

ABSTRACT

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AND NEUROPHYSIOLOGY
11. INFORMATION PROCESSING AND STORAGE IN THE CENTRAL
NERVOUS SYSTEM, WITH EMPHASIS ON NEW
TECHNIQUES FOR MEASUREMENTS AND ANALYSIS

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The growth in the past decade of new knowledge of cerebral anatomy and its possible functional connotation has been stimulated in considerable degree by increasing awareness of intrinsic mechanisms by which information may be coded, transacted and stored in central nervous structures. It has become apparent that the classic notions of neuronal physiology, with conveyance of information exclusively on the basis of pulse-coded phenomena cannot easily provide a realistic basis for discussion of many important aspects of cerebral integrative processes.

Anatomical studies at the level of the single cell have revealed in electron micrographs the striking proximity between dendrites of adjacent nerve cells, and the proximity of nerve cells to neuroglial cells which essentially envelope the nerve cell. These findings have indicated the possibility that information may be conveyed between neurons on the basis of slow, graded wave processes passing in spatiotemporal patterns between adjacent neurons.

Since the neuroglial tissue intervenes between the neuron and its vascular apparatus, it interrelates intimately with neuronal

metabolic processes, and may function other than as a passive support to the neural tissue. Histochemical studies have revealed simultaneous and sometimes reciprocal changes in enzymatic and nucleotide content of neuronal and neuroglial cells in the course of informational transactions (and perhaps storage).

Development of new transducing techniques in the nervous system have been aimed at elucidating some of the long term processes in information storage in which neuroglial tissue might participate. The low membrane resistance of neuroglial cells to small applied impedance measuring currents provides a preferential path by comparison with that through the high resistance of neuronal cell membranes. Our studies have revealed changes in electrical impedance in brain tissue relating to the acquisition of a learned discriminative habit. These studies have revealed that these impedance changes do not occur simultaneously in different brain regions during the learning process.

In constructing a realistic model of the brain system at the cellular level, it appears that electrical brain wave processes may be associated with physicochemical changes in neuronal and neuroglial tissue in laying down of the "memory trace." Changes in electrical impedance loading offered by neuroglial tissue may change the patterns and frequency of EEG waves. At the same time, these studies have emphasized the absence of an equipotentiality in different cortical and subcortical structures in information storage.

In analysis of patterns in electrical brain waves, we have developed an extensive series of computing techniques, including cross-spectral analysis, digital phase-and-amplitude filtering, mutual information analyses, and equivalent-noise bandwidth techniques. These studies have established the characteristics of patterns of brain waves in broad states of consciousness and sleep, as well as dealing with the fine aspects of focused attention in correct and incorrect decision-making.

The model constructed on these computed analyses is both non-linear and stochastic. The probabilistic mode of operation extends beyond the mere pulse-coded firing of the individual neuron to the operation of a cortical domain. It envisages the recall of information as lying in the establishment of a wave pattern in that domain which would resemble, but not necessarily be identical with, previous wave patterns associated with the deposition of information.

I. MODERN CONCEPTS IN
NEUROANATOMY AND NEUROPHYSIOLOGY

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First, may I say how much I appreciate the privilege of participation in this meeting and to have the opportunity to discuss aspects of our rapidly changing knowledge of brain organization. It seemed that a useful way to divide this material would be to consider first some of the anatomical and physiological substrates that have come to a sharp focus over the last five years in studies of brain anatomy and physiology. In the second presentation, I hope to indicate the specific aspects of computer-oriented techniques which have contributed to these developments. In this way, it is possible to consider in detail the physiological basis of cerebral system organization as it may determine use of the computer, and to consider details of techniques that have evolved in relation to specific problems in our own work.

1. Consideration of structural and functional uniqueness of brain tissue.

Let us first determine critically our frame of reference in considering cerebral anatomy and physiology. What are the characteristic things about a brain that distinguishes it from any other part of the central nervous system? May we discover in its anatomy and physiology clues to its unique capacities in processing of information, and particularly in information storage? There is, for example, the curious configuration in the cortical and subcortical brain structures in which cells are very close together, and in

cortical structures exhibit what may be described as a palisade arrangement of cells. This proximity between nerve cells appears to be one of the most fundamental features about the way that the brain is built. I hope to show that it is one of the very fundamental aspects about its functional organization. Probably arising out of this proximity and overlap are forms of physiological activity which are unique to the brain in the occurrence of certain rhythmic electrical processes. It appears that they may have their basis in this physical overlap. The third unique aspect of brain function is its capability to store information rather than merely to transact it or transmit it. Can we usefully consider these notions in a physiological frame which might provide us with some of the basic concepts of how a memory trace could be laid down? I am aware that this is a very ambitious concept if we are to confront it directly at this stage.

a. The concepts of neuronal firing and electrotonic activation.

You are familiar, I am sure, with the general configuration of a nerve cell. It has a cell body, and a very large series of branches, called the dendritic tree, and it has one process which runs away from it, the axon. It has a membrane potential; that is to say, the interior of the cell is negative with respect to the outside. By a process of synaptic bombardment, the membrane potential is moved downwards from its extreme negative value to a point where the cell quite explosively undergoes a massive depolarization. An impulse will then pass at high speed down the axon by similar depolarization. That is the first of the two fundamental electrical phenomena that we will consider. This explosive depolarization produces what is called a "unit discharge." But, as is characteristic of nerve cells in any situation, and particularly in brain tissues, there are slow processes which are believed to be partial depolarizations, or electrotonic phenomena, in which the nerve cell may initiate a wave at the tips of its dendrites and this electric wave creeps relatively slowly towards the cell body, taking perhaps a tenth of a second, or longer. This may or may not be associated with the firing of the cell. Let us then consider the second process, this electrotonic wave, as in some degree independent of the pulse-coded firing of the cell.

b. The role of dendritic overlap in cerebral organization.

How may the profound intermingling of dendritic trees of different nerve cells be detected? Going back to the days of Golgi and Cajal at the turn of the century, it became possible to outline from a very dense population of cells one cell by depositing on its surface silver chromate. One could see the entire cell from the tips of the dendrites, down to the cell body and then out through the axon. This histological method is known as the Golgi technique. This method selectively impregnates about one percent of the nerve cells that occur in any given volume of cerebral tissue. It allows a clear impregnation of all the processes of a particular cell. With this technique, much anatomical system analysis was performed by the older anatomists. Considering the cerebral systems as organized on the basis of aggregates of cells in close proximity (we shall come back to this question of proximity later), what may we learn about the transaction and storage of information by considering separately the activity of single cells and also the activity of populations of cells? This particular topic has led to very intensive investigation, both of the wave phenomena and of the single cell discharges. Let us look first at some aspects of single cell discharges.

2. Major areas of new knowledge of central nervous functions.

a. Patterns of discharge in single cells in response to sensory stimuli.

One of the techniques that physiologists have earnestly endeavored to investigate is the technique of applying a very fine electrode, a microelectrode, to an animal that is conscious. This work was first done effectively by Dr. Hubel, now in the Department of Pharmacology at Harvard University (Hubel, 1959). His technique used a cylinder containing oil which propelled the microelectrodes from a remote drive line. In this way single cell activity could be recorded in the conscious animal, providing it remains reasonably still.

To exemplify the small size of the electrode, which has a diameter of less than one μ at the tip, the process of penetration can be observed under the microscope. It actually indents the cell progressively, and finally penetrates it in an almost explosive entry, which, of course, is not a good thing, and is one of the difficulties and dangers of this technique, since it is necessary to deform the cell before one can actually enter it.

In his initial studies, Hubel was able to show that in the visual cortex the discharge of cells is dependent upon the size and the shape of the object and even on its direction of movement in the visual field. In other words, these cells are indeed extremely sensitive to pattern. For example, during upward movements of a small disc a cell may fire, but reciprocal movements may be without effect on the same cell. In other words, even here at about the fourth or fifth transform of information coming in from the retina there are these very profound dependencies on the pattern of excitation. The effective stimulus is not the presence of a particular stationary object in the visual field, but rather the movement of a particular object in a well-specified direction. This gives some clue as to the complexity of information transaction in the primary visual system.

b. Patterns of discharge in single brain cells during Pavlovian conditioning.

The problem becomes substantially more complex when one tries, as we have done in the last year or so, to examine single cell discharges during the process of learning (Kamikawa, McIlwain and Adey, 1963).

Our own studies have aimed at the study of cells in the central region of the thalamus and to determine if there are changes in firing pattern of the cell in the course of Pavlovian conditioning. Our paradigm was to use light paired with a shock, perhaps one of the oldest and most classical conditioning situations.

The display technique is one in which a cathode ray spot moves slowly across the screen, taking about 5 seconds to do so (Figure 1). Each time the cell fires, a little dot of light appears on the screen and its position, of course, relates to the slow travel of the spot across the screen. The spot is invisible except when the cell fires. One can repeat this procedure every ten seconds. In the course of the passage of the spot across the screen one can expose the animal to a flash of light and then to the sciatic shock. In the interval between the flash and the shock, we noted the progressive appearance of an increased discharge, in what the psychologist would call the CS-US interval. When one then exposes the animal to the flash only, there is a persistence of the increased firing rate in the CS-US interval, not present initially. In other words, there has been a fundamental change in the system. One might call this learning, but it would probably be well to reserve opinion as to whether the cell itself has actually learned anything. But here is what is called an adaptive change, which is the result of the impingement on the cell of something about which it has had previous experience.

This work is very exacting in its technical requirements, since it is necessary to record from one cell for several hours in the face of vascular and respiratory pulsations which tend very soon to destroy the cell membrane by impingement on the electrodes. One can train the cell and extinguish the response, and retrain and extinguish in a sequence of such procedures. During the so-called extinction period -- that is, when one is giving flash only -- the pattern changes progressively with repeated experience of the cell. In the progressive examination of a series of extinction trials, and using the mean firing rate as the basis of the statistical analysis, one may find that at first there is a slight inhibition, followed by an increase in firing during extinction. The inhibition becomes progressively greater and the amount of acceleration decreases progressively (Figure 2). In other words, seen at the level of the single cell, the complexity of pattern change that occurs during these procedures is very profound indeed. It makes one wonder how it would be possible to assess the changes in cell firing in the population as a whole, if one multiplied this process a hundred, a thousand, a million times, to reproduce the complexities of firing patterns in the brain as a whole. In terms of the analysis of this data, we are currently interested in a model based on the hill climbing statistics of G. P. Box (1951), in which the optimization of the process is viewed as the development of a hill arising out of a terrain, or the examination of a terrain containing multiple hills for the hill that is highest. Interest in this particular model of the learning process was engendered in the first instance by a paper by Dr. Samuel of IBM here several years ago, in which he discussed the use of the same technique -- for the checker playing schemes that he has developed out of playing one 7090 against another.

Looking at cellular behavior in this case may well reveal the intrinsic phenomena at the level of the single cell. It would seem very difficult, however, to extrapolate to neuronal populations on the basis of the very wide variations that occur from cell to cell in the one population, indeed in one nucleus between neighboring cells in the course of this learning process. As I have indicated, concurrently with these phenomena in single cells, there is considerable slower wave activity in the same domain of tissue.

c. Electrical waves in cerebral tissue; inequipotentiality in the cerebral cortex.

Let us now turn to the concomitant wave processes occurring in cerebral tissue and ask how these might correlate with the learning

process. The cortical mantle, covering the hemisphere, has been viewed by psychologists as being in many respects equipotential, that is, as having equivalent properties throughout its extent, without major aspects of regional functions. This, of course, was the theory that was most heavily propounded in the late 20's by Lashley, and it has necessarily undergone considerable modification since (Lashley, 1926). There are two cortical regions which do appear to concern themselves very much with the learning process. One is the frontal region of the cortex, and the other is the temporal region. Magoun's early work emphasized the presence of an internal core, or reticular formation projecting widely up to the cortical mantle in an essentially indiscriminate fashion. Our studies and those of others have disclosed descending pathways from the cortex to this reticular core. Both processes appear very important in the intrinsic mechanisms of consciousness. In particular, those projections which come out of the frontal lobe and out of the temporal lobe, and particularly those from the temporal lobe, are those which appear to have some prior significance in the process of learning and memory. That is not to say that either frontal cortex or temporal cortex is the seat of memory, but that those processes which characterize activity in the frontal cortex and in the temporal cortex, and particularly temporal cortex, appear essential to the normal laying down of a memory trace in other brain systems. This may be an oversimplification, but is a useful working hypothesis.

Behaviorally, there are some curious changes following removal or modification of this temporal lobe cortex. Perhaps the most dramatic is the condition known as "psychic blindness," in which the animal is unperceiving of the significance of objects in its environment (Kluver and Bucy, 1939). Additionally, in a classic study by Schreiner and his colleagues (1953), in which they removed or damaged this cortex in a series of animals, one very obvious and striking effect was the ensuing hypersexuality. He cited instances of a cat attempting intercourse with a dog, with a chicken, with a monkey, and of four cats engaged in tandem copulation. I mention this to exemplify what can happen to the global aspects of behavior when one interferes with a small portion of the brain. In this case, also, there are usually defects in learning capability. Our own studies have been mainly concerned with the electrical activities of these temporal lobe regions of the brain. Let us next consider the feasibility of recording brain electrical activity in the freely moving, or at least relatively unrestrained animal and man.

d. Recording electrical wave activity from conscious, performing subjects.

It is possible, for example, to implant electrodes in the brain of a cat, and to place a small connecting plug on its head, and it remains a perfectly normal, healthy animal. In the same way, with a rather more substantial attachment, one can study monkeys on a centrifuge undergoing accelerations up to about 10 or 20 g's sustained over periods of minutes to simulate booster profiles for space experiments, with the animal making an oddity discrimination performance while the brain activity is recorded. We have done many such experiments while recording the brain activity in these animals on the centrifuge facility at the University of Southern California (Adey, Kado, Winters and DeLucchi, 1963).

Most recently our interests have focused on the chimpanzee. We have a unique colony of animals with implanted surface and deep brain electrodes, after two years' work in preparing a stereotaxic atlas. It is possible to perform similar implantations in the brain of man. It is possible to have the man walking along, or sitting, making judgment discriminations and actually learning task performances while the activity is recorded from inside his brain in the same placements that were used in the chimpanzee. These are patients who are suffering from temporal lobe epilepsy and Parkinson's disease, and the placement of these electrodes by our neurosurgical and neurological colleagues is on the basis of a therapeutic need (Rand, Crandall, Adey, Walter and Markham, 1962). Such procedures in man may be made only after full consideration of ethical problems. We have also developed techniques to be discussed below which allow us to record the surface EEG from a subject driving on the freeway at about 65 or 70 miles an hour. The analysis of such data will be discussed in the second presentation.

What sorts of wave patterns can one discern in a simple discriminative test situation in an animal? One may consider a cat in a modified T-maze, where the approach is to one or other side of a T-box, on the basis of a visual cue. The sides are selected in random sequence and the animal must come from behind closed doors to reach the food. Under these conditions, there are very clear changes in the pattern of activity in the temporal lobe region of the brain. One notices that during the period when it is approaching the food with maximum focusing of attention on the problem, there is the appearance of a rhythmic train of waves in the hippocampal system of the temporal lobe. In other words, in the period of decision-making this type of activity appears in the temporal lobe of the brain.

e. Physiological manipulation of the EEG wave process in performing subjects.

However, there are other areas of the brain, such as the core substance, or reticular formation, and the visual cortex, where there are fragmentary but quite definite aspects of this original rhythmic process which appears to arise out of the temporal lobe. How can one best manipulate these wave processes to see how fundamental is their relationship to the performance? I shall describe four that we have used.

i.) Effects of subthalamic lesions. Firstly, one can make small lesions, damaging regions of the brain which are quite remote from the temporal lobe, but which appear to alter rhythms in the temporal lobe itself, and observe what happens to the performance. One can also give drugs, which have effects primarily in this part of the brain. One can introduce physical stimuli, such as vibration or radiation, which disrupt the rhythmic process and again see what happens to the performance.

Let us consider the effects of two small adjacent lesions in deep brain structures, one in the subthalamus and another confined to more dorsal parts of the diencephalon. The first lesion produced a profound disturbance in the animal's behavior, which was reproduced in a series of animals with this type of lesion, whereas the more dorsal thalamic lesion did not (Adey, Walter and Lindsley, 1962).

The animals with subthalamic lesions suffered a curious alteration in perceptive capability. After a lesion restricted to the left side of the brain, the animal ignored objects placed in the opposite half of the visual field. It would watch closely the concealment of food in the opposite half of the visual field, and as soon as the object was concealed it would completely ignore that situation and make its subsequent approach to the left. This defect gradually subsided over a period of ten days. After coagulation on the right side, there was a complete reversal of this picture. This time, the cat no longer approached objects on the left, but gradually recovered the capability to do so. There were characteristic changes induced in the electrical activity of deep parts of the temporal lobe following these lesions (Figure 3). During the approach, control records from the deep parts of the temporal lobe were characterized by regular wave trains, and these were abolished after a bilateral lesion. At this stage the animal was considerably below chance in its performance. After several weeks,

the waves gradually reappeared and the performance improved concomitantly. There is thus a correlation between the presence of a wave train in brain tissue and the ability to perform the required task.

ii.) Effects of psychotomimetic and hallucinogenic drugs.

Another quite dramatic way of investigating the relations between brain electrical activity and behavior is to give drugs known as psychotomimetic or hallucinogenic agents. Drugs such as lysergic acid diethyl amide (LSD) and allied substances have the capability to totally disrupt patterns of electrical activity in the deep parts of the temporal lobe, replacing them with epileptiform seizures which spread into the central core or reticular formation (Adey and Dunlop, 1960; Adey, Bell and Dennis, 1962).

We may consider briefly the effects of another series of drugs, the cyclohexamines and their derivatives, in the cat (Figure 4). Five minutes after one of these drugs is given, the wave pattern in deep temporal lobe regions during the period of approach in the T-maze no longer speeds up to a 6 cycles per second burst. It remains around 3 or 4 cycles per second, and the animal makes an incorrect decision, going to the wrong side. After twenty minutes there is no regularity of wave patterns, and no movement. Twenty-four hours later the animal recovers to an 80% performance level. With correct decisions there is regularization of the waves at about 6 cycles per second. This drug was one that had extensive clinical trial in this country as a general analgesic agent for major surgery. It has the theoretical advantage that it is not necessary to make the patient unconscious in the usual sense. They may be quite cooperative throughout major surgical procedures. However, it has been found to be an undesirable clinical agent for the reason that, associated with this abnormal activity, the patients suffered a severe psychotic experience. They felt that they had died, and that they would go to the place wherever dead people go, being accompanied into this death state by relatives. It was so disconcerting to them, that they were not infrequently homicidal towards their medical advisors following this experience. About three months ago the FDA therefore requested the destruction of stocks of this drug.

Typically, cats under LSD lie purring, but nevertheless have their claws extended. They frequently paw at invisible objects, striking at things that apparently appear as hallucinations in front of them. They stand with a curious wide-legged gait, with the tail up, the fur is erect, and frequently shake their heads violently, as though trying to get rid of something that is in their visual field; or, they will stand for long periods with a curious catatonic posture, not moving at all.

When tested in the T-maze box while under the influence of LSD or Psilocybin, episodes of seizure-like waves in the deep parts of the temporal lobe and spreading to subcortical structures can be seen about an hour after administration of the drug. At these times, the learned performance is severely disrupted. Psilocybin is more transient in its action than LSD, and about two hours later the animal recovers to the extent that it can reach the food. It may still be so disoriented that it may occasionally walk into space if passing on a narrow bridge, but the rhythmic wave patterns characteristic of discriminative approach have reappeared in most trials, and it can reach the food correctly.

iii.) Effects of vibration on EEG activity and performance.

As a problem associated with the booster phase of space flight, the body is exposed to a wide spectrum of vibrations. Very little is known about the effects of vibratory stimuli on the functions of the central nervous system. We have tested monkeys on shakers at the Douglas Aircraft plant in Santa Monica. The shaker is essentially a giant loudspeaker unit, capable of handling 10 kilowatts of audio. For those of you who are interested in hi-fi, you will realize that this is somewhat larger than the typical instrument. Our interest was in shaking the whole body of the monkey over a spectrum from 5 to 40 cycles per second. We observed a driving of brain wave rhythms under these conditions, which disappeared with anesthesia or death, and which was sensitively related to certain frequencies of vibration and was not the result of artifacts in the connecting leads (Adey, Kado, Winters and DeLucchi, 1963).

For example, shaking across the part of the spectrum from 9 to 12 cycles per second induced rhythmic driving in the visual cortex, centrum medianum, midbrain reticular formations and in the deep parts of the temporal lobe. Accelerometers were placed on the head, the torso, and the table (Figure 5). After a barbiturate anesthetic, that driving during vibration at the same frequencies in the same animal was abolished, except for a small amount persisting in some subcortical regions, such as the midbrain and in the centrum medianum of the thalamus, but much reduced by comparison with control records, suggesting that it is a true physiological phenomenon.

This can interfere grossly with the ability to make an oddity test performance. For example, during shaking at 5 cycles per second, there was no driving of cerebral rhythms, and the oddity task was immediately performed correctly, quite unimpaired by the shaking. However, during shaking at 15 cycles per second, there was intermittent driving, particularly in core structures such as the midbrain and in the nucleus centrum medianum. At this stage when the animal is presented with a task, it looks away and pays no attention to the test. This is typical of the distraction that occurs and which appears to relate critically to the initiation of these abnormal wave patterns in the structures of the brain most concerned with discriminative functions, namely, the temporal lobe systems and the core structures of the reticular formation.

iv.) Studies of changing states of consciousness and sleep. So far we have discussed primarily those patterns of waves which relate to focused attention and judgment capabilities. But there is a whole category of processes which relate to the broad states of consciousness and unconsciousness, from extreme alertness through normal attention to drowsiness to light sleep and to deep sleep (Adey, Rhodes and Kado, 1963). For example, records from one of our chimpanzees as he shifts from wakefulness, to napping and light sleep show typical changes. High amplitude wave trains appear in both cortical and subcortical structures during light sleep. When the animal is wakened, there is a fundamental change in pattern, with replacement of the high amplitude waves by low amplitude fast activity. This might serve as our first introduction to these very broad changes about which I will say much more later, relating specifically to the problems of sleeping and wakefulness.

3. The nature of the cerebral wave process.

What is this cerebral wave process? As already mentioned, a likely explanation is that it is an electrotonic phenomenon in the dendrites of nerve cells. Why, then, does its frequency change from moment to moment?

Although there is no definite answer at this time, I would like now to introduce a hypothesis which will figure prominently in the remainder of this discussion. It is suggested that these changes in frequency may result from an electrical impedance loading of the dendritic generators by tissue adjacent to the nerve cell. This adjacent

tissue may be other neurons, but it may also be the neuroglia tissue. This, then, is the basis of the hypothesis: that the change in frequency may in part be induced by impedance loading offered by tissue around the neurons. The tissue elements involved are not necessarily neural, but should be capable of altering their own resistance as the functional state of the brain changes (Adey, Kado and Didio, 1962).

a. The intraneuronal, intraglial and extracellular compartments of cerebral tissue.

If we examine an electron micrograph of the hippocampus in the temporal lobe, we find that the distance between nerve cell membranes may be as short as 100 angstroms (Green and Maxwell, 1961). Where the nerve cells are not in contact (and in other parts of the brain, the so-called neuroneuronal contacts are presumably not so great), the rest of the cerebral volume is essentially filled by the paler substance of the neuroglia. There is very little extracellular space. It is generally agreed that the brain has a very small extracellular space, probably not more than 2 to 4% of the total cerebral volume. Here, then, is the concept of a tricompartmental brain system, with extracellular, intraglial and intraneuronal compartments. We will discuss below the way in which these compartments may possibly relate to one another in the laying down of a memory trace. At this stage, however, we may examine some of the functions which can definitely be attributed to this neuroglia tissue. Here we may turn to the classic work of Holger Hydén in Sweden over the last fifteen years, which has delineated certain separate functions of the nerve cell and the neuroglia and certain ways in which they may interrelate with one another (Hydén and Pigon, 1960). His elegant techniques enable him to examine the nucleotide content of single cells, in both neurons and neuroglial cells. He dissects out the single cell from the living brain and then places it in a small chamber into which ribonuclease is introduced. The depolymerized ribonucleic acid is extracted and examined with ultra-violet light. It is possible to prepare a density scale and to record automatically the content of RNA for that particular cell. This test can be performed on the cytoplasm or on the nucleus separately.

It is also possible to measure the content of the four bases that make up this nucleic acid. The nucleic acids have four bases: adenine, guanine, cytosine, and uracil, and they are the genetic building blocks of all cellular systems. Hydén isolates the

RNA from the cell, places it on a cotton thread, and then separates the four bases iontophoretically by applying a current to the thread. Their density can be automatically determined. On this basis, Hydén has initiated manipulations of physiological activity in certain systems of brain cells, in circumstances which may relate to learning and storage of information. The system with which he has worked mostly is the Deiter's nucleus in the medulla, part of the vestibular system. He has exposed rabbits to rotational stimuli for a period each day for a week, and then examined the nucleotide and enzyme content of the cells after this exposure.

He has found, for example, that during the period of seven days' exposure to rotation, the RNA content of the neurons increased, whereas the RNA content of the neuroglia decreased. Similarly, the cytochrome oxidase increased in the neurons, but decreased in the neuroglia. In the same period, the total protein content of the neuron increased progressively. Thus, in essence, the neuron and its neuroglial envelope constitute a metabolic microcosm. They cannot be considered apart from one another. The changes between them may be reciprocal or mutual, but the functional shifts that have been examined so far support the view that neurons and neuroglia form part of a common metabolic system. It should be emphasized that this neuroglial tissue completely encloses the neuron, that the only way that vascular exchange can occur is through the substance of the neuroglia. There is no direct access by neurons to their blood supply.

b. Use of impedance measuring techniques in cerebral tissue.

Can one, then, assess the functional role of the neuroglia by electrical techniques? For example, if it offers a changing impedance load to the generators in the dendrites of nerve cells, then the changing impedance of the neuroglial tissues might be assessable. It has been found that neuroglial cells conduct electric current from 100 to 1,000 times more easily than do nerve cells, by reason of a much lower membrane resistance (Hild and Tasaki, 1962). If we impose a current on the tissue to measure its impedance, it may pass through any one of three paths. It can go through extracellular fluid; or through neuroglia, or through neurons. The extracellular space is small. One would not expect its conduction to change during physiological phenomena. The neurons have a high membrane resistance and neuroglial cells low membrane resistances. Therefore,

at least as an initial working hypothesis, if one can measure the impedance of cerebral tissue during various functional states in the brain, it might be possible to say something about the functional role of the neuroglia.

We have looked at cerebral impedance changes by a new technique, developed in our laboratories to measure impedance with very small measuring currents. We have used currents of the order of 10^{-13} amperes per square micron of electrode surface, at 1,000 cps, and with a differential amplifying system it is possible to measure changes in current as small as 10^{-15} amperes per square micron. Changes are recorded in approximately 1.0cmm. of brain tissue. Why use such small currents? The earlier work with cerebral impedance measurements used currents that actually stimulated the tissue that was under test, which seems a dubious way of trying to detect the normal dynamics of the tissue being examined.

With this technique it became possible to see in many parts of the brain changes, which result, for example, from the stimulation of the animal by some external event. In the dorsal hippocampus in the temporal lobe, transient decreases in impedance occurred each time the animal saw an observer, or when a female cat saw a male cat, and again when the animal smelt milk, or when a drop of milk was placed on its nose (Figure 6). By this sensitive technique, it became possible to see changes in electrical brain impedance, which relate to changes in functional states of the tissue (Adey, Kado and Didio, 1962).

It is also possible in this way to see changes of a rhythmic nature in impedance, which occur much too slowly to be seen with a conventional EEG. The septal region of the brain, which is intimately related to the deep parts of the temporal lobe, exhibits 3 cycle per minute waves in burst which go on more or less indefinitely during normal sleep. During a barbiturate anesthetic the impedance rises progressively and then reverts to the baseline level on waking. At the full depth of the anesthetic a painful stimulus produces very little impedance change. About two and a half hours later there is a sharp transient decrease with a similar stimulus. This response becomes sharper as the anesthetic wears off. A new step is established in the impedance as the animal wakes up.

Whereas barbiturate anesthesia induced a raised impedance in this brain tissue, by contrast, some of these psychotomimetic drugs that were mentioned earlier, the cyclohexamines, induced a fall in baseline impedance. At the onset of the action of the cyclohexamines, there was a brief fall in impedance briefly as the animal heard a loud click, in a fashion strongly resembling the normal impedance response. Under the full influence of the drug, however, one brief click produced a sudden step in the baseline impedance which took many many minutes to come back to the original baseline level. The concomitant EEG showed only a transient change, much different from this long lasting change in the brain impedance.

Because the impedance measuring system appeared to be quite sensitive, we decided to apply it to the learning situations that we have discussed in detail, particularly the T-maze situation, and to seek changes in the course of the acquisition of a learned habit (Adey, Kado, Didio and Schindler, 1963). A computed average of 30 trials at the chance level of performance showed no inflections relating to the presentation of the situation. With the performance at about 80% correct, there was a small, brief fall followed by a rise. From the same animal, as its performance reached 100% , there was a deep fall in impedance on three averages from three successive days¹ training. This fall maximized during the performance of the learned task. It was found to persist as long as the animal remembers the task (Figure 7). One can extinguish the behavioral response, and the impedance response is lost. In retraining, it reappears. Its full configuration is first a fall, followed by a rise, and then a return to the baseline over a period of 8 seconds, as determined in computed averages.

One of the very interesting findings was that this impedance change does not appear simultaneously in all brain regions. For example, in essentially symmetric placements in the hippocampal zones of the temporal lobe on the left side and the right side, impedance records averaged over a period of five days and four days for the left and right sides respectively at the 80% performance level, already showed a deep change in the right side, but extremely little on the left. When the animal reached 100% performance level, the side which was lagging now showed a change as big as that on the side which was showing the change at the 80% performance level.

4. Summary

In summary, it would appear that we may have in the brain a tricompartmental system, in which we can measure changes in its long term state on the basis of impedance measurements, made here with 1,000 cycle current. This is obviously one end of a very broad electromagnetic spectrum, and there are many other regions of the electromagnetic spectrum that we can use for these impedance measurements. Currently we are very interested in, and have initiated experiments with, various parts of the light spectrum. We are now moving into the infra-red and the ultraviolet segments. It is suggested that it will be through such techniques that we will come to an understanding of the mechanisms underlying the storage of information. In this tricompartmental cerebral system, the intimate relations between nerve cells are associated with a wave process. This wave process, as I hope to show in the following paper, appears concerned in the transaction of the information and is not a noise in the cerebral system. For so long as we have been able to record the EEG, it has been considered by many as having merely the characteristics of a noise. The waves appear, however, to underlie the physicochemical basis of a memory trace, in that the initial deposition of the information appears to be related to the presence in the tissue of a particular spatiotemporal pattern of waves. It also appears that the recall of that information may depend on the establishment of a similar, but not necessarily identical, pattern of waves in that same cortical domain. The frequency of these waves may be a function of the impedance loading offered by the neuroglial tissue to the generators. The loading changes in space and time and is probably non-linear. We have progressed to the point of considering a model of the system, which is non-linear in its characteristics of information transformation and possibly information storage. The final point, and the working hypothesis which we are pursuing very earnestly, is that the physicochemical changes underlying the memory trace may lie at the interface between the neuronal and neuroglial tissue. It is the physicochemical change here that may be responsible for the permanent change in the excitability of the neuronal tissue in what we call memory (Adey and Walter, 1963).

ACKNOWLEDGMENTS

Studies described here from our own laboratories were assisted by Grant NB-1883 and Grant M-3708 from the U. S. Public Health Service, by Contract AT(11-1)-34 (Project 60) with the U. S. Atomic Energy Commission, by Grant AF(AFOSR) 61-81 from the U. S. Air Force, and by Grant NsG 203-62 and Grant NsG 502 from the National Aeronautics and Space Administration.

REFERENCES

- Adey, W. R., Bell, F. R., and Dennis, B. J., "Effects of LSD, Psilocybin and Psilocin on temporal lobe EEG patterns and learned behavior in the cat." Neurology, 12: 591-602, 1962.
- Adey, W. R., and Dunlop, C. W., "The action of certain cyclohexamines on hippocampal system during approach performance in the cat." J. Pharmacol. Exper. Therap., 130: 418-426, 1960.
- Adey, W. R., Kado, R. T., and Didio, J., "Impedance measurements in the brain tissue of chronic animals, using microvolt signals." Exper. Neurol., 5: 47-66, 1962.
- Adey, W. R., Kado, R. T., Didio, J., and Schindler, W. J., "Impedance changes in cerebral tissue accompanying a learned discriminative performance in the cat." Exper. Neurol., 7: 282-293, 1963.
- Adey, W. R., Kado, R. T., Winters, W. W., and DeLucchi, M. R., "EEG in simulated stresses of space flight, with special reference to problems of vibration." EEG Clin. Neurophysiol., 15: 305-320, 1963.
- Adey, W. R., Rhodes, J. M., and Kado, R. T., "Cortical and sub-cortical sleep recordings in the chimpanzee." Science, 141: 932-933, 1963.
- Adey, W. R., and Walter, D. O., "Application of phase detection and averaging techniques in computer analysis of EEG records in the cat." Exper. Neurol., 7: 186-209, 1963.
- Adey, W. R., Walter, D. O., and Lindsley, D. F., "Effects of subthalamic lesions on learned behavior and correlated hippocampal and subcortical slow-wave activity." A.M.A. Arch. Neurol., 6: 194-207, 1962.
- Box, G. P., and Wilson, K. B., "On the experimental attainment of optimum conditions." J. Royal Statistical Soc., 13: 1-45, 1951.
- Green, J. D., and Maxwell, D. S., "Hippocampal electrical activity. I. Morphological aspects." Electroenceph. Clin. Neurophysiol., 13: 837-846, 1961.

Hild, W., and Tasaki, I., "Morphological and physiological properties of neurons and glial cells in tissue culture." J. Neurophysiol., 25: 277-304, 1962.

Hubel, D., "Single unit activity in striate cortex of unrestrained cats." J. Physiol. London, 148: 574-91, 1959.

Hydén, H., and Pigon, A., "A cytophysiological study of the functional relationship between oligodendroglial cells and nerve cells of Deiters' nucleus." J. Neurochem., 6: 57-72, 1960.

Kamikawa, K., McIlwain, T., and Adey, W.R., "Response patterns of thalamic neurones during classical conditioning." EEG Clin. Neurophysiol., accepted for publication, 1964.

Kluver, H., and Bucy, P.C., "Preliminary analysis of the functions of the temporal lobes in monkeys." Arch. Neurol. Psychiat., 42: 979-1000, 1939.

Lashley, K.S., "Studies of cerebral function in learning. VII. The relation between cerebral mass, learning and retention." J. Comp. Neurol., 41: 1-58, 1926.

Rand, R.W., Crandall, P.H., Adey, W.R., Walter, R.D., and Markham, C.H., "Electrophysiological investigations in Parkinson's disease and other dyskinesias in man." Neurology, 12: 754-770, 1962.

Schreiner, L., and Kling, A., "Behavioral changes following rhinencephalic injury in cat." J. Neurophysiol., 16: 643-659, 1953.

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LEGENDS TO FIGURES

- Figure 1 Development of an inhibitory conditional response in a thalamic neuron (see text). Each dot represents a single neuronal firing, and each horizontal row of dots a single trial. Trials are grouped according to stimulus conditions, designated SCIATIC ONLY (stimulation of sciatic nerve), FLASH ONLY (presentation of flash stimulus without shock to sciatic nerve) and FLASH AND SCIATIC ("training trials" where both flash and sciatic shock were presented.) Times of respective presentation of flash and sciatic shock are shown by vertical lines. (From Kamikawa, McIlwain and Adey, 1963).
- Figure 2 Repeated extinctions of inhibitory conditional responses in a habenular neuron. A. histograms depict difference scores (numerical difference between firing rates in pre-flash control period and epoch of 820 msec beginning 820 msec post-flash) for flash control and all extinction sequences. Each column represents average score for 5 trials. Columns are below the abscissa when the CS caused inhibition.
- Figure 3 Recovery from effects of bilateral subthalamic lesions, with correlated EEG records during T-maze performance. Rhythmic 6 cycles per second waves in hippocampal structures in control record (A) were replaced by irregular, slower waves during attempted approach 3 days after lesion completed, but much of this activity had returned 27 days after lesion (C). All EEG traces are from hippocampal (LDH and RDH) and entorhinal cortical structures in the temporal lobe of the brain. (From Adey, Walter and Lindsley, 1962).
- Figure 4 Effects of psychosis-inducing drug Sernyl on the EEG wave patterns in the brain of the cat during attempted discriminative performances. Control record (A) shows typical 4 to 5 cycles per second hippocampal activity in the waiting period, and a speeding to 6 cycles per second during the approach. The drug abolished the correct behavioral performance in 20 minutes, and disrupted hippocampal slow wave activity (B and C). After 24 hours (D), behavioral performance had improved, with return of 6 cycles per second burst during approach. (From Adey and Dunlop, 1960).

- Figure 5 Driving of EEG rhythms by whole body shaking from 9 to 13 cycles per second as part of spectral sweep. By contrast with records from conscious animal (A), records in (B) under a barbiturate anesthetic show essentially no driving in hippocampal regions of the temporal lobe (L. HIPPO. and R. HIPPO) and visual cortical leads (L. and R. VIS. CORT.). Driving in subcortical structures of thalamus and midbrain (L. MB. RF.) was much reduced. (From Adey, Winters, Kado and DeLucchi, 1963).
- Figure 6 Simultaneous impedance and EEG records during various physiological stimuli in the cat. In the top left record, there was a succession of transient falls in impedance each time the cat sees an observer. In the top right record, there was a fall in impedance in a female cat on observing a male cat. In the bottom left record, a fall in impedance was induced by smelling milk. In the bottom right record a drop of milk was placed on the cat's nose. (From Adey, Kado and Didio, 1962).
- Figure 7 Averaged hippocampal impedance record over 8-second epoch, during discriminative approach to food in a cat at the 80% performance level. This is a computed average of 40 trials on 1 day. (From Adey, Kado, Didio, and Schindler, 1963).

UNIT CONDITIONING FLASH AND SCIATIC STIMULUS REPETITION ONE PER 10 SEC.

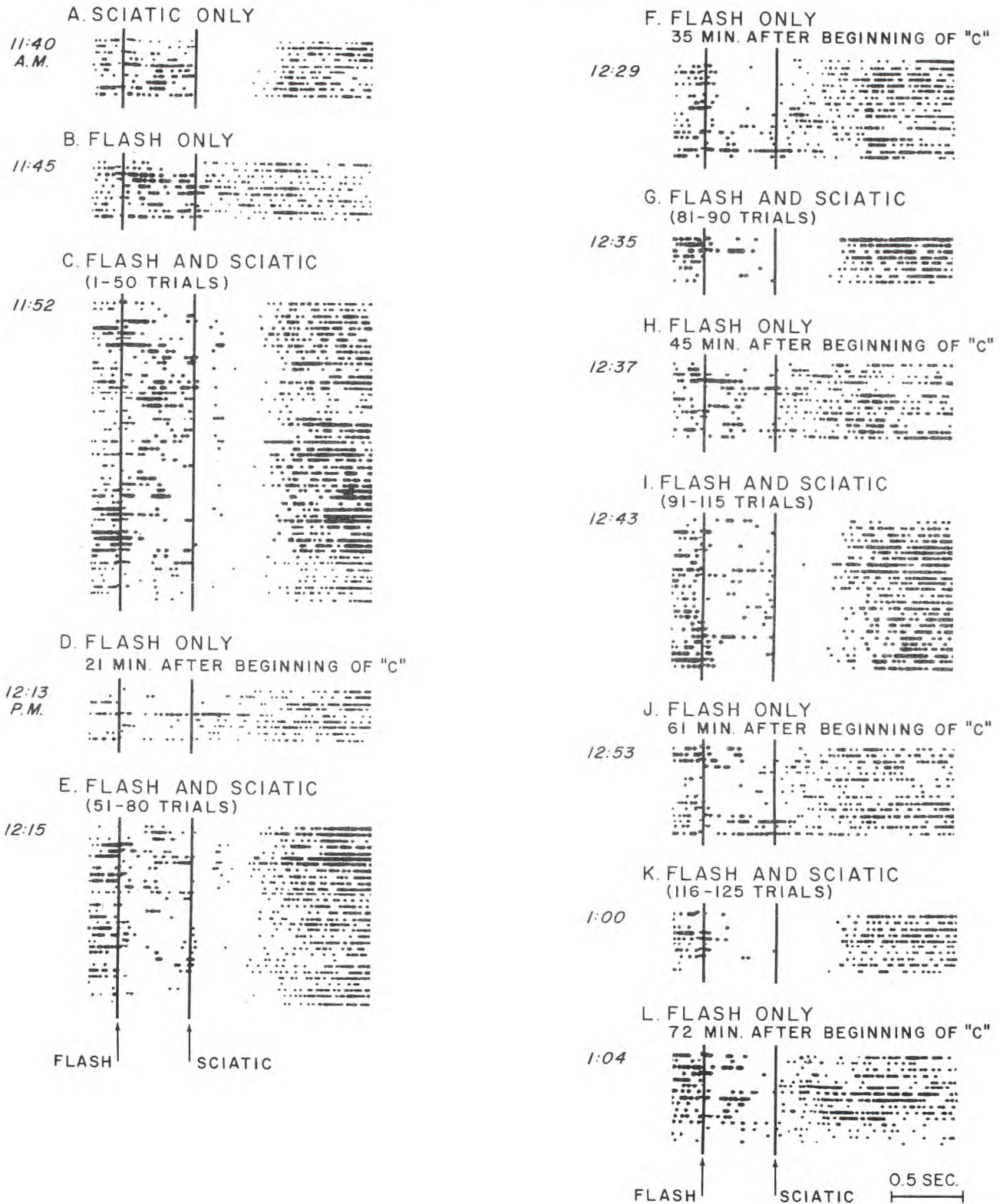
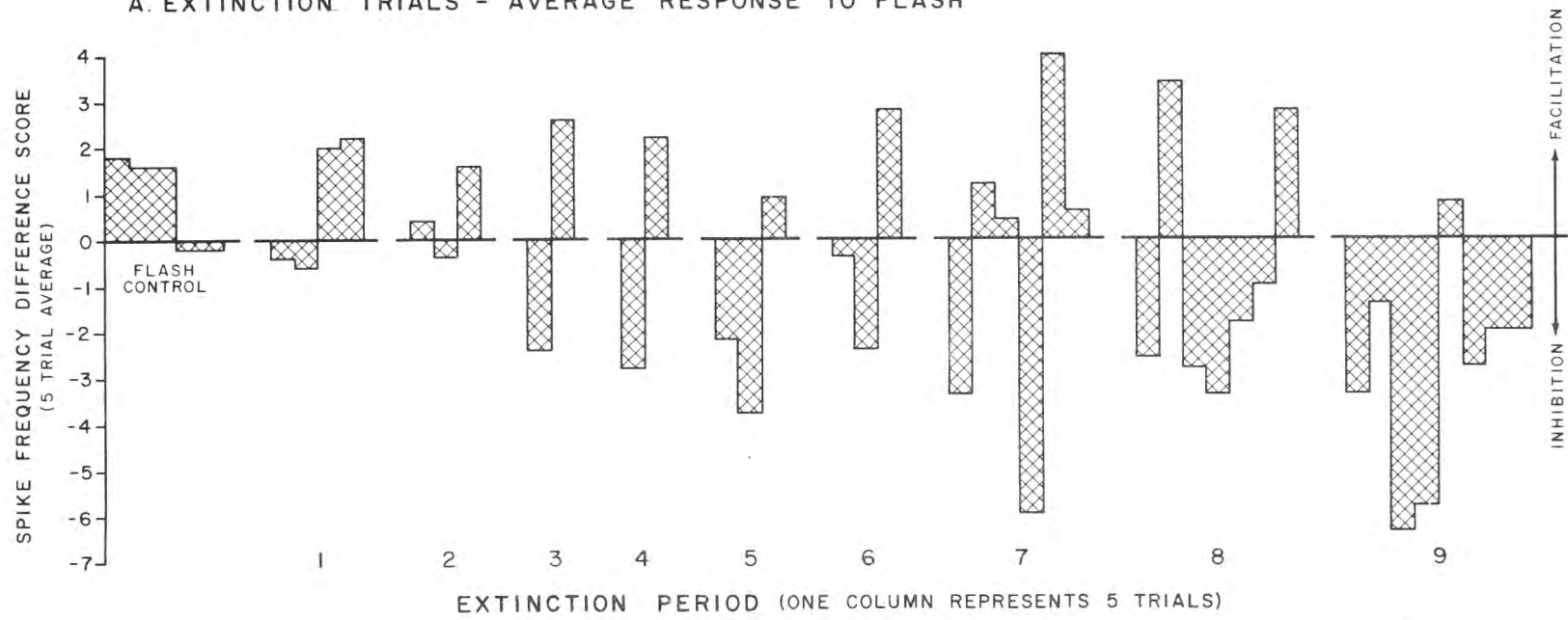


FIGURE 1

UNIT CONDITIONING - RIGHT HABENULAR COMPLEX

A. EXTINCTION TRIALS - AVERAGE RESPONSE TO FLASH



B. FLASH ONLY (EXTINCTION 9)

INTERTRIAL INTERVAL = 10 SEC.

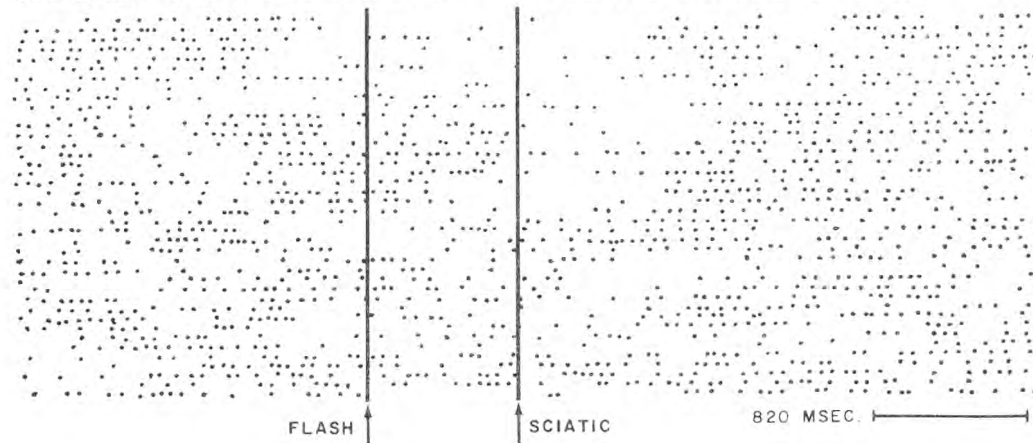
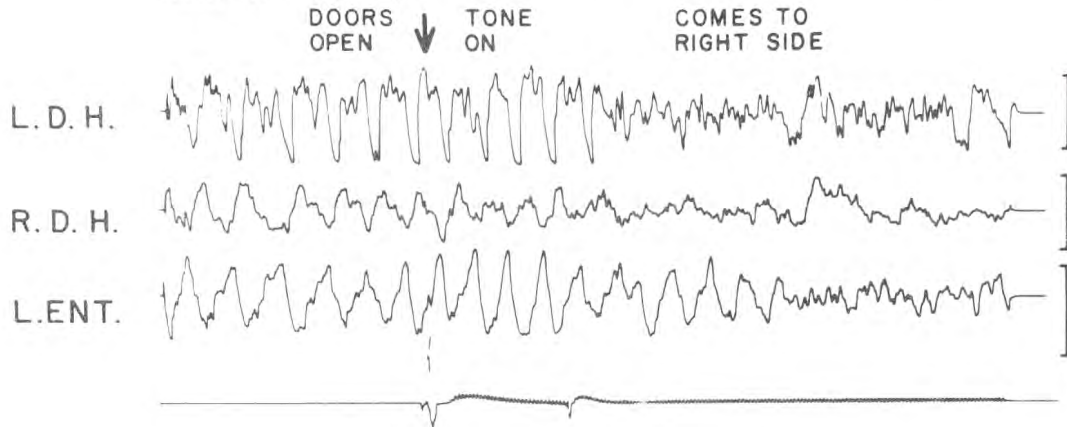


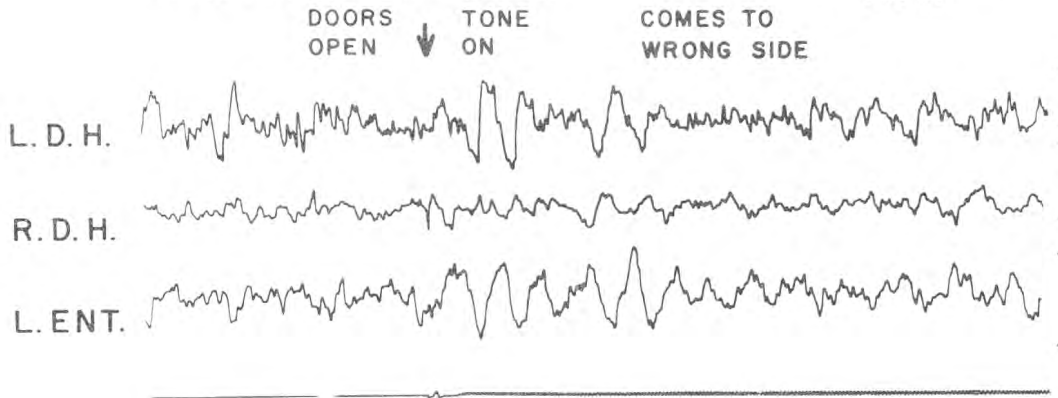
FIGURE 2

EFFECTS OF BILATERAL SUBTHALAMIC COAGULATION - T-MAZE (CAT WL-8)

A CONTROL RECORD - PERFORMANCE 84%
TRIAL 17 - RUN 13



B 3 DAYS AFTER BILATERAL SUBTHALAMIC COAGN.
TRIAL 1 - (POST-OP) - RUN 32 23%



C 27 DAYS AFTER BILATERAL SUBTHALAMIC COAGN.
TRIAL 9 - (POST-OP) - RUN 11 65%

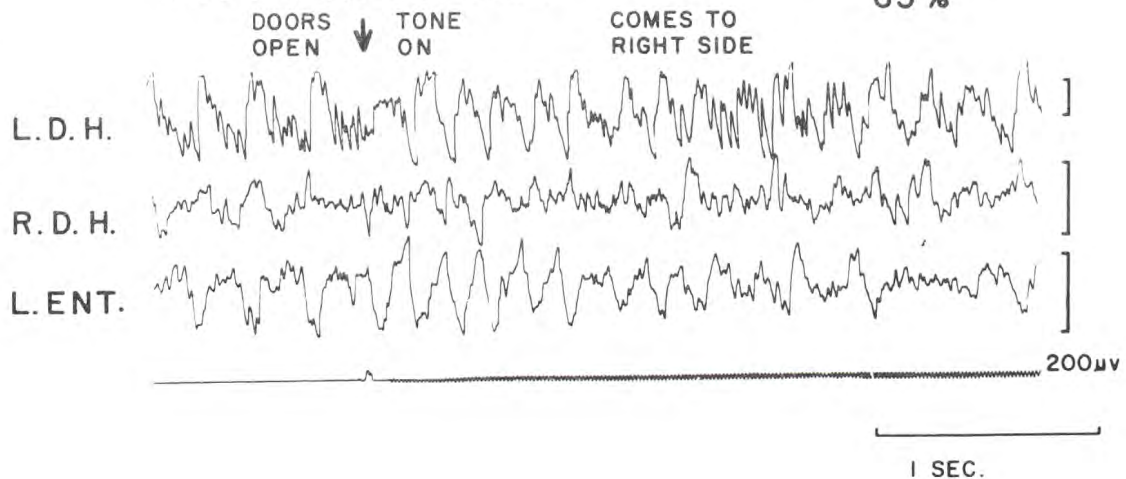


FIGURE 3

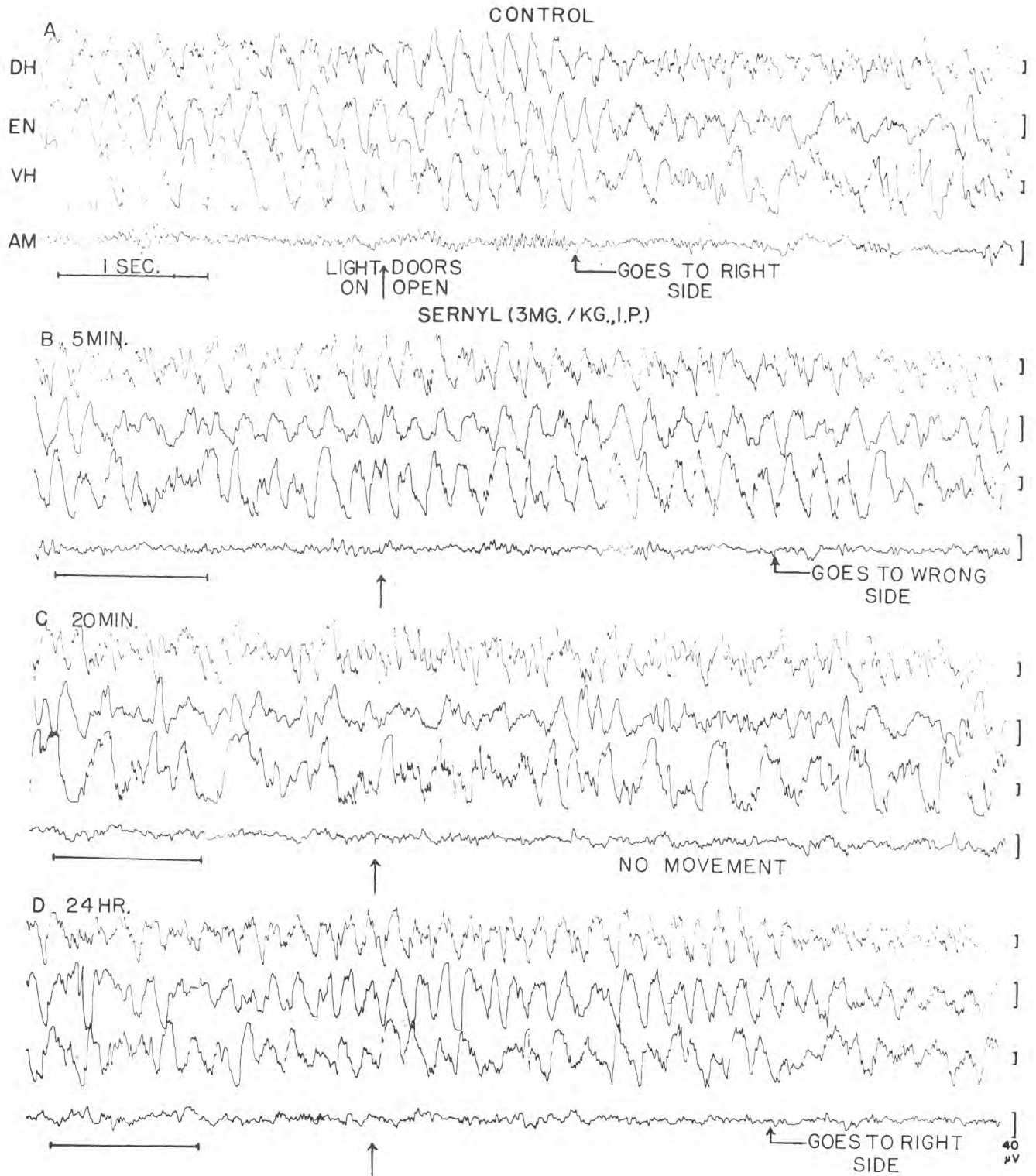


FIGURE 4

SHAKING TEST - LONGITUDINAL AXIS

0.25" DOUBLE AMPLITUDE

A. CONSCIOUS MONKEY

MACAQUE N-3

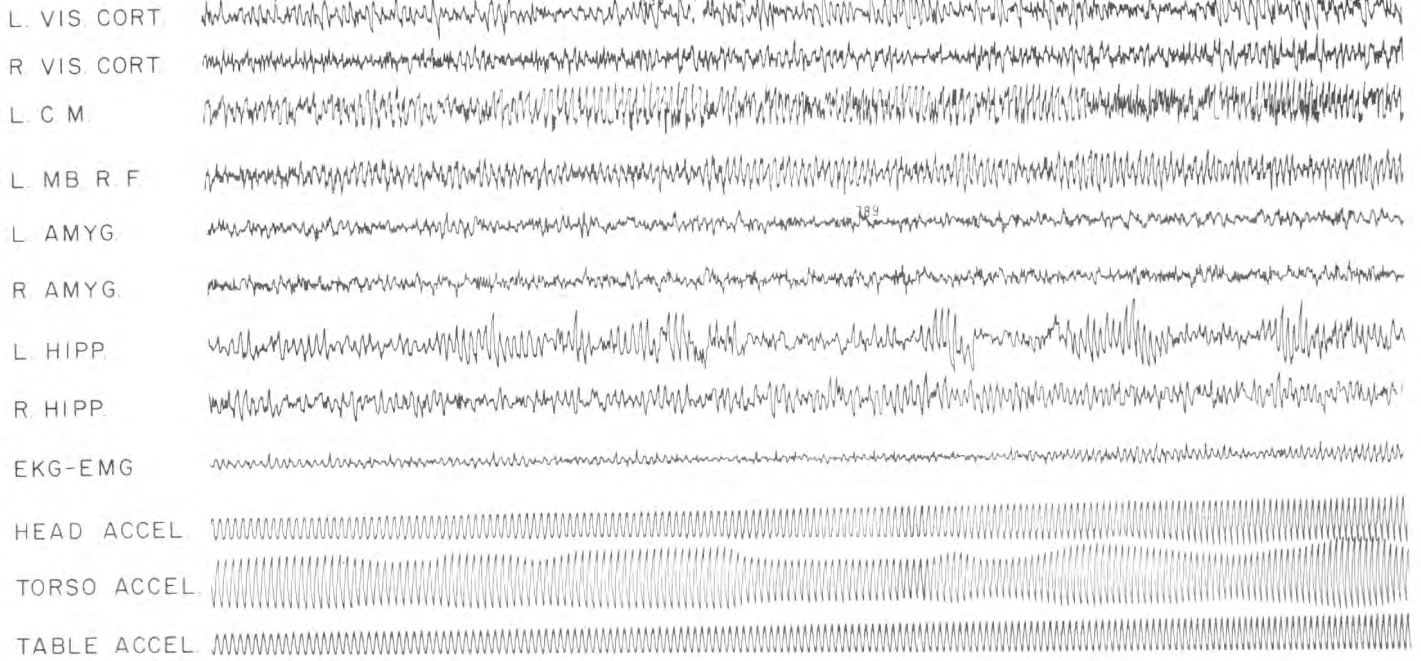
19 MARCH 1962

↓ 9 c.p.s.

↓ 10 c.p.s.

↓ 11 c.p.s.

↓ 12 c.p.s.



1 SEC.

B. SAME MONKEY - BARBITURATE ANESTHESIA 40 mg/Kg I.V. TOTAL

↓ 9 c.p.s.

↓ 10 c.p.s.

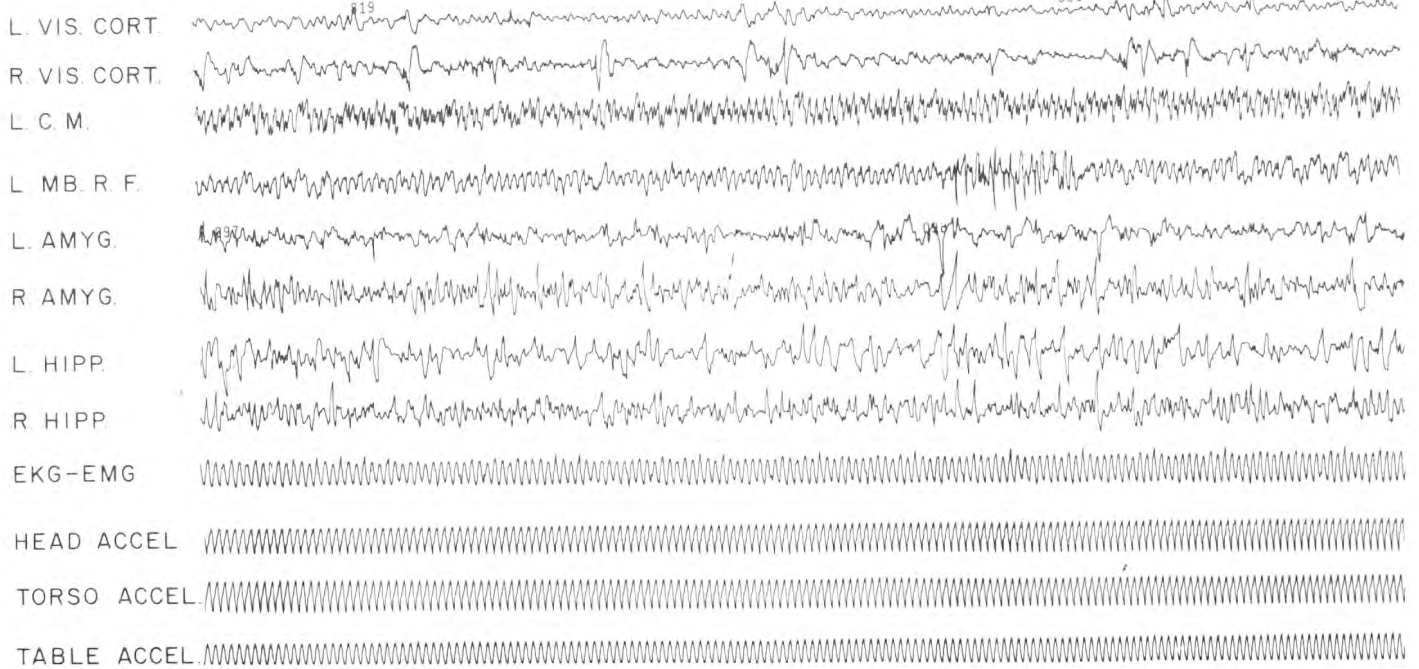


FIGURE 5

IMPEDANCE CHANGES TO PHYSIOLOGICAL STIMULI

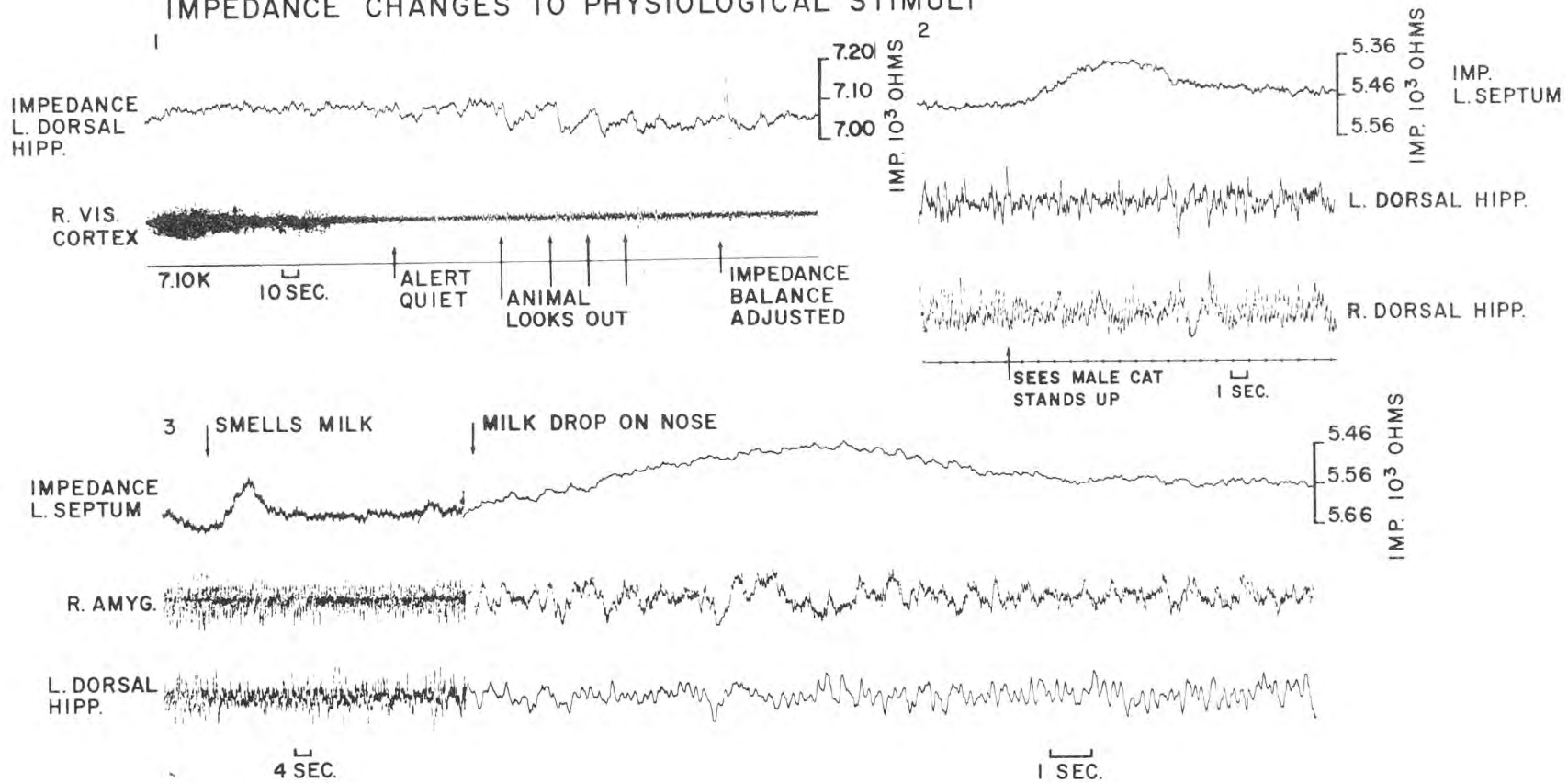


FIGURE 6

IMPEDANCE CHANGES DURING APPROACH

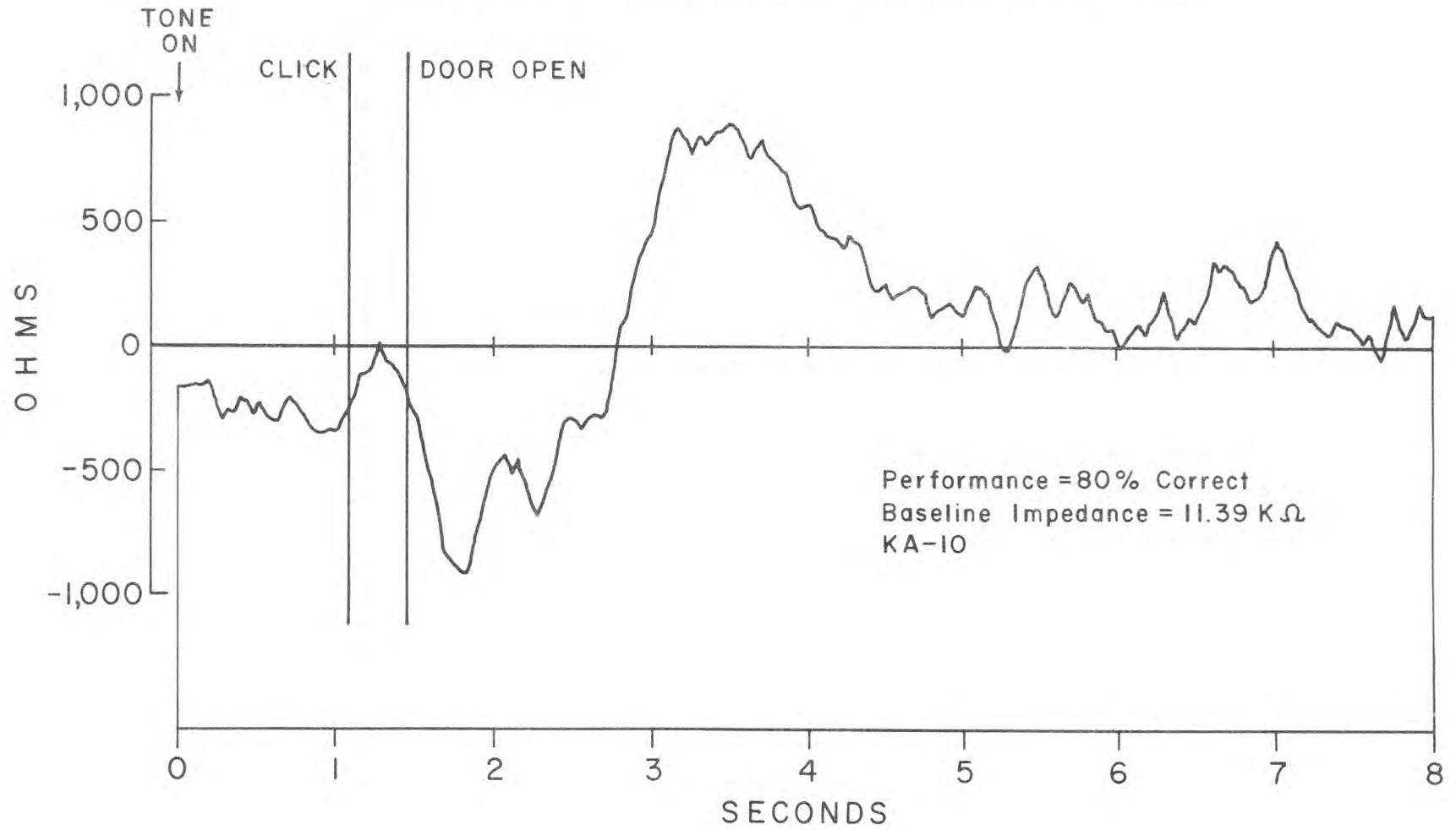


FIGURE 7

Endicott, N. Y.

October 7-11, 1963

II. INFORMATION PROCESSING AND STORAGE IN
THE CENTRAL NERVOUS SYSTEM, WITH EMPHASIS
ON NEW TECHNIQUES FOR MEASUREMENT AND ANALYSIS

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As a point of departure, we may discuss some of the problems of acquisition of data in computable form from neurophysiological experiments. There are special problems in this regard by reason of the number of channels of information that must often be recorded from a single experiment. In this connection, the maximum number of channels available on commercial tape recorders, for example, (usually 14 or 16) is less than often deemed optimal. There are also problems of standardization of the experimental protocol to permit meaningful correlation of results recorded at one stage of the experiment with similar data recorded at other times in the same experiment, or with data from other individuals exposed to similar conditions. Thus, much attention must be devoted to this question of data acquisition.

1. Data collection for computer analysis; utilization of tape recording techniques.

Much of our own data has been gathered in the field, often under quite strange environmental conditions. We have participated in experiments, for example, where biopacks have been launched by balloon from Labrador. The intention was to fly these balloons from Labrador to the Rocky Mountains for collection of data relating to the effects of primary cosmic irradiation in the brain of the primate, in experiments conducted by NASA Ames Research Center in the

summer of last year. Monkeys were prepared with surface brain electrodes and placed in small restraining chairs. It was necessary to collect baseline data prior to flight with a miniature seven-track instrumentation recorder that is fully compatible with full sized recorders using standard IRIG subcarriers. It has the capability to record for an hour and a half at 1-7/8 inches. It is extremely rugged and has many applications. For example, it has been flown on the laps of jet fighter pilots before any permanent installation of a flight recorder was possible. They were prepared to hold the recorder while going through various maneuvers in zero gravity.

As mentioned above, the number of available channels tends to be strictly limited in conventional recorders. We have, therefore, spent considerable effort in FM multiplexing which permits as many as 14 channels of information on one tape track, so that with only a two-track recorder it is possible to have 28 channels, and with the seven-track instruments as many as 98 channels. The standards for the oscillator center frequencies and deviation characteristics are established on the basis of military requirements, so that the apparatus is readily available for both the recording and the demodulation according to these standards not only in this country but throughout the Western Bloc. Providing the permitted frequency deviation is not exceeded, this system is as easy to operate as any other tape recording system, with many advantages in robustness in field conditions (Adey, 1963).

As mentioned at the outset, the standardization of protocol in data collection is extremely important. My colleague, Raymond Kado, has recently developed a system to record the EEG in 200 human subjects for NASA (Figure 1). This is a special study in astronaut and pilot trainees. This project holds the hope that it will become one of the few series of cases where extreme accuracy of performance requirements and of data collection can be imposed. The entire system is under magnetic tape control, and presents various stimuli, including shock, oddity response tasks, and a projector displays various tasks of increasing complexity, and slides of an increasingly stressful nature. We record the individual's EEG, eye movements, and various autonomic responses, including GSR, electromyogram, EKG and respiration, together with the stimulus control signals generated on audio sub-carriers as in the FM multiplex system described above. We have recently installed this device in Dr. P. Kelloway's laboratory in Houston. Similar

devices will be installed at the Salpetriere Hospital in Paris for Dr. A. Remond, and also in our own laboratory. There will thus be the facility for data collection under identical conditions in these different laboratories, and the opportunity for computer analysis by the systems that each group has developed. The principal advantage is that since the command is on a tape, one may retain it indefinitely or modify it at will.

2. Development of sensing electrodes and miniature pre-amplifiers.

To collect this type of data in the field, or under conditions of minimal restraint, we became interested in the construction of microminiature amplifiers. For example, a three stage EEG amplifier can be constructed as a cylinder 1 cm in diameter and 1 cm high (Adey, Kado, Winters and DeLucchi, 1963). It can be mounted in a stainless steel top-hat and made adhesive to the scalp by a flange that will hold it in place for good recording for as long as 36 hours, but for use in the normal helmet configuration of the pilot or astronaut, it was deemed inadvisable where the subject might suffer a blow on the head (Figure 2). Instead, the little amplifiers were arranged as cylinders and incorporated in the helmet liner. At the same time, great effort has gone into the development of a zero contact-potential recording electrode. It is generally recognized that, because of the large contact potential usually present at the metal-to-skin interface with the usual EEG electrodes, slight displacements of the electrode on the scalp produce huge electrical artifacts, which completely conceal the tiny brain wave signals. Removal of the contact potential is possible in a system which we have developed in which a tin wire is in a solution of stannous chloride inside a ceramic cylinder, which constitutes one half of the half cell. By a series of ingenious developments, my colleagues have found ways to retain the stannous chloride solution to stay inside the cylinder for extended periods. On the outside of the cylinder there is a solution of potassium chloride, which is non-corrosive to the skin. Historically, the Russians suggested some three years ago that for their space flights they were going to use a tin electrode and stannous chloride, because it had no contact potential, which is true. However, since the pH of the stannous chloride solution is 2, in a very short time it will corrode the skin. After a year's development, we have managed to obviate the major aspects of this problem. The contact with the scalp is made only with a sponge. It is not necessary to shave the head nor to remove

the hair. Satisfactory records can be secured with a small amount of electrode paste rubbed into the scalp. Moreover, as the man inevitably sweats in pressure suits of current design, the sweat of itself is not disruptive to the performance. In fact, the wetter it gets, the better it works. These are some of the studies that have concerned us in this area of what might be called environmental physiology and data acquisition under rather stressful conditions.

3. Data analysis methods; a hierarchy of computing devices.

In our laboratories, the computations are on the basis of a hierarchy of computing devices. We have used extensively for on-line applications small, special purpose instruments, such as the Mnemotron CAT computer, as a means of primary data collection, together with the primary paper or tape analog record. It is feasible to subsequently take the output of the CAT computer and perform further computations, such as spectral analyses, but the bulk of the analyses have been performed on larger computers. Our Brain Research Institute has a CDC 160A computer, a medium-size digital machine with 8,000 words of core storage. Primary access to this computer is through an optical reader for punched paper tape. The output is by an electric typewriter or a plotter. The most useful readout method is a TV display operating under control of the computer. It is possible to process data from the 160A very rapidly onto the TV display. We view the 160A mainly as an input-output system for interfacing with the IBM 7094 in the Health Sciences Computing Facility adjacent to this laboratory. The 7094 normally reads out through an IBM 1410, using a printer as a plotter, but this tends to be too slow for our special needs, and involves the 1410 for three and four hours a day writing out extensive spectral analyses, which would be better displayed on TV or by the plotter. It may be added that one hour of data collected according to the Houston EEG data collection plan may yield of the order of 10,000 spectra. To be meaningful, this has to be reduced very much before it can be effectively collated by an observer. The display techniques are thus exceedingly important in this, as in almost any other, form of physiological data analysis.

4. Evolution of computational techniques.

Just as there is a hierarchy of computing devices, we have used the hierarchy of computational techniques. Some five years ago, we began EEG analysis by simple correlation methods, as Dr. Rosenblith and his colleagues at MIT proposed.

a. Correlation analysis.

On this basis, it was possible to show, for example, in the relative simplicity of the cat's brain, differences in the functional organization of certain deep parts of the temporal lobe from the beginning to the end of learning. They involved shifts in phase patterns of waves accompanying the discriminative performance. For example, at the beginning of training, it was possible to discern in the essentially concentric shells of hippocampal tissue in deep parts of the temporal lobe, a phase sequence running from the innermost to the outermost shell. After the animal had learned the task fully, the wave trains from the same zones displayed a converse series of phase patterns (Adey, Dunlop and Hendrix, 1960). One could spend much time in a consideration of possible anatomical significance of such findings.

We were encouraged to think that if the animal was fully trained, then perhaps it might be possible to detect differences in patterns of waves that occur when the animal makes one of its occasional incorrect responses, which continue to appear at high performance levels. Our initial approach was by the use of correlation analysis (Adey, Walter and Hendrix, 1961). It was possible to show in data from one day that correct responses were accompanied by hippocampal wave trains with highly consistent phase patterns, and that these phase patterns reversed during an odd wrong response on the same day. Analysis of data from a different day showed identical patterns in correct responses, and a wrong response was accompanied by a phase pattern, which again reversed that in correct responses.

We may thus ask how rigorous is the phase locking in a particular pattern of wave activity, where this electrical activity appears in relation to a particular behavioral performance. One way of examining this problem would be by preparation of many correlograms. In this way, it was found that phase patterns were qualitatively constant in a long series of correct responses, although the absolute amount of the phase shift differed in successive responses, but remained qualitatively the same. The amount of the lead or lag in any pair of lead varied from 25 to 60 msec, but remained constantly in the same direction. If that is the case, one might ask at what stage of learning this rather rigorous phase locking occurs.

We therefore reverted to simple averaging of EEG's from these brain structures during the period of the learned performance at various levels of learning (Adey and Walter, 1963). When the performance was around mid-level, with the correct responses at about 75%, the hippocampal average was completely irregular (Figure 3). Some days later, performance had improved to about 80 or 85%. The wave trains appeared identical with those in previous tests. Now, however, the computed average showed that the wave trains were substantially phase locked to the time of presentation of the situation (Figure 4).

It thus appears that somewhere around the mid-level of training, this phase locking becomes a characteristic feature of the patterns of waves in these deep regions of the temporal lobe. I would like to introduce here some data, which we have recently gathered, to show how sensitive this simple averaging technique is in detecting late effects of some of the hallucinogenic drugs (Adey, Porter, Walter and Brown, 1964). It is well known that, after a single dose of LSD, a tolerance develops rendering further doses ineffective for a period of several weeks. The biochemical basis of this adaptive mechanism remains unclear. One might expect that aspects of it would be revealed by computed averages of EEG wave trains during discriminative performances on days following a single dose of LSD. Control records on days before the drug exhibited rhythmic averages, with gradually increasing amplitude in the waves occurring in the first 500 to 700 msec.

After LSD, there was an increase in the regularity and amplitude of the average for four or five days. The maximum amplitude of the averaged waves now occurred in the earliest waves of the series and declined thereafter (Figure 5). One must remember that these effects were induced by a few millionths of a gram of this substance. As far as is known, it is completely eliminated within a short period of its administration. These findings encourage the view that in the future more delicate histochemical analyses and pharmacological assays will reveal fundamental mechanisms by which substances such as the biogenic amines may be altered in their distribution in brain tissue.

b. Detection of phase and amplitude modulation by digital filtering techniques.

So far, we have discussed only the question of the time-locking of trains of apparently identical waves to the presentation of a stimulus. We may now consider the different question of how rigidly fixed in frequency these wave trains may be. It would be conceivable that, although the wave train, for example, had a 6 cycles per second dominant frequency, it was also characterized by a certain amount of frequency modulation on that 6 cps "carrier" frequency. It is conceivable that this frequency modulation has the capability to carry some form of information. Various techniques have been devised to detect this frequency modulation on a wave train. One such method was devised by Tukey at Princeton and his graduate student, Roy Goodman. Its first application was in the space environment, where by examining the sinusoidal output of a magnetometer on a spinning satellite, it was possible to detect very small variations in the earth's magnetic field by variations in the frequency in the sinusoid coming from the magnetometer. If the satellite is spinning at a constant rate and advancing through a constant field, there will be no such frequency modulation. If the field varies, and thereby produces an apparent shift in the wave front, then the frequency will vary. This is, in fact, what happened.

Our application of the technique was by using the computer as a digital filter, in which the bandwidth of the filter is very accurately specified (Adey and Walter, 1963). It is necessary to specify not only the bandwidth but an extremely flat top characteristic, and shoulder and skirt characteristics within narrow limits. If one does not do this, then there will be ambiguities in the readout between frequency and the amplitude parameters.

In actual application to EEG data, this technique has been very fascinating. For example, in the EEG waves accompanying a delayed response test, it was repeatedly noted that the frequency modulation appeared as a concomitant of the most regular wave trains, which were themselves correlated with periods of maximum attention, as in the actual approach to food. This frequency modulation occurred at about 1/3 of this "carrier" frequency 6 cps.

It is obvious that, if the individual cells were functioning as phase comparators, sensing aspects of shifting frequencies, either against intrinsic rhythms of their own, or in relation to patterns of waves arriving in spatio-temporal configurations in the vicinity of the cell, the addition of the parameter of frequency modulation would greatly enhance the amount of information that the system can carry. We are currently pursuing this more intensely. Instead of the Goodman filter, the Ormsby filter is an easier one to handle in this type of analysis.

c. Use of cross-spectral analysis.

We are now making wide use, essentially as a basic method of analysis, of the technique of cross-spectral analysis (Adey and Walter, 1963; Walter and Adey, 1963; Walter, 1963). This allows one to look at shared components between two wave trains across a spectrum of frequencies, for example, from 0 to 20 cps. As a readout from this program, one can measure shared amplitudes, shared phase angles, and the coherence factor between these traces. This technique was also developed by Tukey for analysis of missile vibration characteristics. There is obviously a substantial similarity between data that one collects from a vibrating missile body and the many simultaneous wave trains occurring in the brain.

We have applied this technique to analysis of wave trains from deep temporal lobe regions during correct and incorrect responses (Figure 6). In a correct response, shared amplitudes between two hippocampal wave trains showed a single large peak at around 6 cycles per second, and the phase angle remained almost constant between these two traces at about +20 or 30 degrees from 2 to 12 cps. The coherence was also relatively high at the time of maximum amplitude. By contrast, similar analyses during incorrect responses showed that the shared amplitude curve had a double peak with the larger one at about 7 cps. The phase angle reversed sharply from +90 to -90 degrees at 5 cps, in confirmation of results of simple correlation analyses. The coherence factor in the vicinity of 6 cps was low, where previously it had been high in a correct response. This method thus confirms fundamental differences in patterns of waves characterizing correct and incorrect responses.

d. Use of cross-spectral analysis in establishment of a stochastic model of brain function.

The next development of this technique was initiated by my colleague, Dr. Donald Walter. He introduced a stochastic factor into this cross-spectral analysis (Adey and Walter, 1963; Walter and Adey, 1963; Walter, 1963). One can display on a polar diagram the phase angle between various brain structures, or between brain rhythms recorded in different brain structures. It is possible to establish the limits of that phase relation at the 50% level of probability for each part of the spectrum, thus establishing a series of fan-shaped sectors for phase relations between different parts of the spectrum. The inner and outer radial limits of these sectors can also be stochastically established, thus establishing the stochastic limits of the amplitude transfer function. As noted above, maximum shared energy lay in the 6 cps band, and the disposition of its "fan" was found consistent in correct responses from different days. Similar analyses of incorrect responses from different days also showed strong resemblances between them, with their location in sectors of the polar plot displaced more than 90° from the location of the 6 cps fan during correct responses.

How reliable is this as a measure of correctness? So far, we have performed a number of analyses from one animal. It appears that some of the incorrect responses produce polar plots iso-phasic with the correct. Polar plots for correct responses have not displayed the phase patterns of incorrect responses, however. Those cases where the incorrect responses duplicate the correct response pattern may be those in which the animal runs very rapidly without appearing to pay attention to the correctness or incorrectness of its decision.

What does this mean in terms of organization? Basically it suggests to us that the brain is functioning in a probabilistic fashion in the large, not merely at the level of the single cell in the discharge of single units, but also in wave phenomena which occur through a whole cortical domain. The recall of information could well depend upon the establishment in that domain of a pattern of waves which resembled, but were not necessarily identical with that associated with the initial laying down of the information. Here we return again to this question of the participation of the waves, both in the laying down of an informational trace and its subsequent recall.

I would now like to discuss some of the new techniques which we have developed to deal with this question from an informational point of view.

e. Use of lagged mutual information techniques in analysis of the multivariate case.

My colleagues, Dr. Donald Walter and Mr. Dan Brown, in the last year have been very active in trying to analyze beyond the linear case, as exemplified by most of these methods that we have used here, in a consideration of non-linear situations. We may discuss first this question of mutual information. The mutual information connecting to partially random functions is a general measure of interaction, and it can be calculated for this type of physiological data. If the processes are gaussian, the only possible relationship is a linear one, and the cross-correlation technique is adequate to define this mutual information. However, the correlation function underestimates the mutual information between two records in a variety of situations; firstly, for distributions of amplitude which are other than gaussian; secondly, where there are non-proportional outputs; and thirdly, where there is modulation of one signal by another (Walter and Brown, 1963). The latter two situations appear to occur in brain tissue with reasonable certainty. Our colleague in the Engineering Department, Professor Ballakrishnan, has for a long time been interested in lagged mutual information, which essentially generalizes the cross-correlation function and allows it to be assembled as a cross-information function.

The mutual information for two series, X and Y, can be calculated on the basis of a simple formulation, and the subsequent estimation of multivariate mutual information can be carried out for several lags at once, and can be expressed in multiple, partial, or in a canonical form. In essence, interrelations in the tissue can be expressed in terms of the presence of inferred generators, at various times during a behavioral performance. For example, one such analysis (Figure 7) has shown that the right entorhinal cortex is substantially displaced in its generator functions from the other three channels during the first half of the delayed response. During the second half of the delay it moved much closer. But during the approach it moved away again, to be essentially independent and quite isolated from these other channels. This approach is one which allows us to detect more efficiently than by the cross-correlation techniques, for example, the presence of shared components, which may be essentially non-linear in their characteristics.

5. New computing techniques for analysis of changing states of consciousness.

The final area that I wish to discuss concerns some of the problems of assessing the on-going EEG record, as it relates to varying states of consciousness. This is work that we have performed on chimpanzee records. These animals are exceedingly intelligent and play tic-tac-toe, often without the need to be rewarded for each correct response. This level of intellectual capability is matched by equal complexity of EEG patterns, closely rivaling that of man himself. In seeking aspects of pattern in these records, it is necessary to use quite sophisticated techniques to deal appropriately with the general pattern of the EEG. One of the more complex problems concerns a record which is well known to those in the EEG field, in a state of sleep that is called "paradoxical." This paradoxical sleep state is one which is apparently associated with dreaming and is associated with big eye movements under closed lids (Adey, Rhodes and Kado, 1963). It has been a fundamental physiological question as to whether this is a deep state of unconsciousness or a superficial state. Opinions have been fairly evenly divided for the following reason. The waking EEG record in cortical and subcortical leads is largely characterized by low amplitude fast activity. The record from paradoxical sleep closely resembles the waking record and it is difficult for even a skilled observer to tell what is the difference between paradoxical sleep records and waking records (Figure 8).

We have noted a type of paradoxical sleep, which we now call the "amygdaloid spindle" phase, in which certain characteristic rhythmic discharges appear in the amygdaloid tissues of the deep temporal lobe. If the paradoxical record looks like a waking record, how easy is it to actually awaken the animal? Animals in this state are at least as difficult to arouse as in any other sleep phase, and some observers have maintained that they are more difficult. Granted that it may be more difficult, does this mean that the animal or man is more deeply unconscious, or rather that he is focusing his dream on some gorgeous blond and he really does not attend to efforts to waken him? If it were possible to specify the pattern of the EEG as a whole during these phases and see how the paradoxical sleep relates to the awake record, it might be possible to establish some electrophysiological criteria as to depth of sleep, or depth of unconsciousness.

Our initial approach was by the use of simple power spectra analysis. These studies in our laboratory were performed by Mr. Dan Brown, a mathematician, and Dr. J. M. Rhodes, a psychophysiologicalist. The power spectra from the reticular formation, for example, showed a large peak in energy in a part of the spectrum frequently neglected in assessment of EEG records, in the region under 5 cps. It was discouraging to find so much energy here, since it is cramped into a narrow part of the spectrum, which makes analysis in fine detail difficult. When we repeated these analyses in other brain regions, including the parietooccipital cortex and the hippocampal system, each exhibited a substantial peak in this region where no specific answer can easily be given about patterns relating to changing states of sleep or consciousness. A technique was therefore devised which we have termed "equivalent noise bandwidth-duration." If one takes the power spectrum and expresses its bandwidth as a function of its variance in an epoch of data of known lengths, then this bandwidth is essentially a function of the square of the variance, and can thus provide a measure of the power involved. The display from this equivalent noise bandwidth technique is one in which, for an epoch of record of a particular length, we plot characteristic noise bandwidth against duration in seconds. Of particular interest, these functions for a particular EEG lead can be plotted as a continuum ranging through alerted states to deep sleep, and observations made as to their possible relationship to the amplitude distributions of a gaussian function.

We may interpret this display in the following way (Figure 9). The more stable sinusoidal events will be in the lower right part of the graph, and spike-like transient phenomena will be collected in the upper left regions. There will be a forbidden area in the lower, left part of the plot, where validity of relationships cannot be established. If we examine chimpanzee records from deep cortical structures, such as the amygdala and hippocampus in a succession of sleep states from the spindling phase, through slow wave activity and paradoxical sleep, and including also awake records, drowsy states and those from waking periods, we find that the amygdaloid spindling and paradoxical records are close together and that they are closely related to the awake records. By contrast, awake records from the surface of the parietooccipital cortex, which are those traditionally cited as closely resembling actual awake records during the fascinating periods of paradoxical or "dream" sleep, can, by this technique, be shown to differ sharply in their activity

patterns in the two states. These differences remain completely undetectable by visual inspection of the original records. One can define a great deal about the overall pattern from many brain regions by this technique, which is relatively simple and makes only moderate computer demands. It gives one, we think, the opportunity for the first time to assess appropriately many of the things which inter-relate different brain regions in those parts of the power spectrum in which most of the energy is located, namely in that part which lies below 5 cps.

As a theoretical point, it is very interesting to note that none of these EEG functions follow a gaussian distribution. I recall with particular fascination that, in the MIT volume on processing neuroelectric data, this question as to whether the EEG could or could not be considered as a gaussian function and thereby examined in a linear fashion, was discussed in some detail two or three years ago. It appears that this type of analysis provides an interesting answer to this type of problem.

6. Summary

The model that we have discussed of the brain system this morning is one that is non-linear and stochastic. This is considerably removed from simple notions of deterministically operating nerve nets as the basis for an over-simplified brain model. Additionally, it offers us a very considerable challenge for the future, in terms of the types of computing devices and techniques to be used in analysis of our data. It is obvious that no one computational method will provide a complete answer. In some degree, we may continue to do much on-line analysis, combined with ever increasing sophistication and ever increasing demands for complex off-line analysis. It may be suggested that new transducing techniques will bear fruit only in proportion to the extent that the data analysis by computational methods is successful. In this regard I am persuaded that the impedance type of measuring technique offers probably just as much, if not more, in the future than the recording of the EEG analysis.

If we might in conclusion turn away from the specifics of detailed analysis to the broad philosophies which may guide us in the future in this area of brain research, we may examine the general purview of the growth of mathematical analysis in physiological research, asking ourselves what would be the most appropriate

frame for the conduct of computer-oriented research in the life sciences (Adey, 1963). It may be argued with some cogency that it is insufficient for the mathematician to participate on a consultative basis, and that it is necessary for him to be a full member of the research team closely associated with every phase of the research on a daily, and even an hourly, basis.

To achieve such an arrangement, it is obvious that the mathematician or computer scientist must find in life science institutions the requisite opportunities which will allow him to shape his own career with full and appropriate recognition of the significance of his role by his colleagues in the life sciences. It follows that life science institutions should accept the responsibility for programs of technological and mathematical research as an integral part of their own research, to be conceived as of equal merit with their fields of special interest in the life sciences. This is indeed a substantial responsibility, but without it there is little reason to suppose that advantageous association with limited facets of the mathematical and engineering sciences can provide the basis for a full and vigorous pursuit of these areas, which are at the very core of future life science research.

It also follows that there is little reason to expect a satisfactory degree of continuing success from programs in these areas of mathematical biology conducted as an appendage to the research programs of institutions whose prime interest is in the mathematical and physical sciences. The substantial nature of the life science requirement in these areas demands that they assume the full responsibility for their development, and that those whom they seek to attract as colleagues from the mathematical sciences should feel that these fields are indeed an appropriate vehicle for their life's work. Without such a frame of reference, our early efforts may face a difficult and even a perilous future.

ACKNOWLEDGMENTS

Studies described here from our own laboratories were assisted by Grant NB-1883 and Grant M-3708 from the U. S. Public Health Service, by Contract AT(11-1)-34 (Project 60) with the U. S. Atomic Energy Commission, by Grant AF (AFOSR) 61-81 from the U. S. Air Force, and by Grant NsG 203-62 and Grant NsG 502 from the National Aeronautics and Space Administration.

REFERENCES

- Adey, W. R. , "Potential for telemetry in the recording of brain waves from animals and men exposed to the stresses of space flight." In Biotelemetry, London, Pergaman, 1963, pp. 287-300.
- Adey, W. R. , "Data acquisition and analysis in a Brain Research Institute." Ann. N. Y. Acad. Sci. , 1963, in press.
- Adey, W. R. , Dunlop, C. W. , and Hendrix, C. E. , "Hippocampal slow waves; distribution and phase relations in the course of approach learning." A. M. A. Arch. Neurol. , 3: 74-90, 1960.
- Adey, W. R. , Kado, R. T. , Winters, W. D. , and DeLucchi, M. R. , "EEG in simulated stresses of space flight, with special reference to problems of vibration." EEG Clin. Neurophysiol. , 15: 305-320, 1963.
- Adey, W. R. , Porter, R. , Walter, D. O. , and Brown, T. S. , "Prolonged effects of LSD on EEG records during discriminative performance in cat; evaluation by computer analysis." Submitted for publication, 1964.
- Adey, W. R. , Rhodes, J. S. , and Kado, R. T. , "Sleep: cortical and subcortical recordings in the chimpanzee." Science, 141: 932-933, 1963.
- Adey, W. R. , and Walter, D. O. , "Application of phase detection and averaging techniques in computer analysis of EEG records in the cat." Exper. Neurol. , 7: 186-209, 1963.
- Adey, W. R. , Walter, D. O. , and Hendrix, C. E. , "Computer techniques in correlation and spectral analyses of cerebral slow waves during discriminative behavior." Exper. Neurol. , 3: 501-504, 1961.
- Walter, D. O. , "Spectral analysis for electroencephalograms: mathematical determination of neurophysiological relationships from records of limited duration." Exper. Neurol. , 8: 155-181, 1963.
- Walter, D. O. , and Adey, W. R. , "Spectral analysis of EEG's in the cat recorded during learning before and after subthalamic lesions." Exper. Neurol. , 7: 481-501, 1963.
- Walter, D. O. , and Brown, D. , "Mutual information of two physiological records." The Physiologist, 6: 293, 1963.

LEGENDS TO FIGURES

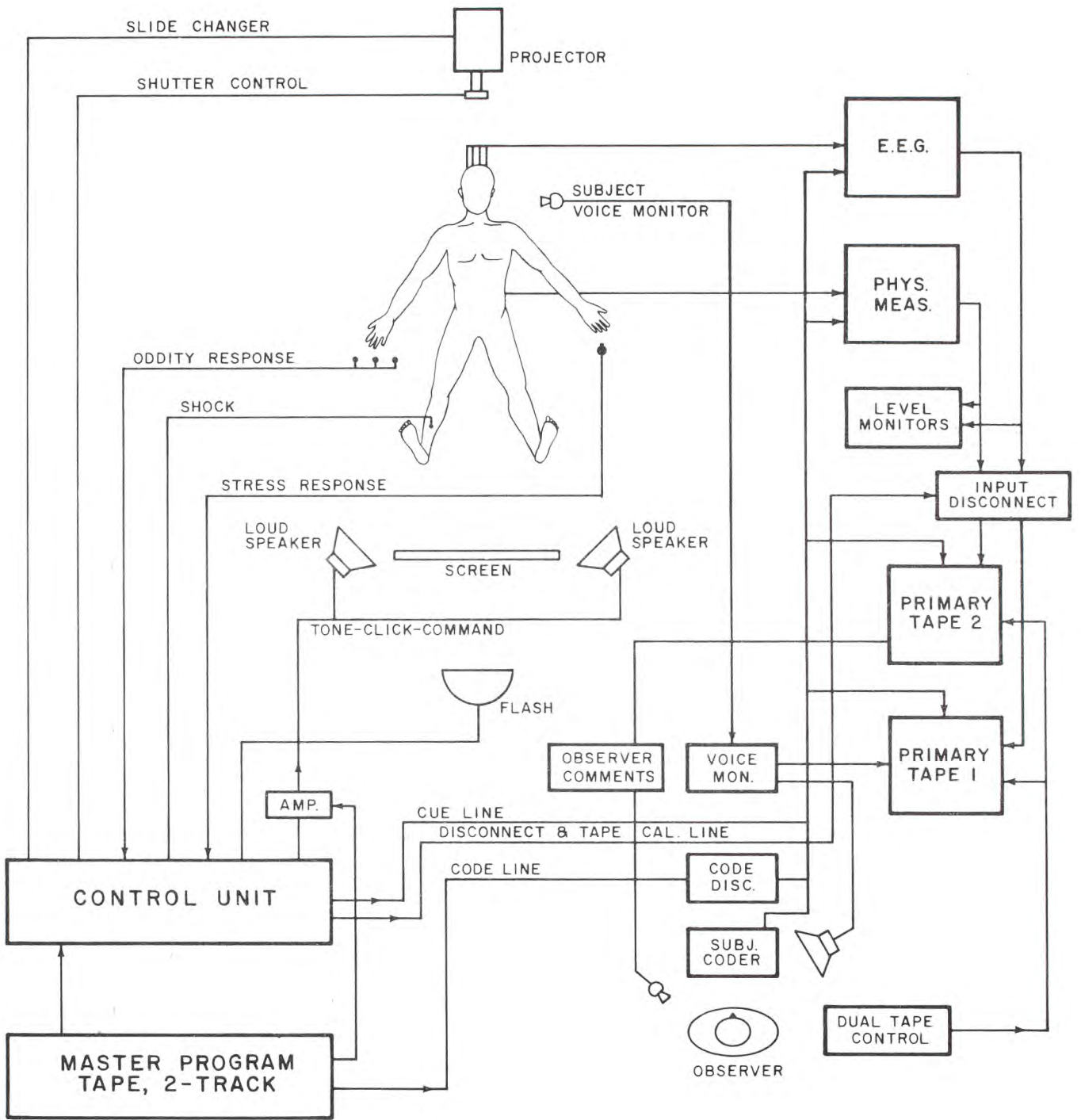
- Figure 1. Block schematic of human psychophysiological test apparatus, developed for evaluation of EEG data from pilot and astronaut trainees, under auspices of Houston Manned Spacecraft Center. The master magnetic tape programmer delivers one hour of test material, including voice instructions to subject and command signals to advance a slide projector, administer learning and perceptual tasks of increasing complexity, as well as trains of simple physiological stimuli. EEG data, together with EKG, EMG, eye movements and GSR are recorded with the command signals on Primary Tapes 1 and 2 for computer analysis.
- Figure 2. Liner of Mercury astronaut helmet fitted with low contact-potential EEG electrodes and microminiature preamplifiers (A and B). Satisfactory scalp contact can be secured through sliding contact with moist sponges surrounding ceramic cylinder at core of electrode (C). The small size of the 3-stage transistorized preamplifier permits their insertion into a stainless steel "top hat" (D) and fixation to scalp through an adhesive flange. (From Adey, Kado, Winters and DeLucchi, 1963).
- Figures 3 and 4. Comparison of averages of hippocampal wave trains during discriminative performance at two levels of training. In 8th trial day (Figure 3) the average is essentially irregular, but by the 17th trial day (Figure 4), a highly regular average has emerged in the right dorsal hippocampal lead (RDH) and in lesser degree in the left dorsal hippocampal lead (LDH). (From Adey and Walter, 1963).
- Figure 5. Averages of hippocampal wave trains during discriminative performances on days preceding and following a single dose of LSD. There was a substantial increase in the regularity and amplitude of the computed average on the 4th and 5th days after the drug. (From Adey, Porter, Walter and Brown, 1964).

Figure 6. Cross-spectral analysis of wave trains in two hippocampal leads during correct and incorrect decisions in a discriminative task. The phase angles and amplitude transfer functions are displayed in a polar plot, with limits of phase angles at the 50% probability level shown in a circular dimension, and the 50% probability limits on the amplitude transfer functions shown in the radial dimension. In cat 15 (A), there is good agreement between correct responses on two different days in the disposition of the 6 cycles per second portion of the spectrum (shaded area), which shows the greatest part of the spectral energy under these conditions. In incorrect responses on the same two days, the phase angle for the 6 cycles per second part of the spectrum has been displaced approximately 90 degrees. Data from another animal (B) shows similar differences between correct and incorrect responses. (From Adey and Walter, 1963).

Figure 7. Determination of interrelations between wave generators in the hippocampal system during the first and second halves of the delay period in a delayed response performance, and during the approach. In the first half of the delay, the right entorhinal generator (R. ENT.) was substantially displaced from the left entorhinal (L. ENT.) and left and right dorsal hippocampal generators (LDH and RDH). All four generators were closely grouped in the second half of the delay period, but the right entorhinal generator resumed its isolation from the other three during the approach epoch. (From Walter and Brown, 1963).

Figure 8. Typical records from surface and deep brain structures in the chimpanzee when awake, and at various depths of sleep. The awake record (A) shows close similarity to the record from "paradoxical", or dream sleep (C). Lead abbreviations: R. AMYG., right amygdala; L. HIPPO. and R. HIPPO., left and right hippocampus; R. C.M., right centrum medianum; R. MB. RF., right midbrain reticular formation; P-O. CX., parietooccipital cortex; E. M., eye movements. (From Adey, Rhodes and Kado, 1963).

Figure 9. Equivalent noise bandwidth-duration diagrams for parietooccipital cortex (P-O. CORTEX), entorhinal cortex (ENT. CORTEX) and midbrain reticular formation (MB. R. F.) in different states of consciousness and sleep, including spindle sleep (SP), spindles and slow waves (SS), slow waves (SL), amygdaloid spindles (AS), paradoxical (P), drowsy (D), waking (W), and awake (A). Note that this technique distinguishes sharply between the parietooccipital record when awake (A) and the paradoxical sleep record (P). Increasingly sinusoidal wave trains are located to the lower right in this diagram, and more irregular, spike-like phenomena in the upper left sector.



PSYCHO-PHYSIOLOGICAL TESTING AND DATA ACQUISITION SYSTEM BLOCK DIAGRAM

FIGURE 1

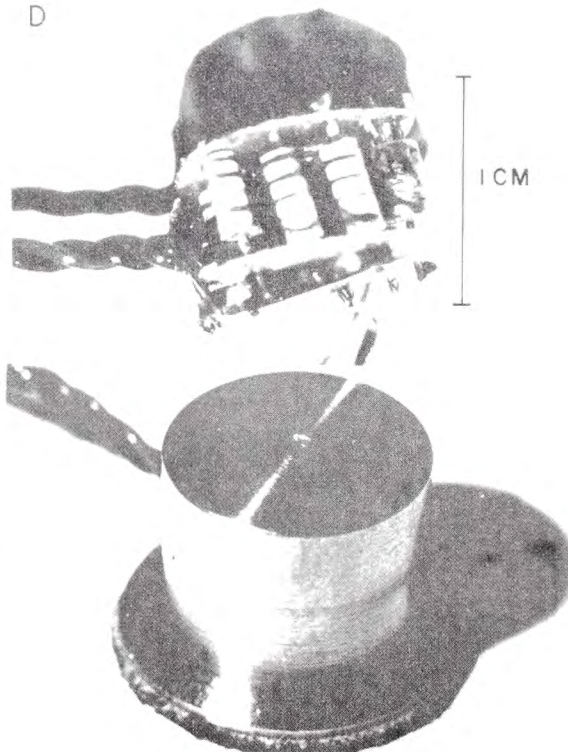
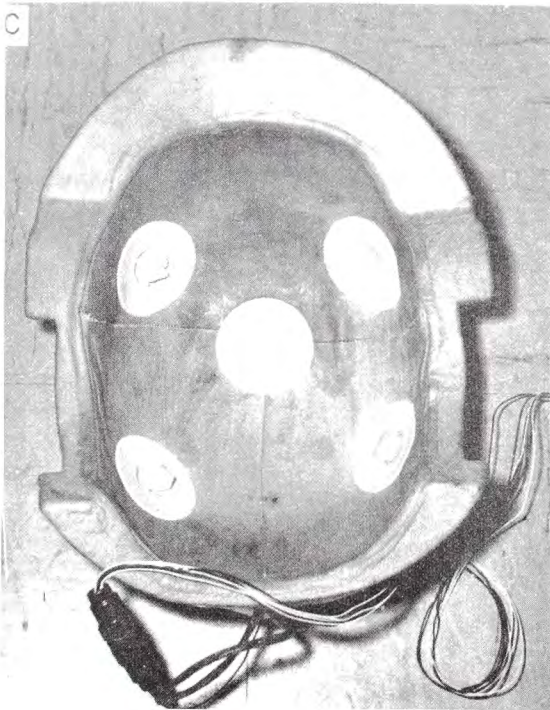
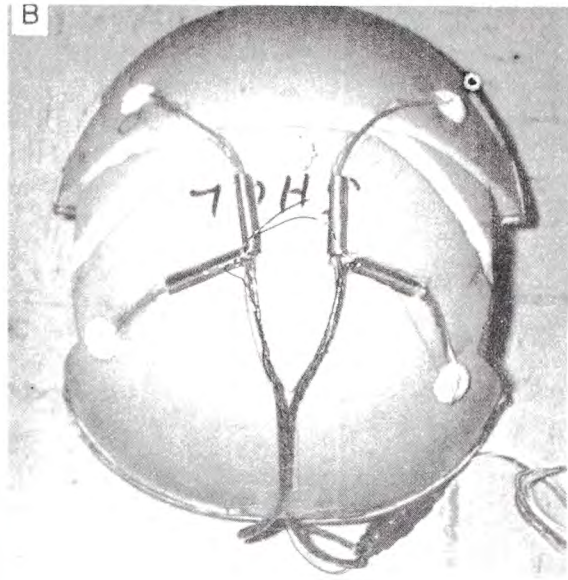


FIGURE 2

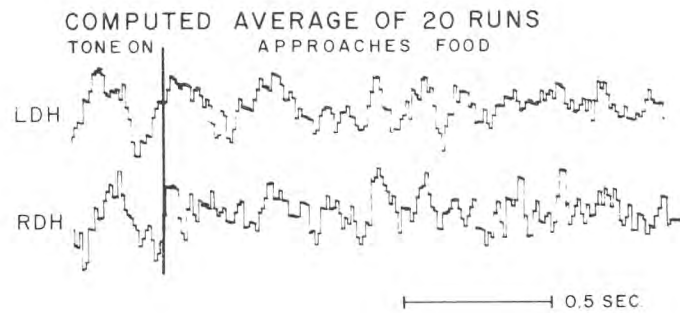
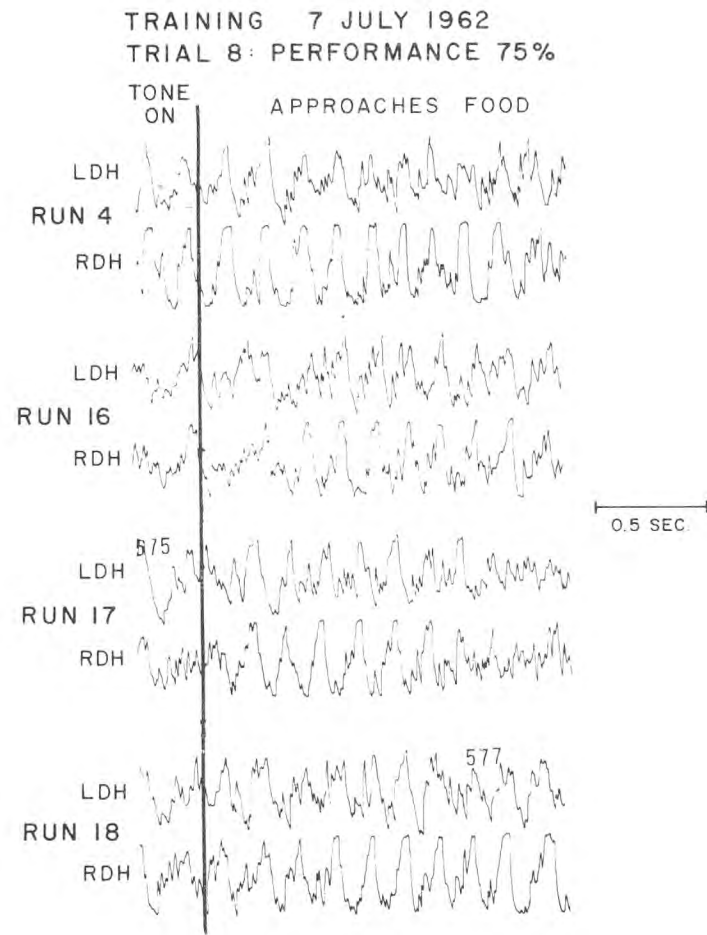


FIGURE 3

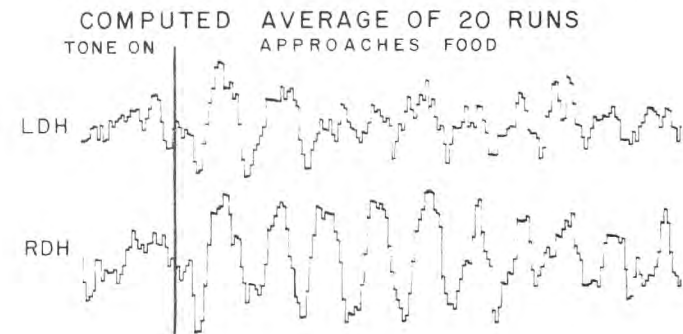
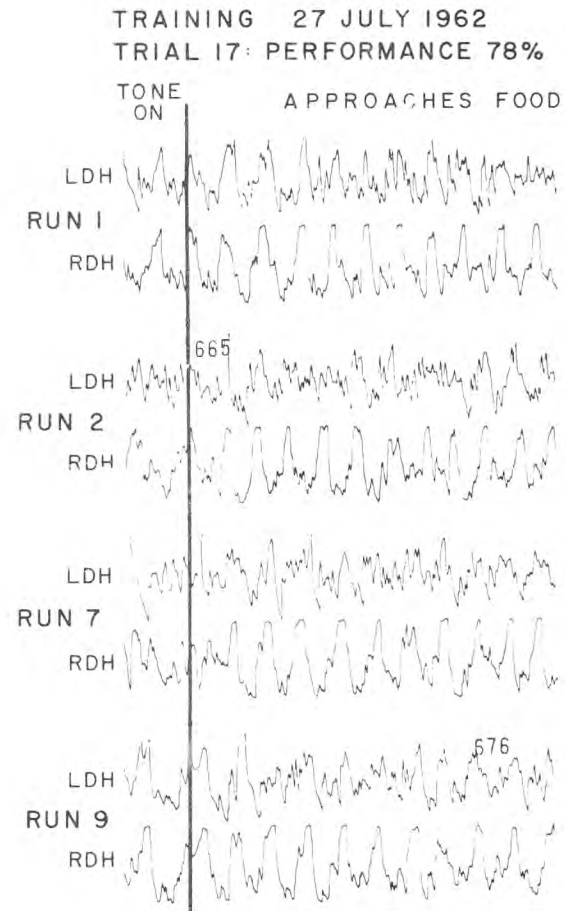


FIGURE 4

LATE EFFECTS OF LSD LEFT HIPPOCAMPAL RECORDS DURING DISCRIMINATIVE PERFORMANCE

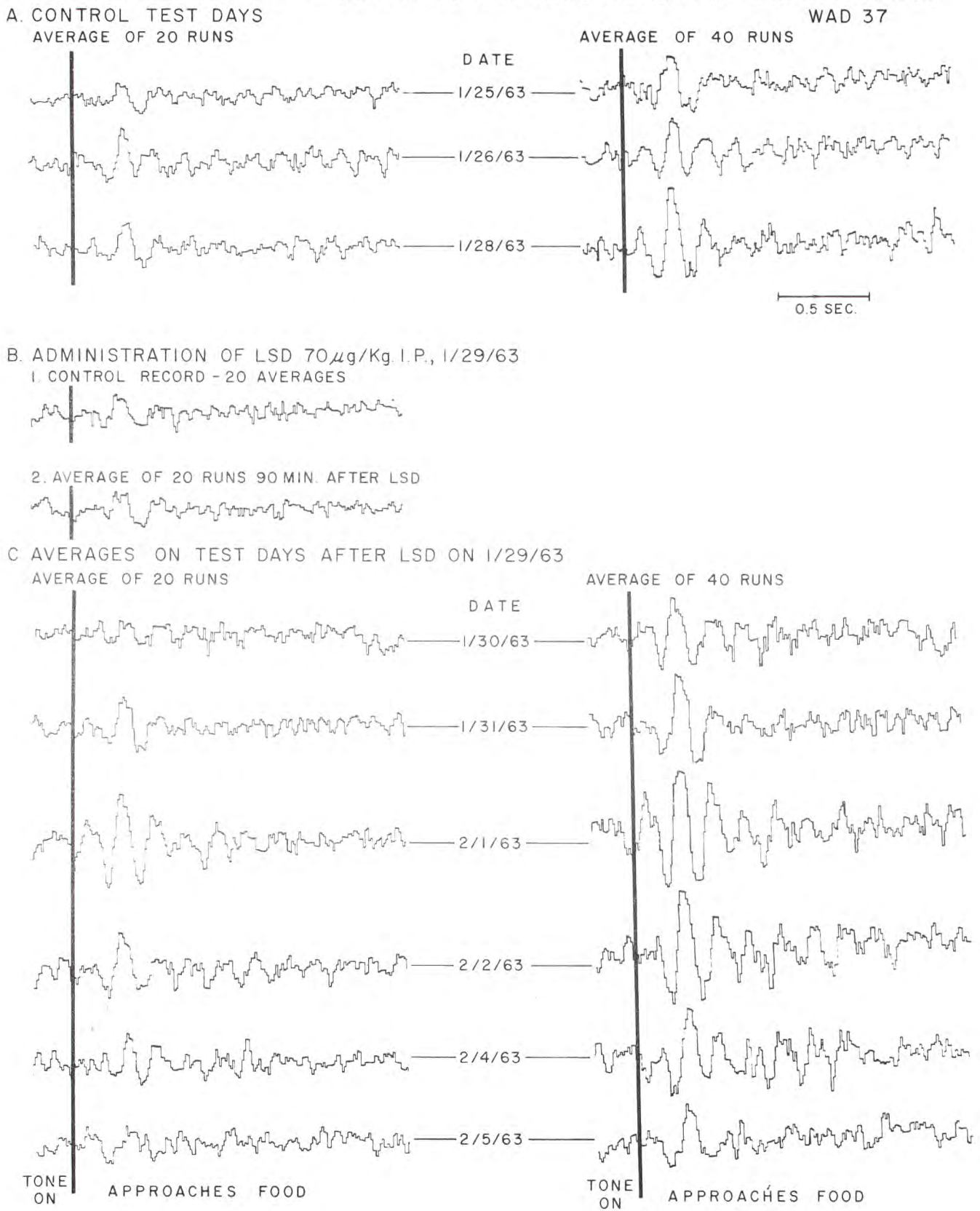
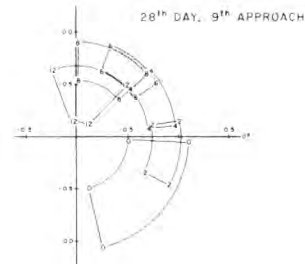
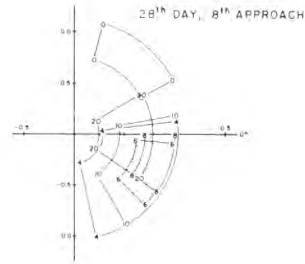
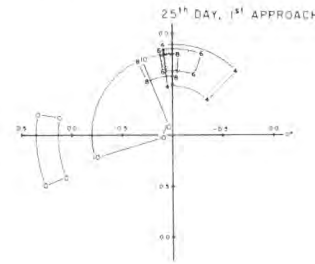
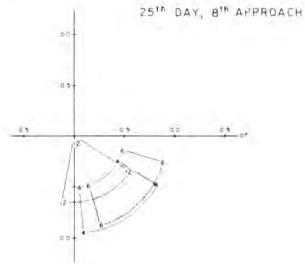


FIGURE 5

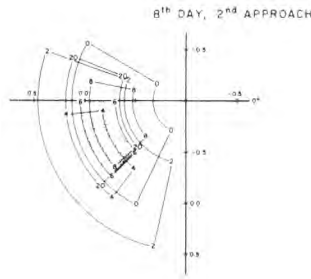
STOCHASTIC MODELS OF EEG

PROBABILITY BOUNDS ON COMPLEX TRANSFER FUNCTIONS,
DORSAL HIPPOCAMPUS TO ENTORHINAL CORTEX

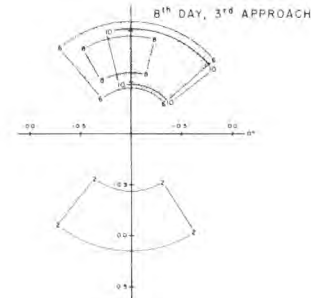
A.
CAT 15



B LC55



CORRECT



INCORRECT

FIGURE 6

EVOLUTION OF GENERATING PROCESSES IN THE COURSE
OF A DELAY TRIAL CAT WAD36, 17th DAY OF TRAINING

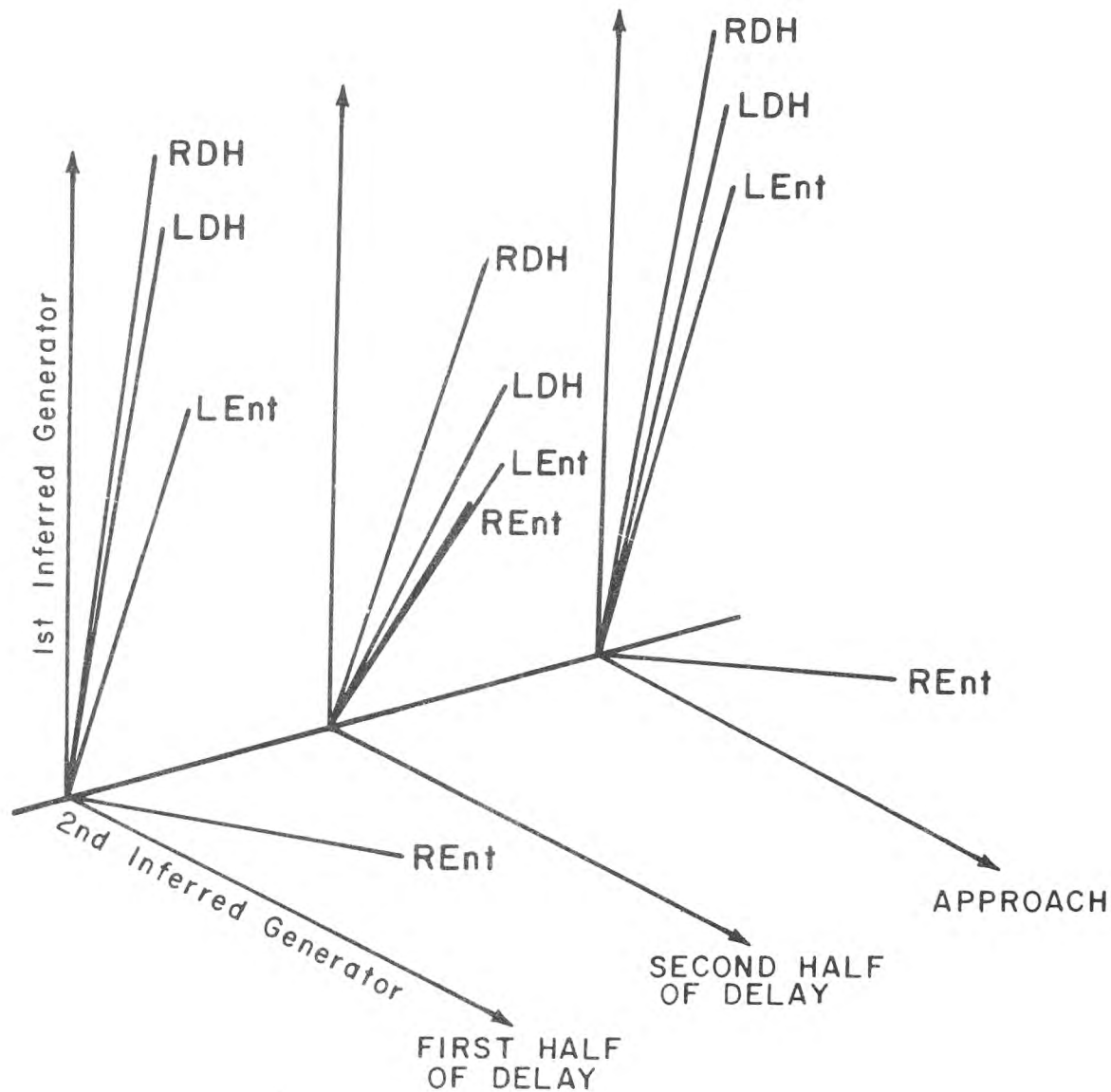


FIGURE 7

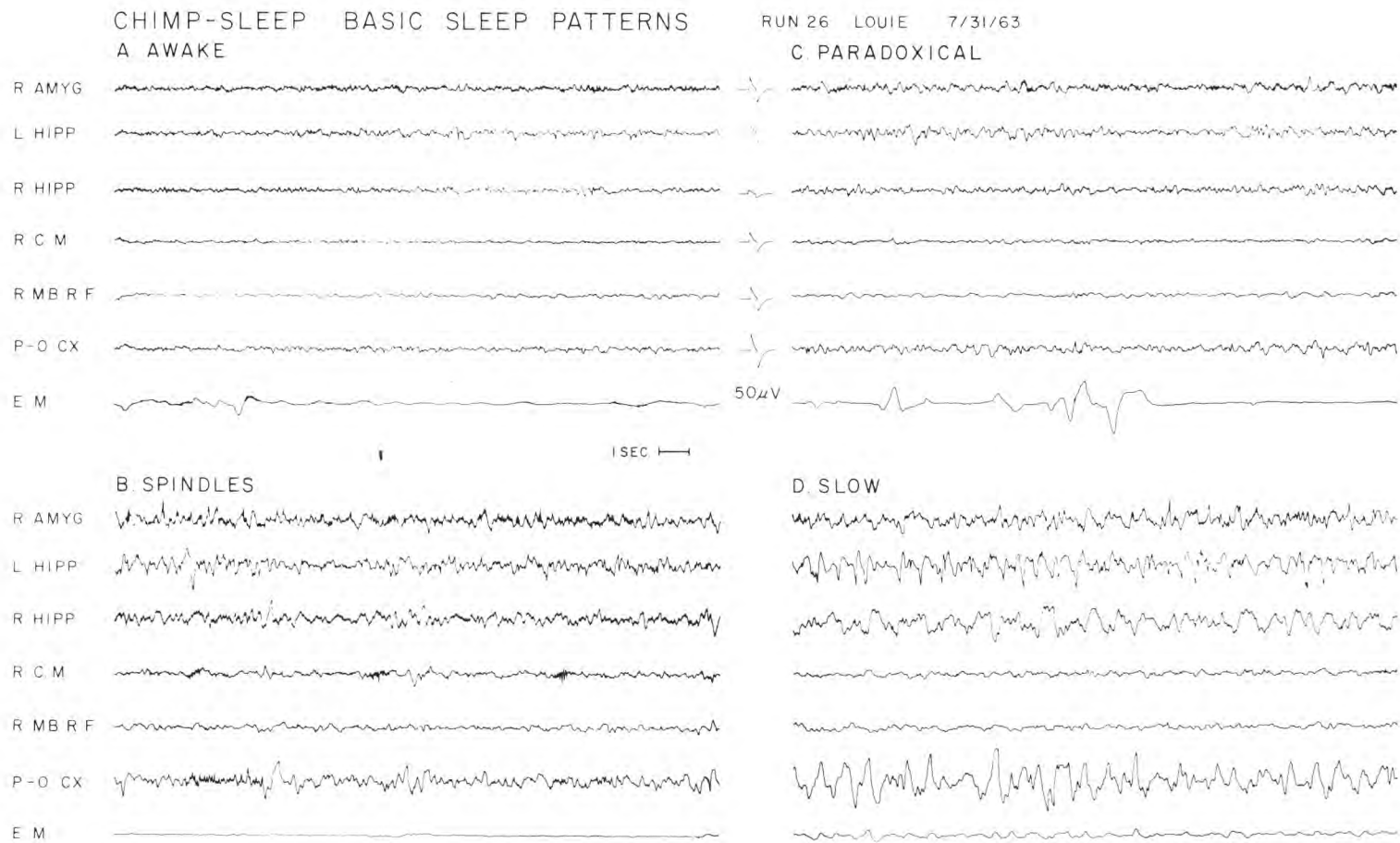


FIGURE 8

EEG STABILITY DIAGRAM

SIGMUND RUN 16 AUG., 1963

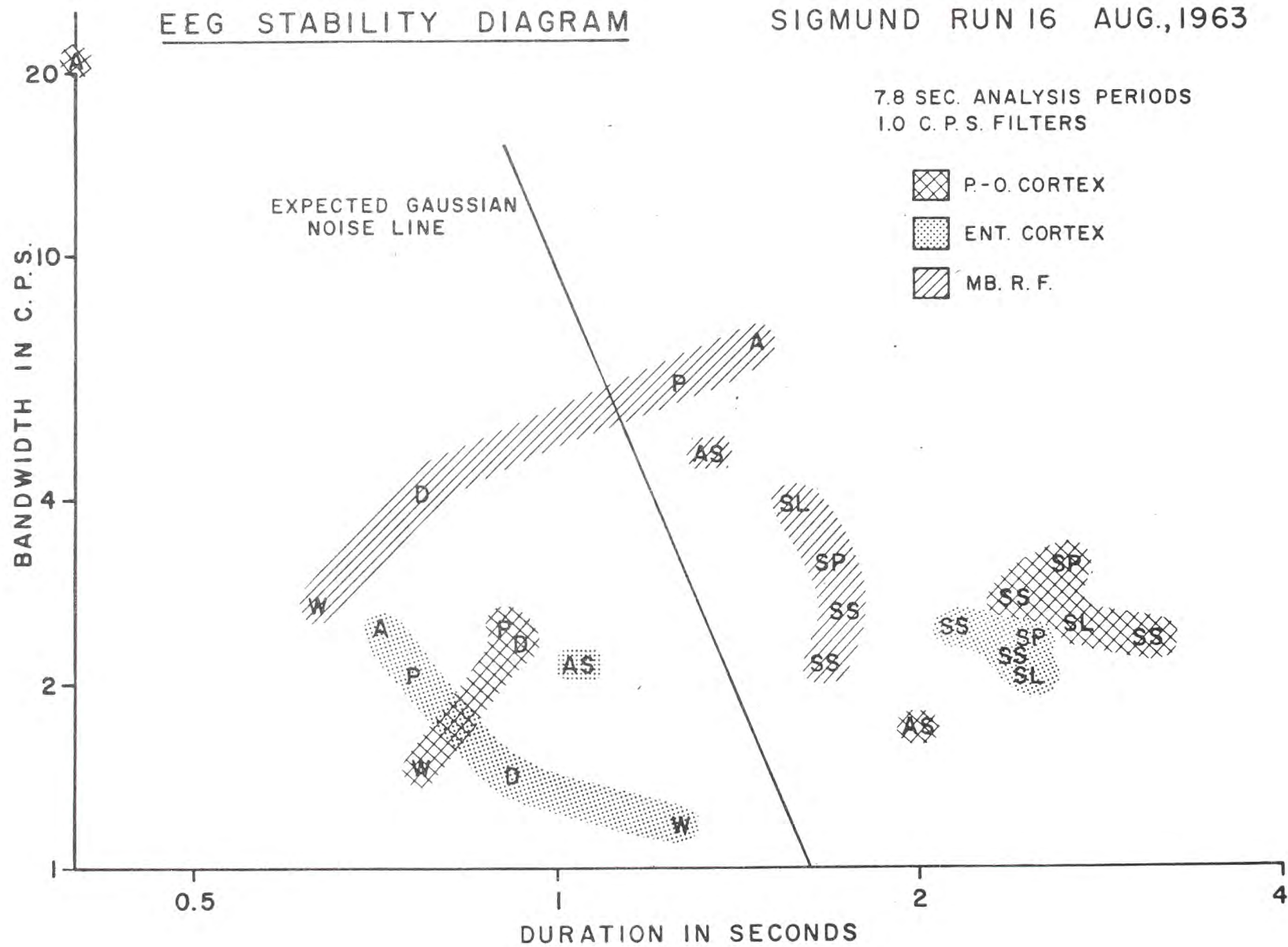


FIGURE 9