported either decreased (Welch and Welch, 1968) or unchanged (DeVanzo et al., 1963). ACh levels in the midbrain-diencephalon areas have been reported as decreased (Sobotka, 1969) which is correlated witht the findings of Giacolone et al. (1968) and of Agrawal et al. (1967) that this area of the brain is associated with decreases in serotonin and brain amino acids in isolated animals. Biochemical findings in the blood stream include a decrease in corticosteroids (DeVanzo et al., 1963) and blood levels of FFA (Giacolone et al., 1966).

Behaviorally, isolated mice are hyperexcitable and more responsive to tactile and arousal stimulation (Yen et al., 1959; Agrawal et al., 1967). This increased responsiveness was inferred when Welch and Welch (1966) found that isolated mice are more sensitive to amphetamine stimulation than aggregate animals. Scott and Fredricson (1951) postulated that isolated animals which are removed from stimulation of other mice would become dishabituated to the presence of other mice and upon introduction of a strange animal would allow aggression-generating stimuli to act to promote fighting. Sobotka (1969) states that decreased levels of ACh in the midbrain-diencephalon areas in isolated animals points to a loss of the cholinergic habituation mechanism first postulated by Carlton (1963). Further biochemical confirmation of this cholinergic mechanism is afforded by Geller et al. (1965) and Rosenweig et al. (1960) who reported decreased subcortical AChE activity in isolated animals.

The present data indicate that in isolated animals there is no signifi-

cant change in latency or duration values (Figure 17). However, once these animals were placed in a cage with another isolated male and ECS induced during the subsequent fighting, the latency was increased and the duration was significantly decreased. Data from this laboratory (Richardson, unpublished observations) indicate that a fighting animal has significantly increased levels of catecholamines. Welch and Welch (1968) indicate that a stressed animal has an increased activity of the adrenergic system and Weiss et al. (1961) report an increase in the activity of the serotonergic system during stress. Additionally, in animals under stress, plasma FFA, pituitary-adrenal activation and urinary catecholamines are all markedly increased (Mallov and Witt, 1961; Michalova et al., 1966 and Moore, 1966). This would indicate a strong sympathomimetic effect both peripherally and centrally. This adrenergic-serotonergic pattern is strongly evident in fighting animals. The lack of change in the ECS pattern in isolates can be attributed to a lack of neurotransmitter change sufficient to result in a change in patterning. However, this lack of response to the overwhelming stimulus of ECS is opposed to the theory of general hyperexcitability of the isolated animal (Welch and Welch, 1966; Yen et al., 1959). It still remains consistent with the theory of habituation of the animal to incoming stimuli which in effect would result in no significant change to the stimulus of the ECS. One would expect, however, some evidence of a correlation of a depression of ACh levels in the midbrain-diencephalon shown by Sobotka (1969) with the seizure pattern.

d. Stressed Mice

Chronic stress of the type used in this experimental design has been used previously as a negative reinforcement in conditioning (Sobotka, 1969). During the stress, the animals show initially a typical alarm reaction i.e. squeaking, wild running and jumping, urination and defecation (Mallov and Witt, 1961). This seems to correlate with the theory of increased central

excitation also confirmed by EEG patterns exhibited during chronic stress which demonstrate a gradually increasing state of arousal (Mindy-Castle, 1951). However, as the stress continues, the animal's behavior changes to a type of freezing or catatonic state followed in time by behavioral depression or withdrawal during the remainder of the intershock interval (Mallov and Witt, 1961; Ruther et al., 1966; Sobotka, 1969). There are several interpretations in explanation of this apparent maladaptive behavior. Winters (1967) observed that this behavior may occur during hyperexcitability while Bovet et al. (1969) stated that freezing behavior is indicative of increased emotional arousal. Okin et al. (1960) theorize that the animal restricts the central reception of the stressing stimulus; the phenomena of freezing could be therefore related to habituation. Winters (1967) proposed a model indicating that sensory input systems show increased modulation during these states of activity.

Malmo (1966) stated a theory of opposing systems, continued stress may suppress optimal levels of neural activity so that the systems cancel one another out. Other theories have proposed that the animal gradually learns that a behavioral act fails to reduce the adverse stimulus; this activates a negative feedback system in the reticular formation leading to the inhibition of the behavior. Continued operation of this system would lead to a reduction of available behavioral acts.

Biochemically, the stressed animal has been shown to have decreased central NE levels (Ruther et al., 1966; Corrodi et al., 1968) which is indicative of increased activation (Scudder et al., 1966). Central and peripheral sympathetic mechanisms are activated as shown by an increase in plasma FFA and an increase in urinary catecholamines (Michalova et al., 1966). Moore (1966) has stated that adrenalcorticotropin release from the pituitary is increased during stress.

As with the adrenergic system, the serotonergic system has been demon-

strated to be activated during stress. Liberson et al. (1964) and Corrodi et al. (1968) found decreased central levels of 5-HT in the cortex and hippocampus which were interpreted by Welch and Welch (1968) to be indicative of an increase in serotonergic activity.

Krause et al. (1964) found increased cholinergic and adrenergic activity in the telencephalic-diencephalic region of the mouse brain during emotional excitation while Sobotka (1969) found an increase in telencephalic ACh level during chronic shock stress. This may point to a cholinergic activation of the phylogenetically younger areas of the central nervous system during stress.

In the case of our experiments, the effect of frustration on ECS showed an adrenergic pattern seen with increased levels of catecholamines (Figure 8) and with low dosages of methamphetamine (Figure 11). Data from this laboratory (Richardson, unpublished observation) have shown that animals that have been exposed to chronic shock stress of the type used in this study have significantly decreased levels of catecholamines with an increase in serotonin levels. This finding would be opposed to the pattern seen in Figure 17 (compare Figures 8 and 17). Quantitatively, the changes were not as great as those seen in fighting animals in regards to latency and duration (Figure 17). The differences may be ascribed to several mechanisms. The decreased levels of catecholamines may point to an active adrenergic mechanism both centrally and peripherally which would account for the seizure pattern (Corrodi et al., 1968). As discussed above (pp. 40-44) serotonin is able to potentiate the adrenergic effects on duration; since 5-HT levels have been shown to be increased during chronic stress (Richardson, unpublished observations) and the serotonergic system has been reported to be activated during stress (Welch and Welch, 1968) it is conceivable that the levels of serotonin in stressed animals is sufficiently high to override the lowered levels of catecholamines and thus result in a pattern which is essentially similar to that

shown with high levels of catecholamines. Serotonin has been described as a modulator and this would correlate with Winters (1967) theory of an increase of serotonin of the modulation of incoming stimuli. With pargyline, α-MPT and 5-HTP given in combination, the levels of serotonin were increased while the levels of catecholamines were lowered (Richardson, unpublished observations) and this may correspond to the pattern of the ECS seen (Figure 9b).

It is also conceivable that an active telencephalic cholinergic mechanism in combination with an activation of the serotonergic system may result in an increase in latency and decrease in duration. With some doses of antiChE's (Figure 14) there has been shown a decrease in latency occurred while with combinations of drugs that result in an increase of serotonin there has been shown an increase in duration (Figure 9a and 9b) Sobotka (1969) found that scopolamine modified the behavior of the frustrated animal while Ruther et al. (1966) using a cholinolytic agent, imipramine, found similar results.

It is possible that the decrease in catecholamines may represent a gradual loss of these amines due to the chronicity of the experimental design. Although with acute stress, the catecholamines are increased (See section on fighting animals), with chronic stress a loss may occur due to a depletion or increased turnover. This would account for the adrenergic pattern seen in Figure 17 with the quantitative decrease in central catecholamines as reported by Richardson (Richardson, unpublished observations).

e. Enovid 10 and Female Control Mice

Comparatively little work has been done on the effect of hormones on the seizure pattern. Toman et al. (1957) first reported that the seizure latency in female mice was not significantly changed from male controls. This correlates well with the data reported in Table 12 as neither the latency nor duration in female mice differed significantly from control males.

However, several authors have found in rats significantly sex-related differences in the electroshock threshold for seizure (Woolley et al., 1961). Kawakami and Sawyer (1959) postulated that the sex steroids affect changes in threshold of an "EEG arousal system" involving the brainstem reticular formation, while Beach (1958) concurred with a theory of a change in the threshold activity of the central nervous system with a change in the level of sex steroids.

The drug Enovid® given in this experiment consisted of 10 mgm of norethynodrel and 0.15 mgm of mestranol, a 3-methyl ether of ethinyl estradiol, the most biologically active of the estrogens (Goodman and Gilman, 1970). Since the combination of the two hormones was given, the effects of each on the electrical activity of the central nervous system will be briefly reviewed.

Estradiol, administered in various forms, has been shown to lower the electrochock threshold in rats (Sitt and Kinnard, 1968) which is due to a specific effect of the hormones itself, as it lowered the threshold in hypophesectomized rats and when administered to male rats (Wooley and Timiras, 1962). Furthermore, the dosage was in the physiological range of 0.25 to 4.0 μgm/100 gm of body weight. Logothetis et al. (1959) believe that the exacerbation of epileptic seizures in females observed just prior to menstruation are a result of increased plasma levels of estrogens at this time leading to a hyperexcitable state. They further report that topical application of water-soluble estrogenic conjugates (Premarin®) on the brain of normal female rabbits results in EEG activation.

Progesterone has been reported to raise the seizure threshold in female rats but not in male rats in doses comparable to those given in the form used in this study (Wooley and Timiras, 1962; Wooley et al., 1960). Kawakami and Sawyer (1959) reported that a decreased EEG arousal was observed approximately five hours after administration of progesterone. Laidlaw (1956) postulated that the exacerbation of seizure activity in females prior to menstrua-

tion was due to a falling of serum progesterone levels resulting in increased excitability of the central nervous system. However, Sitt and Kinnard (1968) report that progesterone has no effect on minimal seizure threshold.

In our hands Enovid® given one hour prior to the ECS caused no significant changes in latency or duration values when compared to female or male controls. If the estrogen compound has been reported to lower seizure threshold and progesterone compounds have been reported to raise the seizure threshold then it is safe to assume that there may be a cancelling of effects resulting in no change. This would be mirrored in the seizure pattern which demonstrated no significant change. However, this finding does conflict somewhat with Sitt and Kinnard (1968) findings that this combination of sex steroids results in a decrease in seizure threshold. These latter findings were demonstrated after a one week period of administration of the drugs, while we used a single dose treatment; moreover, a decrease in seizure threshold may not mean that a significant change in the seizure pattern should occur. In any case, further study into seizure patterns with steroids given alone or in combination may clarify the relationship.

f. Ethanol

Of all the drugs used in this study ethanol induced the largest change of the latency and durational values. The pattern is one of increased latency and decreased duration (Figure 17). The decrease in duration which is in part a measure of the tonic phase of the seizure, may be correlated with McQuarrie and Fingl's (1958) findings that ethanol, given prior to the ECS, gradually abolishes with increasing doses the tonic phase of the seizure. This also may be related to Caspers' (1958) work in which ethanol was injected microphoretically into the reticular formation resulting in a prompt decrease in the spontaneous activity of this structure. This effect is a selective one (Kalant, 1962) possibly due to the fact that the reticular formation consists of groups of nuclei of polysynaptic connections; the effect of

ethanol is most obvious with regards to polysynaptic rather than monosynaptic reflexes. However, the question is open to debate for Kolmodin (1953) found that ethanol depresses equally well both polysynaptic and monosynaptic reflexes in the cat.

The striking increase in latency, which was also seen with increase of catecholamines and serotonin levels and decreases in ACh levels has not been studied in regards to ethanol previously. Kinard and Hay (1960) found that after doses of alcohol higher than the ones given in this study, levels of AChE in the brain were lowered and, as a result, ACh levels were raised. Data from this laboratory (Richardson, unpublished observations) indicate that ethanol in this regimen will result in a highly significant increase in catecholamines after five minutes. However, after one hour the catecholamines returned to mean levels, while the seizure pattern showed a continued increase in latency. This laboratory found no change in serotonin levels after the administration of ethanol.

Although the data given in Figure 17, suggestive of a catecholamine-like effect, the results are inconclusive at the present time as to whether ethanol is related to its role as a modulator of catecholamines in the central nervous system.

Ethanol is known to excite the cerebral cortex at lower doses and to depress it at higher doses (Grenell, 1959; Masserman and Jacobson, 1940). It has been shown also to raise seizure threshold but only at doses that cause general depression of the central nervous system (Goodman and Gilman, 1970). Ethanol has been found to prevent electrically-induced convulsions in mice (Workman et al., 1958). McQuarrie and Fingl (1958) found that a phase of hyperexcitability did occur after an acute dose of ethanol, but only after a five hour latent period. Since ECS was performed five minutes and one hour after the administration of ethanol, and the tonic phase of the convulsion was severely shortened it may be assumed that there is at this time a general

depression of the central nervous system due to ethanol's irreversible depolarization of neurons at this dose level (Gallego, 1948). The EEG has shown that as the concentration of ethanol in the bloodstream rises the brain waves slow gradually which is not unlike the pattern seen in anoxia (Toman and Davis, 1949). This gradual depression may account for the changes seen in the latency values.

In summary, the changes seen in the seizure pattern with ethanol administered I.P. reflect a pattern of ECS as seen with increased levels of central catecholamines and decreased levels of ACh. Although data from this laboratory suggests that catecholamines may function through an adrenergic transmitter, the previous published data would seem to indicate that a general depression of the central nervous system occurs, particularly the reticular formation. This depression is sufficient to explain the striking increase in latency as a result of a lack of reticular activity on the disorganized neuron. The decrease in duration reflects a depression of the reticular substance which is unable to perform adequately and as a consequence the tonic phase is severely shortened and exhibits no clear activity as distinct from clonus.

B. Systems Analysis

1. Introduction

Von Weimann (1951) has stated:

> "Living systems are of enormous complexity and it is clearly necessary to subdivide the problem that they represent into several parts. One method of subdivision... is this: the organism can be viewed as made up of parts which to a certain extent are independent, elementary units. We may, therefore, to this extent view as the first part of the problem the structure and functioning of such elementary units individually. The second part of the problem consists

> of understanding how these elements are organized as a whole and how the functioning of the whole is expressed in terms of elements. The first part of the problem...is closely connected with...organic chemistry and physical chemistry and may indue course be greatly helped by quantum mechanics... The second part is connected with logic..."

The purpose of this section is to deal with the problem of ECS as a whole, the total behavior of the organism serving as the frame of reference. Consequently the language and the thought content shifts from an orderly deductive process to an orderly inductive one. The shift is subtle, yet important for a new intellectual approach which may bring refreshing unity to what is a seemingly disordered and fragmented view of the seizure mechanism.

The introduction of this thesis is, in a sense, a primer for this section. In the introduction the physiological approach to interpret the observations on ECS was presented and expanded to include pharmacological data. This effectively subdivided the phenomenon and allowed for modification of the subunits; the resulting change may be evaluated. However, when dealing with the central nervous system the evaluation of individual sub-systems may be confusing both statistically and pharmacologically. The complexity of interaction and multiple control feedback within the mammalian brain permits an analysis of probabilities rather than of absolutes, in reference to behavior. Consequently, one may not be speaking of the accuracy of the sub-systems; one may only refer to the reasonable probability that given the same variable parameters the sub-systems would function and interact in a predictable manner. Even that would be enough to satisfy the most rigorous of scientists except for the fact that when one deals with behavioral or physiological sub-systems of the central nervous system the term "parameter" is difficult to specify accurately. Herein lies one of the advantages of ECS studies; with induced seizures there can be no subtle interaction between smaller sub-

systems and undefined and unmeasurable complex stimuli. ECS is a massive stimulus delivered in a short period of time. ECS given to a computer would probably cause a complete malfunction; when given to a mouse, the system survives only through the grossest of adaptive homeostatic mechanisms. At this present stage of neuroscience, insight into any real functional system is desperately needed. The term "parameter" then, is effectively restricted in this case, and given the well-controlled and measured secondary variables of drug administration one may modify these more-encompassing sub-systems with relative confidence.

It is important to review the physiological theory of the ECS at this stage. Latency, as has been presented, it s function of the unpattemed firing of neurons in the total brain. It is quantitated initially with voltage, impedance and changes in impedance in the neurons and in the nervous tissue, and to the current passing through the systems. Since the current is a maxima one, large enough to produce full seizure activity, it is assumed that all neurons are discharged and discharged in a random manner. Duration, as a function of the tonic phase of the seizure, is a measure of the length of time that the reticular formation, as a functional unit, is discharging. The flexion and extension phases of tonus may be measures of cerebellar and/or hippocampal sub-system recovery under the modal committments of the reticular formation. The end of tonus and the beginning of clonus is a function of two separate but important systems. The first and probably the major factor is corticol-thalamic reverberation or oscillations as cortical control is re-established after the massive stimulus. The second is the peripheral input to the reticular formation and thalamus establishing secondary feedback factors causing in the hypersynchrony seen in the EEG of this phase. The end of the seizure may be due to full cortical control as balance becomes re-established and as the excitatory impulses are inhibited due to tissue anoxia and to a build-up of metabolic byproducts, e.g. lactic acid, carbon dioxide, etc.

To describe the organism more fully in a systems context several assumptions must be made. The primary one is that the organism regulates itself homeostatistically; adaptiveness implies homeostasis in a systems context. This may seem contradictory but as W. Ross Ashby (1952) states:

> "The primary fact is that all state-determined dynamic systems are selective; from whatever state they have initially, they go towards states of equilibrium. The states of equilibrium are always characterized in their relation to the change-inducing laws of the system, by being exceptionally resistant."

In order for an organism to react adaptively yet homeostatically, Ashby postulates a "step-mechanism" whereby input may result in a series of events dictating a "new" output, "new" in the sense of novel output for this particular input. However, homeostasis implies negative feedback and adaptiveness, at least indirectly, implies positive feedback. These two are reconciled by this "step-mechanism" which is a functional hybrid system. The reticular formation represents in this paradigm a hybrid-step-mechanism.

A second assumption deals with computer analysis of the central nervous system. Although it may be easier to say that any system or subsystem is either discrete or continuous in its function, one can hardly say that this is so for the total activity of the brain. Rather, the brain acts like a hybrid computer containing components which are both digital and analogue. Schade (1970) showed that the neuron itself, is a hybrid computer shifting from digital to analogue and back to digital in its operation. It is not reasonable to fit the brain into one or the other category even though resultant behavior may appear to be either discrete or continuous. This assumption will carry with it a certain flexibility of thinking which will become apparent as the discussion proceeds.

The third assumption deals with the durational values. It is assumed that generally, a decrease in duration has a functional survival value for the organism because the tonus, the period of time the organism spends without active respiratory mechanism, is decreased and, consequently, tissue anoxia will be limited. There is a much greater probability that the organism, other conditions being equal will expire if the ECS duration increases. Thus the attempt by the reticular formation as it becomes activated to shorten the seizure duration. This assumption provides a continuum of action for the organism from one degree of adaptiveness to another even though the output is discrete. Of necessity, when dealing with neurotransmitters, the behavioral output is discrete. Ye t the levels of neurotransmitters fluctuate continuously. Threshold phenomena will not logically provide an adequate explanation when systems are dealth with. The mode, at the same time, must be continuous in function. Duration as it fluctuates from control levels provides the continuity.

2. The Reticular Formation

Scheibel and Scheibel (1958) first postulated a structural basis for a functional theory of the reticular formation. Seen in cross-section and dendrites of the reticular formation appear to be relatively straight, long and radiate widely. However, when viewed in the sagittal plane, the dendrites, although still radiating in the transverse dimension, show little or no projection in the rostral-caudal direction. The authors likened this to a stack of poker chips consisting of a series of flattened dendritic domains; this arrangement results functionally in a significant localization of input because each cell reviewed only a limited series of inputs from a single level of the input continuum. Furthermore, in confirmation of this concept, it was seen that virtually all incoming axonal elements approach the reticular formation at right angles to the long-gitudinal avis and synapse in a parallel fashion on the dendrites.

These same authors theorize further (Scheibel and Scheibel, 1967) that since each reticular cell lies in a matrix of fibers largely core (of the reticular system) derived whose axonal elements give off collaterals at right angles along their path, each cell will have a host of axodendritic feedback synapses which are for the most part, non-specific. The moment-to-moment dendritic input would then represent an integrate of specific and non-specific inputs. Thus:

> "It seems beyond question that the output of each reticular element represents a vector of this type; it therefore follows that specific informational content, intrinsic to each of the afferent sources, is lost in the integrative process. The output of each unit must represent intensity only."

Kilmer et al. (1965) have used this anatomical modular pattern as a paradigm for a new generation of computers which will show increased degrees of flexibility in dealing with data influx and will habituate over time to iterative stimuli. What these authors have tried to do, then, is to define an ultrastable system, i.e. one that will show adaptiveness or degrees of flexibility when presented with a variety of stimuli (Ashby, 1952). The reticular formation has the ability to analyze intensity of input, determine critical states of intensity, and affect a change in the organism's line of behavior (so-called "adapting"). This fits readily to Ashby's concept of a "step-mechanism".

3. Positive and Negative Feedback

In its simplest terms, the step-mechanism must be one that is able to function in a temporal sense, beyond the moment of the initial input. It also must be able to shift modes of operation although the time involved in this shift may be either long, e.g. several hours or short, e.g. several seconds.

There are several control mechanisms familiar to bioengineers which allow for this shifting and time lag; all these control mechanisms contain both

positive and negative feedback mechanisms in various combinations. A discussion of the most pertinent system will be given.

For the most part, the systems in the central nervous system are considered to be dynamic and linear although static and non-dynamic systems can exist. The discussion will deal with linear and dynamic systems. Let us assume that there is a functional element in a system labeled as:

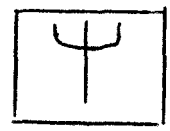

Figure 1

This element has many inputs and one output and for our purposes will be linear in function. We shall represent input and output by arrows:

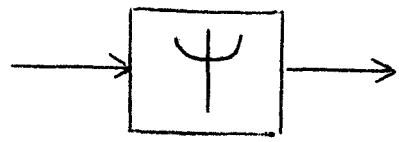

Figure 2

Now, let us assume there is a static linear element equipped for simple summation of feedback signals. Thus we may represent it as:

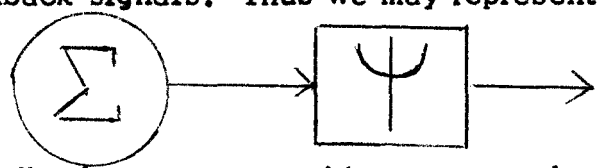

Figure 3

If negative feedback is represented by arrows and a minus sign (-) and positive feedback is represented by arrows and a plus sign (+) we may illustrate a system as follows:

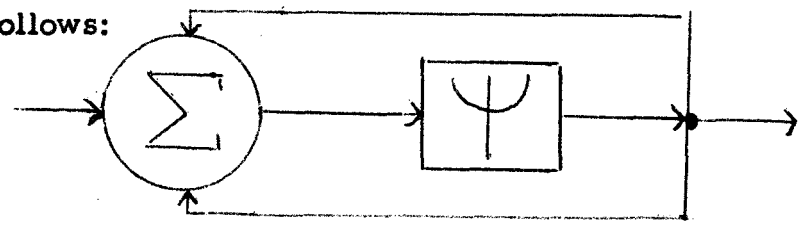

Figure 4

There are several rules for operation of this system involving both types of feedback which will be stated and which may be proved mathematically, the proof will not be provided in this discussion (See Milsum, 1970).

1. The systems basic response pattern is independent of which input produced the initial "kick" to set off the response.

2. For any given systems model, each sub-systems response sequence depends on the whole loop.
3. A positively connected loop is not necessarily unstable; stability depends on the level of input signal.
4. A time interval, Δ t, does not affect the response pattern, only its time scale.

If the basic unit of this synamic linear element is a time delay and it is fed back with a positive regeneration of unity, it will reproduce a response characteristic for the so-called staircase integrator. The integrator may be represented as:

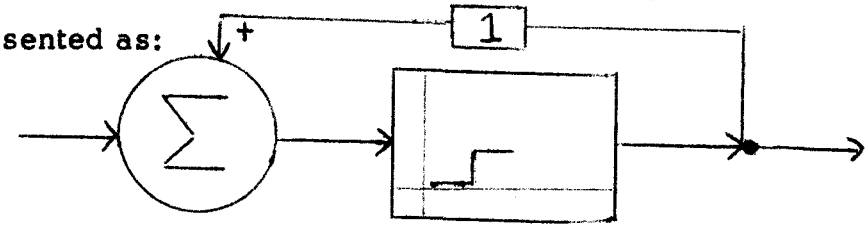

Figure 5

The two-dimensional graph which has replaced the former sign of the functional element represents the values of an independent and dependent variable associated with the element. If this whole response if Figure 5 is alternately fed back into another feedback summator with a negative feedback of the value unity then the pattern of response is represented as:

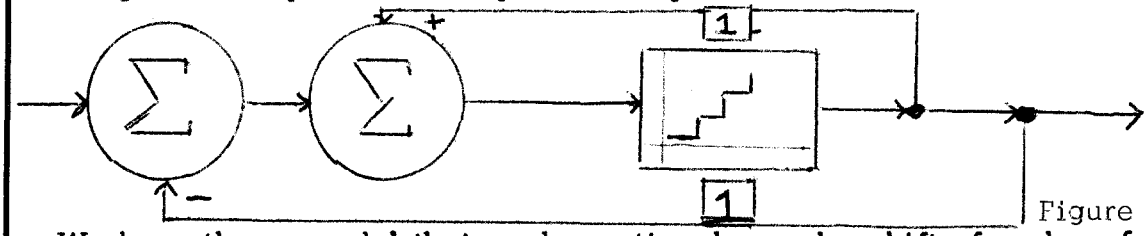

Figure 6

We have then a model that produces time lag and a shift of modes of operations (variables) through feedback. However, this system is one of several that could be illustrated; more important is the recognition that, although the basic element is time delay, the response pattern is, <u>in effect</u>, independent of time. "The values of variables change in time because the values of other variables change, not simply because time flows" (Rapaport, 1970). In the equation:

$$\frac{dx}{dt} = f(x, y, \ldots)$$

$$\frac{dy}{dt} = f(x, y, \ldots)$$

"t" must not appear as an argument on the right hand side. However, solutions of the equation will be given by specifying "x" and "y" as functions of time. This is essentially what this tudy has done by using time as a function of brain operation.

4. Pharmacology of the System

The purpose of the systems approach is to enable the investigator to observe patterns not in the framework of one class of drugs, but in view of the total system. There are two striking patterns that emerge from the data; most drugs and regimens act to increase the latency and decrease the duration in an inverse fashion. These regimens include the adrenergic and serotonergic drugs, most doses of methamphetamine and some doses of scopolamine. However the cholinergic and high dosage of methamphetamine act to change latency and duration in a direct manner, i.e. an increase in ACh levels decrease both values while a block of ACh or high dosages of methamphetamine (cf. Figure 12) results in an increase in both variables.

This may be explained by observing our hypothetical system when the intensity of feedback is altered. If we assume that the levels of ACh affect the gain of the positive feedback loop by decreasing the total loop gain with increasing levels of ACh the initial integrator will act in a more direct fashion to initiate a change of mode. Thus:

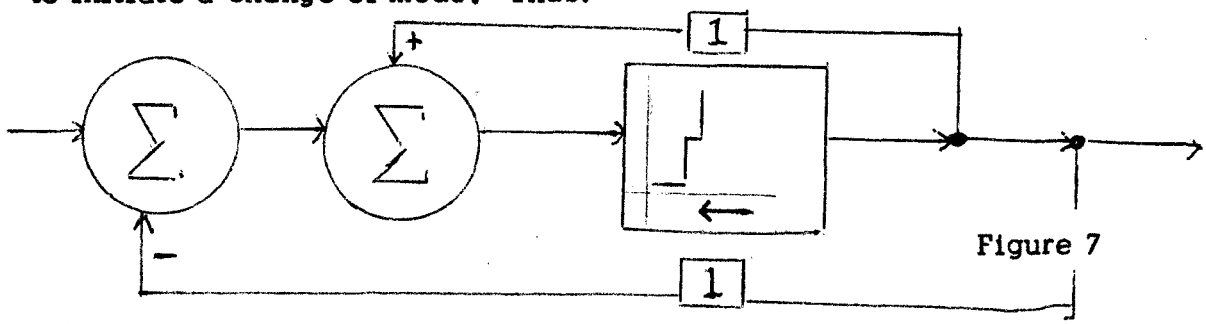

Figure 7

Therefore, when fed into the negative feedback loop the staircase integrator will shift to the left on our graph and will reach a new level more directly than before. If time were one of the measured variables on the two-dimensional graph, we may say that the integrator would act more rapidly.

If we modify both feedback loops by increasing the negative and the positive loop gain we will see that the staircase integrator although decreasing the total time required to reach specific mode of operation will also take longer to perform the initial step. The final result lies in a functionally quicker adaption to the initial stimulus.

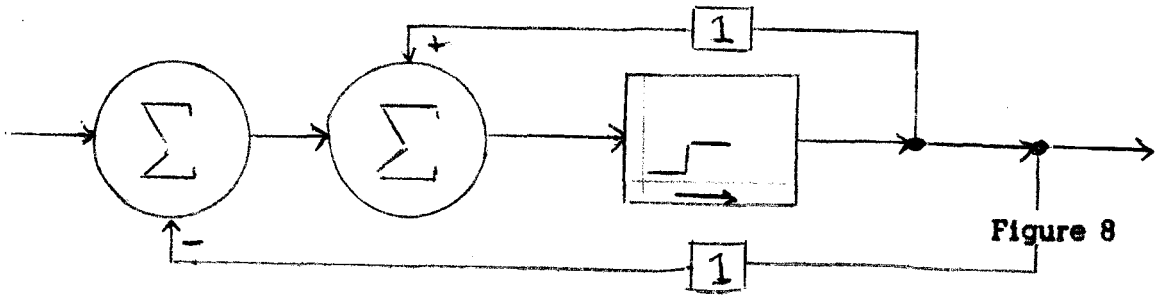

Figure 8

Thus by altering levels of catecholamines, both feedback loops are affected. By increasing the catecholamines, the gains of both loops are increased; in the seizure pattern, this results in an increase in time required for initial reticular performance but also in a more effective homeostatic performance once the latter is initiated. By increasing the levels of ACh only the positive loop gain is increased resulting in a decrease in total seizure duration due to a shorter time required for the initial "kick" by the reticular formation. Notice that the performing efficiency of the reticular formation is not affected by this loop gain; only the initial "kick" of the formation as a result of the stimulus is involved. The terms of the survival of the organism, either high levels of amines or of ACh would decrease the ECS duration and there will be less time for hypoxia to build up.

Thus, when ACh levels are modified by cholinolytic agents e.g. scopolamine, the latency and duration are generally increased (Figure 15); when

ACh levels are changed by the anticholinesterases e.g. DFP or physostigmine (Figures 14 and 16), the latency and duration are generally decreased. As the catecholamine levels are raised by various drugs, the latency is increased while the duration is decreased and as the catecholamine levels are lowered, the opposite effects result (Figures 8, 9a and 9b).

However, to fully explain all the data one must assume a functioning balance between these two neurotransmitter systems as previous authors have done (Carlton, 1963; Aprison, 1962; Karczmar and Scudder, 1966). The two feedback loops are independent in function as the gain or loss of each loop is attributed to the single loop, yet the mechanism of change, i.e. the neurotransmitter level, may be an interpendent one. When levels of one transmitter are decreased, the visible effects on seizure may be a result of the decrease in that transmitter or an increase in the functioning of the opposing transmitter. For example, scopolamine (Figure 15) at lower doses gives a pattern that is consistent with an increase in catecholamines, yet at higher doses, gives a pattern consistent with a cholinergic block. Acute methamphetamine (Figure 10) and low dosages of chronic methamphetamine (Figure 11) give patterns consistent with a sympathomimetic effect, yet at high dosages of methamphetamine (Figure 12) the effect on the pattern is similar to that dose of cholinergic block with habituation to the block. To further substantiate this interdependency, the time-related effect of chronic administration of increasing methamphetamine (Figure 13) shows a pattern of continually increasing cholinergic block.

Data for methionine sulfoxamine (Figures 19a and 19b) shows that at doses low enough to lower the ACh levels (Sobotka, 1969) an adrenergic pattern is seen while after a period of time, a cholinergic block pattern is seen. Teleologically, this may represent a system's effort to adjust the loop gains more efficiently for in all these cases, with increasing doses of any drug, the pattern of the ECS approaches the one pattern that is consis-

tent with a block of the positive loop, i.e. a block of the one loop which when not blocked would lead to instability.

The data for the serotonergic drugs may clarify this interdependency of the two neurotransmitter systems. With 5-HTP (Figure 8) and an increase in serotonin levels, the pattern is one consistent with the blockade of the cholinergic system, yet in many instances (Figures 9a and 9b) the levels of serotonin potentiate the adrenergic pattern. In bioengineering, a differential operational amplifier serves the same purpose, i.e. to control the utilization of a positive and/or negative feedback system. Functionally, its use would appear to be similar to that of Ashby's "null function" (Ashby, 1952) or a mechanism to control independency of system function resulting in a polystable system.

There is one drug that does not fit into the system which we have outlined. Ethanol, although it does give an adrenergic pattern, is unique as it abolishes tonus eventually inducing a seizure pattern that is inconsistent with any adrenergic or cholinolytic drug. This pattern, in all probability, is a result of functional depression of the reticular system itself and of its input circuitry as a whole. Thus, latency is increased greatly because of this depression yet duration is decreased simply because the reticular formation is severely depressed and unable to initiate tonus properly. This represents then not an active inhibitory feedback loop but merely a depression of the entire system.

5. Summary

In summary then, we have given a systems mechanism to explain the data in view of a unified whole. The neurotransmitters function as interdependent modulators of independent feedback loops. The cholinergic system affects primarily a positive loop resulting in directly related latency and duration values, while the adrenergic system affects both a more-encompassing negative feedback loop and the former positive loop resulting in indirectly

related latency and duration values. The levels of serotonin may function as differential operational amplifiers, with these levels determining which of the two former neurotransmitter systems will predominate.

Summary and Conclusions

The two primary purposes of this study were to present a novel hypothesis of the mechanism of electroconvulsive seizure, and to test the effects of various drugs and regimens on the parameters of the ECS; finally, an attempt was made to synthesize a viable theory concerning these drugs' effects on the hypothesized mechanism.

In the introductory sections, the mechanism of the ECS was described and predicated on the assumption that the organism reacts homeostatically to the overwhelming stimulus of induced ECS. It was seen, that the maximal tonic-clonic seizure has four well-defined stages: latency, tonus, clonus and postictal depression. The core of the theory involved the reticular formation which when activated by the electrical stimulus resulted in tonic neuromuscular manifestations. Physiological and biochemical data was given to build on this reticular activation concept in an effort to clarify the four stages of seizure activity. Correlation was drawn between the EEG, musculoskeletal observations, and functional patterning of the organism when ECS was induced.

The drugs have been shown for the most part to induce changes in the central nervous system neurotransmitter levels. Various drug regimens as well as non-drug experimental situations were included for completeness. It was seen that the adrenergic and cholinolytic drugs and low dosages of methamphetamine induced similar changes in the latency and duration of the seizure. It also was demonstrated that cholinergic drugs and high dosages of methamphetamines caused similar ECS patterns distinct from the former groups of drugs. The data for the serotonergic compounds and methionine sulfoxamine were inconclusive while the data for ethanol demonstrated a unique effect on the seizure patterns.

An effort was then made to synthesize both the mechanism of the homeostatic organism with the various neurotransmitter systems demonstrated in the

data. It was seen that the bioengineering concepts of the staircase integrator and differential operational amplifiers clarified the diverse evidence in a new frame of reference for the ultrastable organism's reaction to the overwhelming stimulus. A positive feedback loop enclosed in a negative feedback loop both acting independently whose loop gain would be affected by two interdependent opposing neurotransmitter systems was seen to adequately explain the data in a unified manner.

Finally, in an effort to justify the assumptions of the science of systems analysis, it was stated that in order to clarify by means of verifiable theories the mechanisms involved in organismic behavior be it as a reaction to massive or to complex subtle stimuli the neurosciences must not stop at the sub-system level. The data must be utilized with the assumption that the whole is greater than the sum of its parts; and to view the whole, the context of systems analysis provides a readily understandable general and mathematical framework.

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APPROVAL SHEET

The dissertation submitted by William Charles Hanigan has been read and approved by three members of the faculty of the Graduate School.

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

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 24, 1971
Date

Charles L. Schuster
Signature of Adviser