SOME ADRENERGIC AND CHOLINERGIC FACTORS IN
THE ACTIVATION OF THE ELECTROENCEPHALOGRAM IN CAT

by

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A method is reported for the bilateral, synchronous distribution of injected drugs to both sides of the brain simultaneously. Arguments have been made for the advantages of this approach over unilateral intracarotid injections for studying the direct effect of drugs on central synaptic sites.

Using this method, preliminary studies have been made of the effects of both adrenergic and cholinergic drugs on the activation of the electroencephalogram in both unanesthetized curarized cats and in cats subjected to mid-brain reticular coagulation. These studies indicate the possible co-existence of both adrenergic and cholinergic components in the mid-brain reticular formation and suggests that anatomically these sites probably are not identical.
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Department of **Pharmacy**

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Date **October 4, 1957**.
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## FIGURE

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I. INTRODUCTION

When the EEG of a normal cat is followed from wakefulness to sleep, the low voltage fast activity characteristic of the alert and waking state gives way to a slower and more wave-like discharge during drowsiness, and subsequently, when the animal is undoubtedly asleep, large slow wave and spindle bursts are characteristic of the recording. This changing pattern is reversed as the animal awakens. Under such conditions the EEG has appropriately been described as being desynchronized or synchronized in the contrasting states of wakefulness and sleep.

In addition, if the sleeping animal is suddenly awakened by an afferent stimulus, such as a "whistle-blast" or "hand-clap", again one notices low voltage, fast activity in the record, and in addition one's attention is drawn to coincident motor activity in the animal, manifested by opening of the eyes and movements of the head, suggesting an electro-cortical change associated and coincident with behavioural alertness (Magoun, 1950).

This transition from sleep to wakefulness, or from the less extreme states of relaxation and drowsiness to alertness and attention, has been attributed to bombardment of the cortex by asynchronous afferent volleys from peripheral receptors. Evidence (Moruzzi and Magoun, 1949) has pointed to the presence in the mid-brain stem of a system of reticular synaptic relays whose direct stimulation exerts a general effect on the cortex which is mediated in part by the diffuse thalamic projection system, first described by Dempsey and Morrison (1942, 1943), and later in more detail by Jasper and his collaborators (Jasper and Droogleever-Fortuyn, 1946; Jasper, 1949; Jasper and Ajmone-Marsan, 1952; Hanberry and Jasper, 1953) and by Starzl and
FIGURE 1

Diagrammatic representation of lemniscal and extra-lemniscal sensory pathways through the brain stem.
Magoun (1951) and Starzl and Whitlock (1952).

The central area in the brain stem which is intimately associated with the arousal mechanism, and accounts for electrocortical and behavioural features characteristic of the waking, and/or alert state, is known as the reticular activating system (area R.F. in the figure), and includes the reticular formation of the oblongata and pontine tegmentum, extending from bulbar to mid-brain regions.

As shown in Figure 1, impulses which follow the classical afferent pathway from peripheral sources synapse in the spinal cord or dorsal column nucleus, then enter the medical lemiscus in the lateral brain stem, and synapse once again in one of the relay nuclei of the thalamus, before finally passing by way of the internal capsule to specific cortical areas.

In contrast, a secondary extra-lemniscal pathway carries impulses via collateral fibres (French et al, 1953) from the lemniscus into the central brain stem, from which impulses are rostrally dispatched to cause widespread and generalized alterations termed the "activation" or EEG arousal response.

This area also receives projections from several areas of the cerebellar hemispheres (Baumgarten et al, 1953), and still more recently the cortex has been found to project from certain discrete areas down into the reticular formation (French et al, 1955). The places of origin of this corticifugal projection are the frontal eye fields, the sensory motor cortex, the par-occipital cortex, the first temporal gyrus, the orbital surface of the frontal lobe, the cingulate gyrus, the tip of the temporal lobe and the medio-basal portions of the temporal lobe. Each of these cortical fields appears to project down into the reticular formation to very much the same brain stem areas which receive collaterals from all the afferent systems of the body.
(Livingston, 1957). Indeed the descending cortical influences are seen to interact in the reticular formation with impulses generated along sensory pathways (Hernandez-Peon et al, 1955).

Livingston (1957) has shown that sensory projections e.g. from the sciatic or the trigeminal nerves, will interact in the reticular formation with each other, and with each of the projections descending from any one of the cortical areas which project into the reticular formation. Thus it seems that the reticular formation constitutes a sort of central integrating switchboard for the interaction of impulses generated in remote and varied parts of the nervous system. In addition, not only is there interaction between descending projections from the cortex with sensory input, but each of the cortical fields interacts with each other, and appears to have a rather special pattern of influence within the brain stem reticular formation. (Adey et al, 1957). For example, some of these fields will augment intrinsic activity of the brain stem while others will diminish it, giving rise to a complex sequence of alternating excitement and depression—a sequence which may last from several tenths of a second to several seconds.

Thus it seems that the cortex is not simply the victim of demands made on it by the reticular activating system, but that the cortex itself possesses corticifugal regulating mechanisms which in turn can influence the level of activity within the reticular formation (Livingston, 1957). This level of activity, which reflects input and output relations, taken together with the brain stem's own contributions to these relations, is important to the total organization of behaviour (Adey et al, 1957). Using implanted electrodes in unanesthetized animals Hernandez-Peon, Scherrer and Jouvet (1956) have shown that the sensory paths are remarkably plastic in relation to the animal's past history and current experiences. Thus, at the first sensory relays and
at cortical levels (Morrel et al, 1956), sensory adaptation or habituation to reiterated stimulation occurs. Moreover when the animal shifts the focus of its attention, the amplitude of impulses down the sensory paths is correspondingly affected. That these dynamic alterations along the sensory pathways are related to activity in the brain stem reticular formation is made clear by the effects of pharmacological agents and of brain stem lesions, since in fact, neither the effects of habituation or focus of attention, nor of conditioning, appears to survive after brain stem lesions, and in the case of anaesthetics, these effects tend to return only after the anaesthetic has lost its effectiveness.

Observation of the effects of numerous pharmacological agents which may be involved directly and indirectly in producing variations of the electrical activity of the brain, suggests that this system is an area wherein numerous drugs act, and points to its multineuronal polysynaptic character as playing a major role in central drug action. Bremer (1937), Forbes and associates (1949), and Barany (1947) have emphasized the susceptibility of complex neurone systems to anaesthesia. Larrabee and Posternack (1952) have shown that the effect of central anaesthetics on nerve fibre conduction proceeds without reference to fibre diameter or velocity of fibre conduction, and that synaptic transmission is a limiting factor, which can be blocked long before fibre conduction is affected. French et al (1953) have observed a differential block of ascending conduction in the reticular activating system upon administration of pentobarbital or ether, and have proposed that this may be of importance in the production of the anaesthetic state. Brazier (1955) has discussed the effects of thiopental on the evoked cortical potential and on the EEG, and similar studies have been reported for chloralosone by Munroe et al (1957). In addition, King et al (1957) using low and high concentrations of barbiturates have confirmed the functional block of
ascending influence on the brain stem reticular formation in response to low concentrations, and moreover have demonstrated a depressant effect of larger concentrations directly upon the thalamic relay nuclei.

Conversely it has been demonstrated by Arduini et al (1954) that conduction in the reticular formation of the brain stem can be markedly enhanced by a so-called "excitant" drug Strychnine, and by Metrazol. Purpura and his co-workers (1957) have recently shown that strychnine produces excitation in bulbar reticular systems due to inactivation of the inhibitory "braking-effect" upon excitatory activity; this drug, classified as "a stimulant of the C.N.S." (Drill, V.A., Goodman and Gilman), is in reality a blockading drug whose overt excitant action is produced in the presence of both excitatory and inhibitory electrogenesis, and seems to be the result of selective blockade of the latter (see also Eccles et al).

Similar overt results have been produced for Metrazol, which, however is truly a synaptic excitant, with convulsive activity directly related to this mechanism. It is effective when applied topically to the cerebral and cerebellar cortices, whereas strychnine is without effect on the cerebellum since the latter is relatively lacking in hyperpolarizing synapses, (Grundfest, 1957).

In another theatre of activity, it has been shown that dexedrine and pipradrol (Meratran) produce an alerting reaction (Rinaldi et al, 1955). Whereas, moderate doses of Phenothiazine derivatives can block the alert electroencephalographic reaction (Himwich et al, 1957, Ingvar et al, 1957, Bradley et al, 1955, Hiebel et al, 1954, and Longo et al, 1954), studies with comparatively high doses of chlorpromazine seem to provoke arousal and produce an alert EEG similar to that produced by reserpine (Himwich et al, 1957).

Of unusual interest is the preliminary report, (Marrazzi, 1957; Marrazzi
et al, 1956) that mescaline, LSD-25, serotonin and Bufotenine, adrenaline and adrenochrome, all are cerebral synaptic inhibitors, as tested in the two neurone intercortical transcallosal system of the cat. Bufotenine is the most potent inhibitor of this group being twice as potent as serotonin, which in turn is reported to be about eight times as potent as LSD-25 and twenty-five to thirty times as potent as adrenaline. Moreover, Marrazzi (1957) has demonstrated that central synaptic inhibition induced by a psychotogen, mescaline, can be prevented by tranquillizers.

The activity of complex neural patterns seems to reflect the working of series and parallel neuronal units, and the details of their synaptic organization and associated pharmacological properties must be analyzed in order to better understand the mechanisms by which various agents produce their central effects. The hypothesis that synapses in the central nervous system are chemically mediated is receiving increasing support (Feldberg, 1954). The present investigation was undertaken in order to try to determine the nature of the chemical mediator or mediators having important actions on the medial portions of the brain stem, and to further delineate the various pathways involved, as well as to establish sites of actions for these compounds.

However, the major objective in the work reported here is the development of methods adequate for the analysis of this system. This task has been accomplished, and preliminary results using some adrenergic and cholinergic agents as well as "blockers" of these agents have been obtained which appear to justify the approach employed.
"Cerveau Isole"

An important and primary objective of this program involved the use of the "cerveau isole", since this preparation, produced by transection of the brain stem just rostral to the trigeminal nerve and its major sensory nuclei, has a number of important advantages for studying desynchronization of the electrocorticogram. Effective somatic de-afferentation, muscle relaxation, and pain relief are achieved by this neurosurgical procedure. If initial general anaesthesia is accomplished with a volatile agent, recovery from anaesthesia permits the study of a preparation which is free from extraneous pharmacological effects (anaesthetics, curari-form agents), and should provide optimal circumstances for producing stable, desynchronized patterns of cortical electrical activity. However, olfactory and visual pathways for physiological "activation" remain intact.

Initially this preparation was performed following a technique slightly modified from the original method of Bremer (1936). The occipital portion of the cranium overlying one hemisphere was removed, and that portion of the cerebral hemisphere exposed. The occipital lobe was then elevated, and a chordotomy knife inserted over the rostral edge of the ossified tentorium. An attempt was then made to transect the brain stem blindly by slicing movements of this instrument. This procedure suffered from three major drawbacks. In a large proportion of trials uncontrollable hemorrhage developed, usually as a result of tears in venous sinuses, and there was evidence of cerebral edema despite the application of temporary manual compression at the base of the skull at the moment of section (Le Beau et al, 1938) Bonvallet et al, 1939). Furthermore, post-mortem examination revealed that the transection of the brain stem was usually incomplete.

Another approach was adopted. Exposure was accomplished by removing
part of the cranium on one side, immediately caudal to the bony tentorium. Part of the cerebellar mass was suctioned off in order to expose the brain stem, and transection was then performed just ventral to the bony tentorium. This procedure also suffered drawbacks. Hemorrhage always developed and loss of cerebro-spinal fluid, though controlled, still left much to be desired for the physiological nature of the preparation. Moreover, in a large percentage of preparations, increasing instability of the blood pressure with time and experimental usage (6-8 hrs) tended to make this preparation unsuitable for extended use. Another procedure had to be sought.

**Stereotaxic Coagulation**

Initially this procedure was performed in the following manner. Anaesthetized cats were placed in a Johnson Stereotaxic Instrument Model No. 210, which was calibrated to conform to co-ordinates of "A Stereotaxic Atlas of the Diencephalon of the Cat" by H. Jasper and Cosimo Ajmone-Marson. The muscles overlying the skull were reflected from the mid-saggital line. Bilateral points of entry into the brain were established by the stereotaxic carrier from the precise working co-ordinates which were to be later used in coagulating the mid-brain reticular substance.

Trephined holes were cautiously and carefully made to reduce hemorrhage and leave the dura intact. Some small degree of bleeding does occur (greater with ether than with thiopental in the majority of cases), but hemostasis can readily be produced by the use of bone wax. The dura was next carefully lifted by means of a dural hook, and incised at the point of electrode entry with a sharp ganglion knife. Electrodes mounted on a stereotaxic carrier were then introduced, using working co-ordinates specific for mid-brain reticular coagulation. First one side was coagulated, and the electrodes removed and inserted into the contralateral side for coagulation of the homologous area. The current necessary for coagulation was obtained from a high frequency source.
Current Source

An effort was made to use a Portable Bovie Electro-surgical Unit, Model 0-4 with modifications of its power output as a high frequency current source. Detailed tests proved this "set-up" unsatisfactory, due mainly to 60 cycle modulation, and our attention was directed towards the Heath Kit Amateur Transmitter Model DX-20. The output of this instrument was attenuated by means of a variable output control, consisting of a series lamp and potentiometer, calibrated for different types of electrodes, and for various spacing of electrodes. This calibration was achieved by observing the time necessary to produce a definite amount of coagulation of egg albumin at a temperature of 20°C.

A series of investigations with the oscillator set at 3.5 megacycles have shown that this instrument, with its adjustable output control, is satisfactory for producing discrete lesions in the reticular formation of the mid-brain tegmentum.

This technique produces the very minimum of hemorrhage, and leaves cerebrospinal fluid circulation intact and functional and the blood pressure well maintained so that the animal can be considered more physiological than in the above mentioned procedure. It also provides the investigator with a preparation exhibiting a consistent and reliable deactivated electro-encephalographic pattern, against which drug-induced changes towards activation can be studied comparatively.

Simultaneous Bilateral Drug Administration

A satisfactory technique for making simultaneous bilateral intracarotid injection of drugs presented another procedural problem. We were interested in obtaining direct central effects of drug action, and preferred the intra-arterial route, since direct effects could be inferred if immediate changes coincident with pupillary dilation were to occur without simultaneous marked
fluctuations in blood pressure. When drugs are administered intravenously in preparations in which one ascending pathway may still be intact, the possibility exists that indirect reflexly mediated effects are sometimes elicited.

Unilateral intracarotid injection, though informative seemed to have certain disadvantages. The cerebral circulation on the side of the injection may be temporarily embarrassed during the canulation procedure. Distribution of injected drugs does not reach the same sites bilaterally, equally and simultaneously. As Marrazzi (1957) has pointed out "a drug injected into the carotid artery would act somewhat like a close arterial injection and produce initially a higher concentration in the brain on the ipsilateral side, while when it got into the systemic circulation and got diluted with the overall blood volume it would then be insufficient lower concentration to prove sub-threshold for the periphery and the contralateral hemisphere. The amounts that get through the Circle of Willis ordinarily are small under these conditions."

A satisfactory technique for simultaneous bilateral drug administration now has been worked out. By introducing a small bore polyethylene catheter directly into the right subclavian artery, it has been proved possible to make injections directly into the innominate artery. This catheter is introduced until the tip touches the caudal aspect of the aortic arch - a distance of approximately 10-10.7 cms. for cats weighing approximately 2.5-3.5 kg - and then the catheter is withdrawn approximately 1.5 cms where the tip should then be in the correct position. The location of the tip, with reference to its ability to distribute injected material bilaterally and simultaneously may readily be ascertained by a test injection of 1 cc. of 1-epinephrine containing 5 μg/cc (expressed as 1-epinephrine base). The administration
of the latter produces immediate, simultaneous, equal and bilateral temporary
dilation of the pupil when the catheter is properly placed. By this technique
repeated injections by way of both carotids can be accomplished without
compromising the cerebral circulation, while ensuring equal and simultaneous
distribution to the presumed sites of action.
APPLICATION OF METHODS

Experiments were performed in cats with an average weight of 2.4 kg. Some were anaesthetized with ether and others with thiopental (Pentothal sodium, Abbott, 50 mg/cc). A total of 75 mg. of thiopental was used for the average weight mentioned above.

Animals anaesthetized with ether were prepared in the following manner; a tracheotomy was performed and ether anaesthesia provided and maintained by means of an attached vapour bottle connected to a Palmer respirator. The ether anaesthetized animals were studied in two groups. Some were curarized with gallamine triethiodide (Flaxedil, Poulenc), given intravenously in doses of 10 mg. as needed (Bonvallet, Dell and Hiebel, 1954). Mid-brain lesions were produced in the second group.

Animals anaesthetized with thiopental were prepared in the following manner: Animals were constantly handled prior to drug administration to increase familiarity and reduce apprehension. Injection of thiopental was then made into "vena cephalica antibrachii" of the forelegs. Only mid-brain lesions were produced in these animals.

In all animals the blood pressure was recorded from the right femoral artery with a mercury manometer. In some animals the right femoral vein was cannulated with a polyethylene catheter whose tip was introduced into the inferior vena cava, for venous administration of drugs. The right subclavian artery was cannulated with a small bore polyethylene catheter, whose tip was positioned into the innominate artery to permit distribution of injected material into both carotid arteries. In the other end of both the venous and the arterial catheters, needles were inserted and connected to a two-way stop-cock, so that saline or various drugs could be introduced alternately without
disturbing the animal. The sinuses were unroofed and electrodes (brass screws) were introduced through the skull to the dura. One pair of screws (electrodes) were positioned anteriorly over the motor cortices. The middle pair were inserted approximately 1 cm. behind the coronal suture and 2-3 cm. to each side of the mid-line. The posterior pair were located behind them, just anterior to the origin of the bony tentorium and 2-3 cm. to each side of the mid-line. The dura leads were connected to an AC amplifier and the EEG recorded on a 4-channel Dynograph ink writer. Bipolar recording was carried out between "right frontal-right central", "right central-right posterior", and between corresponding positions on the contralateral side. Most records were taken at night when the laboratory was quiet. Destruction in whole or in part of the mid-brain reticular formation was produced by passing a coagulating current between a pair of steel electrodes, insulated to 1.0 mm. of the tips, and separated 4 mm. apart on the stereotaxic carrier, using the Heath-kit high frequency generator as a source of current. Macroscopic examination of all sections have been performed, and microscopic localizations of all lesions is being carried out. One hour was allowed to elapse following operative procedures before experimental recordings were made on ether anaesthetized animals, and 2 hours for cats anaesthetized with thiopental.

Precautions were taken to avoid arousal reactions caused by external environmental influences during the injection, and repeated controls were made by injecting saline. Acetylcholine (ACh), and epinephrine were injected in the form of the bromide and bitartrate salts respectively; atropine and dibenzyline in the form of sulfate and hydrochloride salts, and eserine in the form of sulfate. All doses are expressed as weight of free bases, and are reported per kg. body weight of the animal.
FIGURE 2

Samples of various types of EEG activity observed in the unanaesthetized cat, ranging from rapid, low voltage activity ("A") to high voltage slow waves and spindles ("D"). Preceding each pattern is an arbitrarily assigned letter of the alphabet, by which that pattern is referred to in the text.
FIGURE 3

CAT STEREOTAXIC COAGULATION.

<table>
<thead>
<tr>
<th>Left Side: Frontal 0</th>
<th>Right Side: Frontal +1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal -4</td>
<td>Horizontal -4</td>
</tr>
<tr>
<td>1st coagulation</td>
<td>2nd coagulation</td>
</tr>
<tr>
<td>L. Lateral 38.2</td>
<td>L. Lateral 38.2</td>
</tr>
<tr>
<td>R. Lateral 32.2</td>
<td>R. Lateral 32.2</td>
</tr>
</tbody>
</table>

Note: appearance of characteristic deactivated pattern following second coagulation procedure.
IV RESULTS

Interpretation of the Cat EEG (Rothballer, 1956).

Under different experimental conditions, a variety of electroencephalographic patterns were observed, ranging from low voltage fast activity to high voltage slow waves and spindles. Almost all the names which one might assign to such patterns carry with them behavioural or analytical implications which at some time or other are undesirable or inaccurate. Consequently, those patterns which it has proven useful to distinguish in the succeeding presentation, have been arbitrarily assigned letters of the alphabet and are characterized below, including reference when possible to the correlation of the EEG pattern with behaviour or states of consciousness.

Pattern "A" Immediately after a noxious stimulus, such as sciatic nerve stimulation, the EEG was characterized by very rapid activity (30-50/sec.) of intermediate voltage (50-100 /µV) and the complete absence of any slower superimposed frequencies. It was also seen in most of the animals just as they came out of ether anaesthesia. This corresponds to the "activation pattern" of Rheinberger and Jasper (1937), which was described as consisting of low voltage, fast activity and was observed after a variety of external and internal stimuli all of which tended to arouse or alert the animal. For this reason, it is often referred to as the "arousal pattern", but it should be mentioned that its correlation with behavioural arousal, while good, is nonetheless not perfect (Wikler, 1952; Hess, 1954). It is also called "desynchronization" or "EEG asynchrony", but objections may be raised to both these terms, since they imply mechanisms in the generation of the EEG which have yet to be demonstrated.

Pattern "B" Ordinarily the EEG of the conscious and alert animal was slightly slower in frequency (25-35/sec.) and lower in voltage (20-50 /µV)
than that described under "A". The superimposition of slower frequencies
(8–20/sec.) of low voltage occurred at irregular intervals. It was seen in
the intact, curarized cat after milder arousing stimuli (tactile, auditory,
or visual), and was usually maintained continuously when the animal was
mounted in the stereotaxic apparatus under local anaesthesia. It corresponds
partly to the "activation pattern" but contains the irregular 8–16/sec.
superimposed activity described by Hess, Koella, and Akert (1953) as
characterizing the transition from alertness to rest, the 12–15/sec waves
observed by Rheinberger and Jasper (1937) in the relaxed animal, and the
10–15/sec. low amplitude activity reported by Clark and Ward (1945) as
associated with rest.

**Pattern "C"** With the animal arranged as comfortably as possible and
undisturbed, brief trains of low voltage slow waves (5–8/sec.) appeared in
the EEG record. This state was associated with pupillary constriction, and
as it progressed the amplitude of the slow waves increased, the frequency
decreased, and these waves occupied more and more of the record. The
underlying faster frequencies (25–30/sec.) still persisted however, but
were of very low voltage and easily overlooked. Finally the record was
interrupted intermittently with high voltage spindle bursts (10–14/sec.).
This pattern has been described by others as characterizing rest or
drowsiness (Hess, Koella, and Akert, 1953) or beginning sleep (Clark and
Ward, 1945). We have observed it under comparable circumstances, as well as
after small lesions of the reticular formation, and following the administration
of chlorpromazine.

**Pattern "D"** This pattern never occurred spontaneously in our animals
but could be produced consistently by making a lesion in the reticular
formation at the pontomesencephalic junction. After the making of a suitable
lesion, the EEG record became converted into an endless succession of spindle
bursts, between which occurred intervals of high or low voltage activity of varying frequency. The record resembled the picture seen in light sleep (Hess, Koella, and Akert, 1953). Often it differed slightly from the characteristic record of sleep in that the spindle bursts were more conspicuous and the slow waves less so.

In its strictest sense, the term "EEG activation" refers not to any one specific EEG pattern, but to the conversion of one pattern into another — specifically from "D" towards "A", or towards the left, if the patterns are imagined as being arranged in alphabetical order. Conversion in the opposite direction is "deactivation". Thus although the end result would not necessarily be the EEG of alert wakefulness, a change from pattern "D" to "C" is still "activation". So is the conversion from "B" to "A"; even though both of these latter patterns might fall into what is generally considered the "activation pattern" to begin with.

EEG Following mid-brain coagulation of the reticular formation.

Immediately after making the lesions on both sides, bilateral miosis of a maximal degree usually appeared. The EEG showed marked activation (pattern A), and over the ensuing 5-15 min., the degree of EEG activation gradually lessened, the record passing through patterns "B" and "C" to "D". (Rothballer, 1956). These observations have been confirmed for the ether anaesthetized animals in this laboratory but in the animals anaesthetized with thiopental, barbiturate "bursts" make their frequent appearance on the EEG, and the transition from one pattern to the other is not only more obscure but also of a longer duration. Once the thiopental has worn off, pattern "D" appears and presents the same configuration for both the ether and thiopental anaesthetized preparations. This pattern, characteristic of reticular formation coagulation, differs somewhat from that seen in drowsiness and sleep, in that high voltage spindle "bursts" appear rather abruptly, often
before the appearance of much slow activity. If the desired degree of deactivation was not obtained, it was sometime necessary to enlarge the lesion or move the electrodes slightly anteriorly (Frontal 1.0 to Frontal 2.0; Figure 3). Moreover when the electrographic activity of the two hemispheres was not symmetrical, further coagulation on the side showing less cortical deactivation was indicated. Although a lesion which produced EEG deactivation always produced miosis (provided the oculomotor nucleus was not damaged), the converse was not necessarily true. Once the spindle activity has been properly established, the preparation showed remarkable stability. (Rothballer, 1956).
Unanesthetized Curarized Cats

**Acetylcholine (ACh)**

In the small number of animals tested (three), the EEG became desynchronized following intra-innominate and intravenous injections of ACh. Effective doses varied in different animals and ranged from 2.0 μg/kg to 5.0 μg/kg intra-arterially. Although some flattening of the EEG and reduction in spindles could be observed with smaller doses (0.5 to 1.0 μg/kg) given intra-arterially, no change in the pattern of the EEG could be noted with these small doses following intravenous administration. Records of the blood pressure show that whether the i.v. or i.a. route was used, only very small modifications of the B.P. could be observed after small doses, but that higher doses of ACh always resulted in a profound but temporary fall in blood pressure.

**Atropine**

The administration of atropine sulfate intra-arterially seems to be definitely antagonistic to the activating action of both intra-innominate and intravenous ACh. This antagonism becomes manifest with doses of 0.25 - 0.5 mg/kg when there appears a stability in the consistency and constancy of the amplitude of large slow waves and of the spindle activity. Subsequent to atropine administration, the injection of i.v. or i.a. ACh in previously effective doses does not produce the usual alerting reaction nor are there any changes or modifications in the blood pressure.

**Epinephrine**

The effects obtained with both the intra-arterial and intravenous injections of epinephrine have been rather capricious, despite typical marked changes in blood pressure. Smaller doses (0.5 to 1.0 μg/kg) by both these routes produced no detectable change in the EEG. A large dose i.a. (2-10 μg/kg) produced some change towards activation, whereas the same dose i.v. seemed to stabilize the already existing pattern.
Cats with coagulation of the mid-brain reticular formation

The effects of drugs were studied only after at least two hours had elapsed since coagulating the brain stem, in order to permit the effects of anesthesia and surgical procedures to wear off, and to allow the deactivated pattern to acquire stability.

Acetylcholine and Eserine

ACh was administered in doses of 2 μg to 5 μg/kg and eserine in doses of (10-20 μg/kg). Both drugs produced desynchronization when administered i.v. or i.a. but the activation produced by eserine was more intense and of a much longer duration (more than 40 minutes) than ACh, and could be recognized more readily against a background of "not-too-well" defined activity. In fact there seemed to be little difference between the desynchronization produced by eserine in the cat with mid-reticular coagulation, and in the unanaesthetized curarized animal. Moreover, the EEG arousal following the administration of both ACh and eserine was generally accompanied by some degree of motor activity of the head and upper trunk, and by changes in the rate of respiration. These effects were not noted in the unanaesthetized curarized preparation.

Both these drugs produced a rapid fall in blood pressure with average values ranging from 110 mm. Hg to 62 mm. Hg, but this fall was of a transient nature only, and the blood pressure readily returned to its original level. In the case of eserine, EEG activation clearly persisted after the blood pressure had returned to normal.

Atropine

The same dose was used in the cat with mid-reticular coagulation as was used in the unanaesthetized curarized cat, and very similar results obtained. Atropine has a definite blocking effect against the fall in blood pressure produced by ACh and Eserine. It also inhibits the desynchronization usually produced by ACh and eserine.
Epinephrine

There has been no consistent or uniform pattern in the EEG effects obtained in this laboratory, from the administration of epinephrine. A single dose of 2 μg/kg i.a. has produced "flattening" of the EEG and an increase in frequency in some preparations, whereas doses of a larger order of magnitude have had no demonstratable effect in other animals similarly prepared.

In some animals in which the background activity was comparable to pattern "D" of Rothballer, (1956) (characterized by high voltage spindles and slow waves) activation appeared soon after the beginning of the i.a. injection of 2 μg of epinephrine and lasted 45 sec., whereupon contrasting re-occurrence of the "spindles" and slow waves, developed.

Animals with mid-reticular lesions which have been anaesthetized with ether seem more sensitive to the effects of epinephrine than those which have been anaesthetized with thiopental. Intravenous doses as high as 10 μg/kg have not produced any discernable change in the electroencephalogram, of the latter group. Blood pressure records reveal that the response of the vascular system to epinephrine in this preparation is quite consistent with known pharmacological actions of this amine, and moreover is quite similar in both the "cerveau isole" and in the unanaesthetized curarized cat.

Dibenzyline

Preliminary results with 1.0 mg/kg of dibenzyline given i.a. at a fairly rapid rate has yielded conflicting results. B.P. rose in some animals and fell or was unchanged in others. Regardless of the cardiovascular response, activation of the EEG was evident. The administration of epinephrine i.a. in doses which previously had evoked a change in the EEG and a pressor response, produced only EEG activation when given after a previous injection of dibenzyline which blocked the pressor response to epinephrine.
DISCUSSION

EEG activation is not only a concomitant of behavioural arousal but may be produced specifically by electrical stimulation of certain well-circumscribed portions of the central nervous system, in particular the bulbar reticular formation, the pontine and mesencephalic tegmentum, subthalamus and dorsal hypothalamus, (Marruzzi and Magoun, 1949). It is only natural that attention should be drawn to these regions, collectively called the reticular activating system, as a possible site of action of adrenergic and cholinergic drugs. Since synaptic transmission in the reticular formation may appear to be exclusively cholinergic on one hand or adrenergic on the other, both acetylcholine and epinephrine have been advocated as the natural mediator of this system.

Some investigators have provided definite evidence suggestive of the cholinergic nature of the reticular activating system. EEG arousal has been obtained with intracarotid acetylcholine (Bonnet and Bremer, 1937; Bremer and Chatonnet, 1949; Longo, 1955; Rinaldi and Himwich, 1955) and cholinesterase inhibitors such as DFP and eserine have likewise produced activation (Funderburk and Case, 1951; Bradley and Elkes, 1953; Longo, 1956, 1957). Accordingly these authors favour cholinergic transmission in the reticular formation of the mesencephalon, an opinion which is not shared by Desmedt and La Grutta, (1955), who suggest a cortical site of action. These investigators have observed that activation can still be produced in the "cerveau sans reticulee" preparation (complete removal of the reticular formation) following the intracarotid administration of an anticholinesterase drug (dimethylcarbamate 2-hydroxy-5-phenyl benzyl triethylammonium, RO 2-0683).

Opposed to the cholinergic viewpoint, are the proponents for adrenergic
activation. Bonvallet et al (1954) have demonstrated that in both the curarized cat and in the "cerveau isole", desynchronization of the EEG could be obtained by intravenous epinephrine, and this activation was similar in degree and in intensity to the activation produced by an external stimulus. Dell et al (1954) had suggested an adrenergic component in their observations on the adrenalin sensitivity of the descending bulbar facilitatory system - a suggestion, which has recently been supported by Rothballer (1956).

The observation that in the cat this same region contains 7% epinephrine, and 93% norepinephrine is pertinent to this hypothesis; the content of these substances is labile and falls after noxious stimuli such as anoxia or ether anaesthesia and it has been proposed that these compounds participate actively in the functions of these organs (Vogt, 1954). It is obvious that there is a lack of agreement as to the exact nature of the receptor sites in the mid-brain reticular formation and this may be occasioned in part by species variation; some investigators using cats, and others rabbits. In addition there also has been variation in the type of preparation used to obtain a consistently reliable deactivated pattern, against which drug induced action could be unmistakably noted; "whether or not any EEG change could be detected after intravenous adrenaline depends primarily upon the background activity, and to a much lesser extent on the dosage. Working against a background of arousal, it was quite difficult to detect any effect at all;" (Rothballer, 1956). Our experience has borne out this view.

Variations, too in the plane of sectioning or site of coagulation may explain different results reported. Bonvallet et al (1954) have shown that intercollicular section of the brain stem, passing to the ventral surface just rostral to the pons, completely suppresses the cortical activation in response to a peripheral sympathetic discharge. Similarly Rothballer (1956) has
demonstrated that progressive coagulation from Frontal 0 to Frontal + 7, proportionately reduces the sensitivity of the reticular formation to epinephrine, and that rostral to Frontal + 7 it was extremely difficult to obtain any response regardless of dose. The difficulty in obtaining an adrenergic activating component in etherized animals may be due to the adrenergic stimulating effects of the anaesthetic agent, which may produce and maintain a continually activated pattern until the adrenergic component has been surgically eliminated. In any event, activation following the administration of epinephrine was extremely difficult to demonstrate, and its inconsistent appearance in cats prepared under thiopental may be related to the extent of the coagulated lesion.

Preliminary results in this laboratory suggest that both adrenergic and cholinergic components may be existent in this area, and although there may be fundamental differences between them, they may be closely related functionally. At present it is tempting to speculate that the reticular activating system may possess both adrenergic and cholinergic components in series or in parallel, with the cholinergic portion situated rostral to the adrenergic, and perhaps functionally interdependent. This may explain the fact that cholinergic activation can often be demonstrated when adrenergic activation cannot be elicited.

It is hoped that a more precise definition of these components will permit an evaluation of the possibility that numerous pharmacological agents which affect the state of wakefulness or alertness may act by enhancing or inhibiting one or the other of these components. Work along this direction is actively under way.
SUMMARY

(1) A method is reported for the bilateral, synchronous distribution of injected drugs to both sides of the brain simultaneously.

(2) A preliminary report is made of the effects on the EEG of acetylcholine, eserine, epinephrine, atropine and dibenzyline in the unanaesthetized curarized cat and in the cat with coagulation of the mid-brain reticular formation.

(3) In each of these preparations, both eserine and acetylcholine produce desynchronization of the EEG. Atropine may block the production of desynchronization by either acetylcholine or eserine.

(4) The effects of epinephrine and dibenzyline are not yet as well defined. They seem dependent to some degree on the extent of coagulation, as well as on the amount of existent background activity.
BIBLIOGRAPHY


