Running title:

ADRENERGIC COMPONENT OF RETICULAR ACTIVATING SYSTEM

STUDIES ON THE ADRENALINE-SENSITIVE COMPONENT OF THE RETICULAR ACTIVATING SYSTEM

by

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A THESIS PRESENTED TO

THE FACULTY OF THE GRADUATE SCHOOL OF ARTS AND SCIENCES

OF McGILL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

1 9 5 5

From the Department of Neurology and Neurosurgery

ACKNOWLEDGEMENTS

I should like to express my appreciation to Dr. Wilder Penfield and Dr. Herbert Jasper for making it possible for me to spend the past year in the Department of Neurophysiology, to Dr. Jasper for his invaluable suggestions and criticisms during the course of this project, and to Dr. Robert Naquet who participated in many of the earlier experiments.

My gratitude is also due Miss Mary Roach and Mrs. Beatrice Kung Von of the Neurophysiology laboratory for help with the experiments, Mrs. Joan Engel and Miss Barbara Nuttle of the Neuroanatomy laboratory for the preparation of the histological material, and all my colleagues in the research laboratories of the Montreal Neurological Institute for their interest and suggestions. Lastly I should like to thank Miss Petrea Jensen for her patience, care and skill in preparing the manuscript.

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I. INTRODUCTION

Activation or "arousal" of the EEG is ordinarily thought to be brought about by stimulation, usually by various sensory modalities, of the reticular activating system; this in turn exerts a diffuse influence upon the electrical activity recorded from the entire cortex (Magoun, 1955). A change in cortical electrical activity has been observed, however, in the isolated cortical slab after direct stimulation of the brain stem reticular formation (Ingvar, 1955), and EEG activation has been produced by stimulation of the cut peripheral end of the splanchnic nerve (Bonvallet, Dell, and Hiebel, 1954). This evidence has suggested the possible presence of a humoral or vascular phase in "arousal" or EEG activation. The possibility is further supported by the observations that both adrenaline (Bonvallet, Dell, and Hiebel, 1954) and acetyl choline (Rinaldi and Himwich, 1955) can bring about EEG activation when introduced into the circulation. Since acetyl choline is not ordinarily present in the blood stream, our attention was first directed to the role of adrenaline in the humoral phase of EEG activation.

The study was subsequently extended to include other sympathomimetic amines and related compounds, and adrenergic blocking agents. After the investigation was under way it

soon became apparent that although a humoral phase to EEG activation mediated by adrenaline does exist it is probably of little importance under most circumstances. Nonetheless observations on EEG activation produced by adrenergic substances have yielded interesting information about certain pharmacological and physiological properties of the arousal mechanism itself; it is principally about these that this study will concern itself.

Investigations of the effect of adrenaline upon the human EEG have yielded conflicting results. Some observed no effect (Gibbs, Gibbs, and Lennox, 1937; Gottschalk, 1952); others noted excessive beta activity (Grinker and Serota, 1941) or, when frequency analysis was employed, a shift to faster frequencies (Gibbs and Maltby, 1943); still others reported the appearance of slow waves (Greenblatt, Funkenstein, Miller, and Rinkel, 1947; Faure, 1949).

In the anaesthetized cat intravenous adrenaline produced a slight "increase in the electrical activity" of the cortex (Jasper and Erickson, 1941), while in the unanaesthetized cat it produced definite EEG activation (Bonvallet, Dell, and Hiebel, 1954). In the cat anaesthetized with cyclopropane increase in the electrical activity confined to the posterior hypothalamus has been reported (Porter, 1952), while in the unanaesthetized guinea-pig, no change was noted (Green, 1953). Given

systemically, adrenaline (and noradrenaline) prolonged the duration of electrographic seizures induced by applying a shock locally to the cortex (Minz and Domino, 1953); applied locally to the cortex, however, it produced a marked diminution in the amplitude of the spontaneous cortical activity (Minz and Rémond, 1953). Lastly, adrenaline has been observed to exert an inhibitory effect upon certain cortical synapses (Marazzi, 1950).

It is apparent that adrenaline can produce a whole range of central effects, many of them apparently contradictory, depending upon dose, mode of administration, particular electrical phenomenon under observation, and the manner of interpreting them.

In the following studies only a restricted group of central adrenaline actions were examined but it is hoped that they will resolve at least some of the apparent contradictions referred to above while at the same time adding to our understanding of the role played by adrenaline or adrenergic substances in certain functions of the central nervous system.

II. METHODS AND MATERIALS

The experiments were carried out on 35 cats. With a few exceptions, each animal was weighed and then anaesthetized with ether. A tracheotomy was performed and ether anaesthesia

maintained by means of an attached vapor bottle. In the earlier experiments bilateral vagotomy was performed, but this was subsequently abandoned since it did not appear to modify the results.

The right sciatic nerve was prepared for stimulation, which was accomplished with a square wave stimulator attached through a stimulus isolation unit.

The left femoral vein was cannulated with a polyethylene catheter whose tip was introduced into the inferior vena cava. In the other end was inserted a needle clamped to a two-way stop-cock so that saline or various drugs could be introduced alternately into the circulation without in any way disturbing the animal.

The left femoral artery was tied distally and cannulated with a blunt-tipped #18 needle. This was then attached through a short length of polyethylene tubing to a strain gauge.* The strain gauge was connected through a strain gauge control unit to a DC vibrating reed type amplifier** the output of which was used to drive the recording pen directly.

The muscles overlying the skull were reflected and electrodes (brass wood screws) introduced through the skull to the dura. After the sinuses had been unroofed one pair

*Pressure Transducer, Model P23-4G-255, Statham Laboratories, Beverly Hills, California.

**Dynograph Amplifier, Type 131, Offner Electronics Inc., Chicago, Ill.

of electrodes was placed anteriorly on either side of the mid-line approximately over the motor cortices. The second pair was inserted through the coronal suture 2-3 cm. to each side of the mid-line while the posterior pair was located behind them just anterior to the origin of the tentorium. Bipolar recording was carried out between the right frontal-right central, right central-right posterior, and the corresponding positions on the opposite side. The dural leads were connected to an AC amplifier and the EEG recorded by an ink writer.

When subcortical lesions were to be made, the head was mounted in the stereotaxic apparatus after local anaesthetization of the points of contact. Otherwise the head was supported in a cloth sling in as natural a position as possible. Once preparation was completed, the ether was discontinued and the animal immobilized with a curarizing agent; artificial respiration was carried out for the remainder of the experiment.

Subcortical lesions were made by passing a coagulating current between a pair of steel electrodes, insulated to within 1 mm. of their tips and mounted 4 mm. apart on the stereotaxic carrier. The Wyss high frequency generator was used as a current source.

Three cats were adrenalectomized beforehand. This was carried out in two stages, employing a lumbar, sub-costal

approach. After the second stage, each animal was maintained on cortisone (10 mg./day, i.m.) until the day of the experiment.

Section of the spinal cord was done under local (Nupercaine) anaesthesia. After suitable exposure, the cord itself was injected with Nupercaine and the segment between C1 and C2 destroyed sub-pially with small cotton pledgets.

Histological localization of all lesions was carried out. The blocks were fixed in formalin, mounted in paraffin and sectioned semi-serially. Alternate sections were stained with cresyl violet and Luxol blue-cresyl violet (Klüver and Barrera, 1953).

Drugs

Epinephrine hydrochloride (Adrenalin, Parke, Davis & Co.). This preparation contains approximately 99.9% pure L-epinephrine and less than 0.1% primary amine compound.* In conformity with current usage (von Euler, 1951) it will be referred to as adrenaline. Ordinarily, the commercial preparation was diluted 1:100, so that 1 cc. contained 10 µg., although further dilutions were sometimes necessary. An attempt was made to keep the volume of solution injected relatively constant. The higher the dilution, the more rapidly the adrenaline solution was apt to deteriorate. Preservatives (such as ascorbic acid) were not used, but when working with high dilutions fresh preparations were made up frequently from the stock solution.

*Parke, Davis & Co., Detroit, Michigan. U.S. Patent No. 2,602,818.

Levarterenol bitartrate (Levophed, Winthrop-Stearns). This is a solution of levarterenol bitartrate monohydrate but dosage is expressed as the equivalent amount of levarterenol base. Again in conformity with current usage (Von Euler, 1951), it will be referred to as noradrenaline.

Phenylephrine hydrochloride (Neo-synephrine, Winthrop-Stearns). This solution was diluted with saline until 1cc. contained 20 µg. and was administered intravenously.

Chlorpromazine (Largactil, Poulenc)*. The preparation was given intravenously, intramuscularly, and intraperitoneally, diluted in saline usually in a dosage of 1mg./kg.

Gallamine triethiodide (Flaxedil, Poulenc)*. This drug was chosen in preference to other curarizing agents for the reasons outlined by Bonvallet, Dell, and Hiebel (1954). It was given intravenously undiluted in doses of approximately 10mg. as needed.

Phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French)** This drug was administered either intravenously or intramuscularly in doses of 1-6 mg.

Methamphetamine hydrochloride (Desoxyn, Abbott Laboratories). The preparation was given intravenously in doses of 10-1,000 µg.

*For which the author is indebted to Mr. W.L. Jeffry, of Poulenc Ltd., Montreal, Canada.

**For which the author is indebted to Mr. W.R. Featherstonhaugh, of Smith, Kline and French I.A.C., Montreal, Canada.

Serotonin creatinine sulfate.* The crystals were weighed out and dissolved in a solution of physiological saline so that 1cc. contained either 10 or 100µg. of the salt.

Cocaine hydrochloride. This was employed in an aqueous solution containing 50 or 500µg. per 1cc. and was given intravenously in doses of 20-1,000 µg.

III. RESULTS

A. Interpretation of the Cat EEG.

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 behavioral 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 behavior or states of consciousness (Fig. 1).

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

*Sandoz Chemical Works Inc., New York, N.Y.

(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 behavioral 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 frequency (25-30/sec.) still persisted however, but was 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 ponto-mesencephalic

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 varying considerably in 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, "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.

B. Effect of Reticular Formation Coagulation

It is apparent that to study EEG activation such as is produced by intravenous adrenaline it is necessary to work with a preparation that is not already activated. Frequently the intact cat was unsatisfactory in this regard and

it proved helpful to induce "deactivation" or a shift in the EEG toward the slower, higher voltage activity artificially by partly destroying the reticular formation at the ponto-mesencephalic junction.*

This was done by inserting the coagulating electrodes into the brain stem (stereotaxic coordinates: Frontal 0; Horizontal -4, the tips being 2mm. to either side of the mid-line) and carrying out bipolar coagulation of the area described. Immediately after making the lesion, bilateral miosis of a maximal degree (greater than that seen after section of the cervical sympathetic chain) usually appeared; the EEG showed marked activation (pattern "A"). Over the succeeding 5-15 minutes the degree of EEG activation gradually lessened, the record passing through pattern "B" and "C" to "D". The succession of events differed somewhat from that seen in spontaneous drowziness and sleep, in that the high voltage spindle bursts appeared rather abruptly, often before the appearance of much slow activity.

If the desired degree of deactivation was not obtained, it was sometimes necessary to enlarge the lesion or move the electrodes slightly anteriorly. 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 deacti-

*The author is indebted to Dr. Robert Naquet (Marseilles, France) who introduced the preparation described and demonstrated its usefulness.

vation always produced miosis (provided the oculomotor nucleus was not damaged), the converse was not necessarily true.

Once the spindle activity had been properly established, the preparation showed remarkable stability. EEG activation was not produced by ordinary stimuli (touch, sound, light; olfactory or gustatory stimuli were not tried), and was produced only transiently, when at all, by sciatic stimulation, in which case the activation often barely outlasted the stimulus. Because of the preparation's insensitivity to external stimuli, it was possible to conduct the experiment under the usual laboratory conditions and without undue precautions as to noise or other disturbances, with the assurance that changes in the EEG were a consequence only of experimentally imposed stimuli and not of accidental environmental occurences often beyond the experimenter's control. It should be added that the utilization of this stable but sensitive preparation made possible semi-quantitative estimations of the effects of various drugs and procedures upon the EEG.

C. The Effects of Sciatic Nerve Stimulation

1. In the intact, curarized animal (Fig. 2)

Approximately coincident with the application of a brief (1-5 seconds duration) sciatic shock, a monophasic,

high voltage deflection appeared in all EEG channels and the EEG pattern was converted to that described under "A" from whatever pattern had previously been dominant. The EEG pattern faded imperceptibly into that shown under "B" over the next 10-30 seconds. In those instances in which the cat ultimately reverted to the record characteristic of "drowsiness" ("C") slow waves or spindles rarely appeared before at least 40-50 seconds had elapsed. Not infrequently EEG activation persisted for several minutes, when the record was often seen to shift back and forth between patterns "A" and "B" at 10-20 second intervals.

A rise in the blood pressure was detectable between 2 and 3 seconds after the shock; it rapidly rose to a peak and then gradually fell off over the subsequent 30-60 seconds. Sometimes a second blood pressure peak or a prolonged plateau was superimposed upon the falling phase. When the rise in blood pressure occurred in two distinct phases (which was infrequent even with shocks of high intensity and short duration) a second peak of EEG activation might also be discernible coincident with the second blood pressure rise. Since both rises in blood pressure occurred within the first 20-30 seconds after stimulation - when EEG activation was still marked - it usually was difficult to separate the EEG pattern into distinct "phases" with any assurance.

2. In the adrenalectomized, curarized animal

The pattern and sequence of events in the adrenalectomized animal was very similar to that described for the intact cat. The EEG activation was as intense and lasted for about as long. The blood pressure rise occurred promptly and was fully as great. However the superimposition of a second peak or a prolonged plateau upon the falling phase was never observed; nor was a tendency observed for the EEG activation pattern or the pressor response to break up into two or more phases.

3. In the animal with mid-reticular coagulation

Coagulation of the reticular formation at the ponto-mesencephalic junction was apt to have variable effects upon the response to sciatic stimulation, depending upon the exact size, symmetry and location of the lesion. (a) When the coagulation was small and the effect upon the EEG incomplete, i.e., conversion to the pattern described as "C" but not "D", the response to sciatic stimulation might remain quite intact. (b) Frequently, the EEG response was only somewhat shortened, while the pressor response was completely abolished. (c) With more extensive lesions, the pressor response was abolished and the EEG effects were reduced to a brief effect not outlasting the stimulus or were abolished altogether. (d) Rarely, the pressor response would remain quite intact, but the EEG

effects were delayed. In this last instance, the EEG activation usually appeared 12-13 seconds after the sciatic shock and lasted 15-30 seconds (Fig. 3).

D. Effects of Intravenous Adrenaline Administration

1. In the intact, curarized animal

Adrenaline was administered intravenously through a polyethylene catheter introduced into the inferior vena cava, so that the drug would enter the circulation at approximately the same site as it does normally when discharged from the adrenal glands. It was given rapidly in approximately 1cc. amounts followed with an equal amount of physiological saline. In those instances where the animal appeared to be roused by the injection itself, saline injections were repeated until the animal ceased to respond to the procedure.

Whether or not any EEG effects could be detected after intravenous adrenaline depended primarily upon the background activity and to a much lesser extent upon the dosage. Working against a background of "arousal" it was quite difficult to detect any effect at all; the most noted was a slight increase in frequency and a disappearance of some of the slower (8-20/sec.) frequencies that occur from time to time. It was often difficult to be certain about changes of so slight a magnitude and quite impossible to time or otherwise measure them. It should be emphasized that although sciatic stimulation was always able to convert

"B" into "A" and thus to impose a clear cut activation upon an already "aroused" record, adrenaline could not do so, no matter what the dosage.

Upon a background containing a certain amount of EEG "synchrony" (pattern "C"), adrenaline was capable of causing EEG activation, i.e., it produced a conversion to the "B" pattern (but never to "A"). This effect usually appeared 10-11 seconds after the beginning of the injection and was 10-40 seconds in duration.

Although EEG activation was the most conspicuous effect of adrenaline, it gradually became apparent that this was not the only effect. Under the proper circumstances, it was possible to observe the opposite effect from adrenaline, i.e., "deactivation" or an increase in high voltage slow activity, even with the appearance of spindles.

Fig. 5 shows that pattern produced by 4 γ. of adrenaline in a suitable preparation. Between 5 and 9 seconds after the injection there was disappearance of the 5-8/sec. slow waves, revealing an underlying fast frequency of 27/sec. The slower frequencies then returned and remained until 17 seconds, at which time EEG activation again supervened and prevailed until 48 seconds after the beginning of the injection, when the record reverted back to that seen before the injection. While this particular pattern might appear fortuitous, it was repeated with minor variations in timing four times in the succeeding half hour.

Although the very early (within 5-9 seconds) excitatory phase of adrenaline was not often seen, an inhibitory phase both before and after the main excitatory phase of adrenaline was almost the rule when dealing with the intact animal. Its

detection however requires a preparation in just the right degree of "arousal" - if the animal is too aroused (pattern "B") the inhibitory effect of adrenaline is generally too feeble to affect the record while if the animal is quite "drowsy" and shows considerable EEG hypersynchrony, a slight increase in slow waves and spindles may easily pass unnoticed. In some instances adrenaline produced a series of alternating phases of activation and deactivation gradually approaching the pre-adrenaline level of EEG activity. In cats with a background activity of the "B" type, where it is difficult to detect much further activation from adrenaline, the deactivation or inhibitory phase appeared quite clearly 40 or 50 seconds after the injections, ushered in with sudden spindle bursts and slow waves.

The smallest amount of adrenaline able to produce a clear-cut EEG effect was 2μg./kg.; the usual dose was 5-8μg./kg., and little was to be gained by exceeding this. Since, as mentioned above, minimal responses are difficult to quantify in the intact animal, no attempt was made to find the threshold dose.

Under the above circumstances, adrenaline initiated an abrupt rise in blood pressure beginning 7-8 seconds after the onset of the injection, and lasting a variable period thereafter, depending upon the dose. Smaller amounts of adrenaline produced a rather small rise in blood pressure followed by a longer fall.

In summary, intravenous adrenaline produces a series of alternating periods of EEG activation and deactivation. Shortly after the injection, when the blood and brain content of adrenaline are presumably rising rapidly, these phases are passed through quickly and may be missed, the record assuming the dominant activated pattern. Then as the adrenaline level slowly falls, the activated pattern gives way to one of alternate activation and deactivation and/or that of deactivation alone.

Although the phase of activation usually follows by 4-5 seconds the rise in blood pressure, occasionally a brief early phase of activation precedes the blood pressure rise (see Fig. 5). Likewise, although the early phase of deactivation is usually synchronous with the onset of the blood pressure rise, the late phase of deactivation often occurs when the blood pressure has returned to normal or is subnormal. Thus it is seen that neither activation nor deactivation bear any fixed relation to the level of the blood pressure or changes in it.

2. In the Adrenalectomized Animal

The response to adrenaline of the adrenalectomized, cortisone-maintained animal did not differ from that of the intact animal.

3. In the Animal with Mid-reticular Coagulation

(a) Pattern of the Response

Depending on the extent of coagulation, such animals showed either the EEG pattern described under "C" or, more frequently, that under "D". When the amount of coagulation was small and pattern "C" predominant, the effects of adrenaline were in all respects comparable to those observed in the intact, spontaneously drowsy animal showing a similar EEG background; both the activation and deactivation phases were observed.

With more extensive lesions in the reticular formation, the background pattern was that described under "D". In such animals it was impossible to detect any phase of deactivation, presumably because the background was already that of maximum high voltage slow activity. As a result, the phase of EEG activation stood out very clearly, being marked by a rather abrupt cessation of spindles and slow waves and the appearance of fast, low voltage activity (usually pattern "B"). Activation characteristically appeared 8-14 seconds after the beginning of the injection of a moderate dose of adrenaline and lasted from 40-80 seconds. At the end of the response, spindles and slow activity abruptly reappeared, so that there was no difficulty detecting the response or timing it (Fig. 6).

The effect of adrenaline upon blood pressure in the animal with mid-reticular coagulation was no different from

that in the intact animal, and the coagulation procedure itself did not change the blood pressure.

(b) Adrenaline Threshold

Because of the clear-cut nature of the activation response and the stability of such a preparation over many hours, it was possible to reduce the adrenaline dosage progressively in an attempt to determine the threshold. Typical results are illustrated in the protocol from an 4kg. male cat (RC28) subjected to mid-reticular coagulation:

Adrenaline Dose Total	Beginning and end of EEG Activation (in seconds after onset of injection)	B.P. response
15 µg.	9-60 sec.	rise at 7.5se
5 "	10-47 "	" "
2.5 "	10-45 "	" "
2.0 "	13-42 "	" "
1.0 "	10-28 "	" "
0.5 "	9-24 "	" "
0.25 "	12-23 "	fall at 18se
0.20 "	23-38 "	" " 20 "
0.15 "	no effect	

Thus as smaller and smaller doses of adrenaline were given, the period of activation characteristically became shorter and shorter but its time of onset for any one cat changed very little; this was true until the threshold is approached, when the time of onset suddenly shifted and occurred later.

The above protocol also illustrates several other characteristics of the adrenaline response of the cat with

mid-reticular coagulation. The threshold for adrenaline was often surprisingly low (in this instance, 0.05 μg./kg.) and EEG activation still occurred with doses of adrenaline too small to produce any rise in the blood pressure, when activation often preceded a fall in B.P. The threshold illustrated above was the lowest observed, but thresholds for similar preparations of 1μg/kg or less were not unusual. It appears then that the sensitivity to adrenaline of the animal with the brain stem lesion can be greater than that of the intact cat. At least part of this is due to the greater ease of detecting a response against a deactivated EEG background.

(c) Intensity of Response

Although the usual effect of adrenaline is to convert the EEG pattern from "D" to "B", occasionally, it is converted to "A", the degree of activation (in frequency and amplitude) being quite comparable to that observed immediately after a painful stimulus in the intact cat. This is illustrated in Figure 7. EEG activation from adrenaline this intense (to pattern "A") has never been seen in the intact animal and has been most frequently observed in cats with rather extensive reticular coagulations. It seems necessary to conclude then that not only may mid-reticular coagulation lower the apparent threshold to adrenaline, but it may increase the intensity of the response as well; however these two circumstances have never been observed simultaneously in the same cat.

(d) Effect of Further Coagulation of the Mesencephalic Tegmentum upon the Threshold and Pattern of Adrenaline EEG Activation; Unilateral Electrographic Arousal

It was observed that, in general, the more extensive the coagulation of the brain stem necessary to produce the desired EEG background, the higher the threshold to adrenaline. Since the adrenaline sensitivity, even in intact animals, is seen to vary somewhat, further evidence was sought on the correlation between the amount of mesencephalic reticular formation left intact and adrenaline sensitivity. This was done by progressively destroying the mesencephalic tegmentum, advancing the coagulating electrodes rostrally in 2mm. steps, unilaterally or bilaterally. The following is abstracted from the same experiment cited above (RC28):

> Some hours after the original adrenaline threshold was determined (the noradrenaline threshold having been measured in the meantime), the adrenaline threshold was again determined and found to lie between 0.20 and 0.25µg., i.e., scarcely different from the original determination. Brain stem coagulation was then performed 3 mm. anterior to the original lesion and on the left side (Coordinates F + 3, L2, 6 (left), H-2), coagulation performed and the threshold again determined. Five µg. of adrenaline produced only right-sided EEG activation, but 15 µg. produced a bilateral response. Coagulation was repeated (at F + 7, L2, 6, (left), H 0), whereupon it was found that 15 µg. of adrenaline produced activation, but on the right side only, and that it was impossible to produce bilateral activation with any reasonable dose. An illustration of this unilateral EEG activation to adrenaline is given in Figure 8.

The above data, along with the results from other experiments in which lesions were made in the mesencephalic tegmentum in similar fashion or bilaterally, allow one to

conclude that the adrenaline sensitivity of such a preparation is roughly directly proportional to the amount of mesencephalic tegmentum left intact, and that by progressively destroying this region in a rostral direction, the threshold is progressively raised. Furthermore, if the lesion is made unilaterally, the threshold will be raised higher ipsilaterally than contralaterally, i.e., if the lesion is confined to the left side of the brain stem, it will require a higher dose of adrenaline to produce both left and right-sided cortical activation than right-sided activation alone. Lastly, if the bilateral lesions are advanced sufficiently rostrally, the response will disappear altogether; a comparable unilateral lesion will abolish the electrographic response to adrenaline of the ipsilateral cortex only, although it will raise the threshold of the contralateral cortical EEG response somewhat.

E. Effect of Anaesthetic Agents on Adrenaline EEG Activation

The intact cat often shows the EEG characteristic of arousal, with predominantly fast, low voltage activity, against which it is difficult to detect further EEG activation from adrenaline. It was hoped that a better background could be established by administering small amounts of an anaesthetic agent and changing the EEG pattern to that of "C" or even "D".

In practice it turned out that adrenaline EEG activation is itself extremely sensitive to anaesthetics - as was emphasized by Bonvallet, Dell, and Hiebel (1954) - and it disappears even before the anaesthetic agent has improved the background particularly. A number of different agents were tried but they all behaved the same way in this particular respect.

Doses of thiopental as small as 1 mg./kg. abolished adrenaline EEG activation for 30-60 minutes. Some of the earlier animals were prepared under thiopental anaesthesia and received a large total dose over the course of several hours. Such animals never showed EEG activation from adrenaline even long after the agent had apparently worn off and the EEG was that of alert wakefulness.

Pentobarbital had the same effect as thiopental. It required slightly larger doses but the effects lasted longer. In an animal with mid-reticular coagulation, 1 mg./kg. had no effect, 1.5 mg./kg. caused a diminution of the response, 2 mg./kg. almost abolished it, and 2.5 mg./kg. abolished it completely. As the barbiturate dose was increased, the adrenaline EEG activation was not delayed but became shorter and fainter until it was no longer distinguishable amongst the slow waves and spindles.

Adrenaline EEG activation was likewise abolished by 33 mg./kg. of intravenous alcohol (2cc. of a 5% solution; 3 kg. cat), and 8 mg./kg. of chloralosane. Furthermore the

response would not appear when the animal was still under the influence of ether. As had already been noted (Schneider, Woringer, Thomalske, and Brogly, 1952), when barbiturates are administered to a preparation showing EEG deactivation (in our cases, because of reticular coagulation) an increase in the spindles and slow waves may occur but the phase so characteristic of barbiturate induction, high voltage fast activity (18-25/sec., predominantly frontal), does not appear.

F. Effects of Other Sympathomimetic Amines

1. Noradrenaline

Early in the course of these experiments it was observed that noradrenaline had an EEG activating effect indistinguishable from that of adrenaline. Subsequently attempts were made to measure the adrenaline and noradrenaline threshold in the same preparation in order to compare the two substances quantitatively. Within the limits of error of the method (probably no greater than 50%) it is possible to state that equal weights of the two substances have approximately equal effects upon the EEG. Furthermore by using the two drugs alternately and gradually reducing the dose towards the threshold level the minimum effective dose capable of producing EEG activation was also found to be the same for the two drugs.

The effect upon blood pressure was of course not the same. The pressor effects of noradrenaline were greater in magnitude and duration than those of equal doses of adrenaline. While the minimum dose of adrenaline producing EEG activation sometimes produced no blood pressure change or a fall, the equivalent dose of noradrenaline always still produced a slight rise.

The effects of noradrenaline were comparable to those of adrenaline in the intact animal and the preparation with mid-reticular coagulation. Lesions which abolished the EEG response to adrenaline either unilaterally or bilaterally abolished the response to noradrenaline likewise. Since most of the experience with noradrenaline has been confined to animals with reticular coagulation and since quantitative estimation of the inhibitory or deactivating effect of adrenaline or noradrenaline is unsatisfactory, no comparison of this effect of the two drugs was attempted.

2. Phenylephrine (Neo-synephrine)

Since this drug is employed clinically principally for its vascular effects and is considered to be practically devoid of central nervous system stimulating activity, it was anticipated that it would lack EEG effects; this however was not the case. It was administered to animals with mid-reticular coagulation and produced an EEG activation indistinguishable qualitatively from that of adrenaline. Comparisons

of the vascular effects of equal amounts of the two drugs have shown that phenylephrine produces less intense effects than adrenaline; however, if equipressor doses of the two drugs are given, the effects of phenylephrine last longer (Goodman and Gilman, 1955). Consequently the EEG effects of the two drugs were compared two different ways.

The drugs were given alternately, and the dose manipulated until the two drugs gave EEG activation of approximately equal duration. Under these circumstances it required more phenylephrine than adrenaline, in a ratio of 5:1.

The minimum effective EEG activating dose for each drug was tested separately and again more phenylephrine than adrenaline, but in a ratio of 10:1. Thus it is seen that phenylephrine is uniformly less potent, weight for weight, than adrenaline, but that the ratio of potencies varies, depending on whether one employs duration or intensity as a basis for comparison; in this respect the EEG effects of the two drugs resemble their vascular effects.

3. Methamphetamine

Since methamphetamine is used clinically for its central stimulating effect and has relatively little influence upon the blood pressure, it was anticipated that it would have the most marked effects upon the EEG of all the sympathomimetic drugs; again, this was not the case. It was possible to distinguish three separate EEG effects of intravenous methamphetamine:

(a) It produced EEG activation similar in pattern and timing to that of epinephrine, but this required much larger doses. The smallest dose ever observed to elicit EEG activation was 2.5 µg./kg., but ordinarily higher doses (10-25 µg./kg.) were required. Furthermore the EEG response showed tachyphylaxis so that after the first dose, larger amounts were required to produce an equal response, and after three or four doses, no amount would produce any discernible effect. Consequently, it was impossible to measure the threshold.

(b) It lowered the threshold to adrenaline for long periods. In each of a series of experiments, the adrenaline threshold was carefully determined before methamphetamine. The protocol of RC35 will serve as an example:

> The threshold to adrenaline (lowest dose producing definite EEG activation) during the control period was 5 µg., or (2 µg./kg.). Immediately after 50 µg. of methamphetamine it fell to 2.0 µg. (0.8 µg./kg.); after an additional 100 µg. of methamphetamine, the threshold was found between 1.0 and 1.5 (0.4 and 0.6 µg./kg.); and after still another 100 µg. the threshold was less than 0.5 µg. (0.2 µg./kg.). The lowest threshold ever observed in any animal was 0.013 µg./kg. of adrenaline (after 180 µg. of methamphetamine). Between three and four hours after the last dose of methamphetamine, the threshold had returned to the control level.

(c) It produced long-lasting EEG activation in large doses. It was noticed that after rather high doses of methamphetamine had been administered to lower the adrenaline threshold, the background showed a gradual change, the spindles occurring at less frequent intervals. This limited the degree to which one could carry the lowering of adrenaline

threshold, since as the background of spindles and slow waves diminished the preparation became a less and less sensitive test object. With very large doses (0.5-1.0 mg./kg.), the spindles vanished altogether and the EEG record was indistinguishable from the normal waking cat (pattern "B"). Furthermore the cat actually appeared to wake up. Since the animals with mid-reticular coagulation are apparently unconscious, only very small doses of curarizing agent are needed to immobilize them, often none at all. However, when the EEG was reconverted to the normal waking pattern with methamphetamine, the animal began to move and had to be immobilized with Flaxedil. After administering a small dose of pentobarbital intravenously (12 mg./kg.), the spindles and slow waves returned.

No sudden rises in blood pressure, such as those that follow administration of adrenaline, were seen after methamphetamine. Rather there occurred a gradual, sustained increase in blood pressure over many minutes, but of low amplitude and difficult to measure. On the other hand, methamphetamine seemed to potentiate the blood pressure effects of adrenaline in the same fashion as it potentiated the EEG effects, and to roughly the same extent. Thus a dose of adrenaline too small to produce any effects before methamphetamine elicited both EEG and blood pressure effects after, and to much the same degree. The responses to a small dose

of adrenaline after methamphetamine were indistinguishable from those of a much larger dose given before.

4. Serotonin

No attempt was made to carry out a detailed investigation of the central effects of serotonin. However it was administered to several animals (with and without mid-reticular coagulation) in order to see whether its effects were in any way comparable to those of adrenaline. On a weight basis it required between 20 and 40 times as much serotonin (5-30 μg./kg.) to produce any EEG effect, but the comparison is inexact since the central effects of serotonin differ both qualitatively and quantitatively from those of adrenaline. The first effect noted was a period of EEG activation, often quite brief, followed by a period of deactivation sometimes resembling normal sleep or pattern "D", but often including a great deal of high voltage delta activity; when the dose was high enough, this was followed by a second period of activation, after which the record returned to its resting state. The observed blood pressure changes were only slight and included a brief rise followed by a longer fall.

5. Cocaine

Although not a sympathomimetic amine, cocaine is known to sensitize receptor sites to the effects of adrenaline applied locally or intravenously, or to the effects of adre-

nergic nerve stimulation. Furthermore, it has notorious central nervous system stimulating properties. Given intravenously to preparations with mid-reticular coagulation, it produced no immediate changes in the EEG record in doses ranging from 7-100 μg./kg., and thus differed from all the sympathomimetic amines tested in having no direct EEG activating effect. However, it had the same effect as methamphetamine in lowering the adrenaline threshold.

Thus in one experiment (RC39), the control adrenaline threshold was 2 μg. (.66 μg./kg.); it was unaffected by 100 μg. of cocaine, but after a total of 200 μg. of cocaine it had dropped to 1 μg. (.33 μg./kg.), after a total of 700 μg. it fell to 0.5 μg. (0.165 μg./kg.), and after a total of 1.2 mg. (0.4 mg./kg.), it had fallen to 0.25 μg. (0.085 μg./kg.) of adrenaline. Further doses of cocaine failed to lower the threshold but produced the same effect as large doses of methamphetamine, i.e., gradual diminution in the frequency of spindles and replacement with the EEG pattern of alert wakefulness.

The blood pressure effects of cocaine also resembled those of methamphetamine since the drug produced only a sustained rise of low magnitude, but sensitized the animal to the blood pressure effects of adrenaline in a fashion parallel to the EEG effects.

G. Effects of Adrenergic Blocking Agents

1. Chlorpromazine

Since chlorpromazine is a potent adrenergic blocking agent and at the same time has a prominent depressing action of a rather special sort upon the central nervous system, a

study was made of its direct effects upon the cat EEG and upon the adrenaline activation pattern and threshold. The drug was given in doses ranging from 1-4 mg./kg., either intravenously, intraperitoneally, or intramuscularly.

a. In the Intact Cat

The effects of chlorpromazine upon the EEG of the intact, curarized cat are somewhat variable, depending upon the dose and mode of administration. Small intramuscular doses often produced no discernible effect, especially if the head was mounted in the stereotaxic instrument, when EEG patterns "A" or "B" predominated. Larger intramuscular doses, intraperitoneal, or repeated intravenous doses produced a shift in the dominant EEG pattern from "A" or "B" to "C". This came about rapidly after intravenous administration of from 2-4 mg./kg., and appeared gradually over 2-3 hours after intramuscular injection of 1-2 mg./kg. In the latter instance, the change to the EEG pattern characteristic of "drowziness" was probably induced rather than spontaneous because the pattern always reverted back to that of "arousal" once the effects of the drug had worn off (4-5 hrs.).

During the period of EEG synchrony, it was always possible to produce EEG activation with sciatic stimulation or even light touch or sound, although the arousal period was shorter than during the control period. All of the EEG patterns observed in the animal under the influence of chlor-

promazine resembled those observed in the intact "awake" or "drowsy" animal (pattern "B" or "C"); abnormal rhythms or wave forms were never encountered except immediately after a large intravenous dose, when slow waves appeared following the precipitous fall in blood pressure. In summary, chlorpromazine produced mild sedation as evidenced by a shift from the EEG patterns of "arousal" ("A" or "B") to that of drowsiness ("C"). It produced no abnormal activity nor did it interfere with EEG activation from sensory stimuli. The intensity of the effects were dependent upon dosage and route of administration, and amounts too small to produce any noticeable changes in the EEG still had definite effects when measured by a different criterion (adrenaline activation pattern).

Since determinations of adrenaline threshold are inaccurate in the intact animal, the technique of gradually lowering the adrenaline dose to threshold was discarded in favor of administering the same dose of adrenaline both before and after chlorpromazine and noting any alteration in the response. This was first done after intravenous chlorpromazine. An abstract from the protocol of RC10 will serve as an example:

During the control period, 20 µg. of adrenaline (6 µg./kg.) produced EEG activation within 9 seconds, and a substantial blood pressure rise within 10 seconds. After 2 mg./kg. of chlorpromazine intravenously (causing a permanent fall in

B.P.), the same amount of adrenaline produced a distinct EEG activation 27 seconds after the injection, and the blood pressure rose only after 35 seconds. Sciatic stimulation produced immediate EEG activation and a marked rise in blood pressure. The dose of chlorpromazine was repeated (causing a further drop in blood pressure). Administration of adrenaline produced the same results, and sciatic stimulation was only slightly less effective.

Since both the EEG and blood pressure responses to adrenaline persisted in clear-cut fashion but appeared only after 3 times the normal latent period, it was first assumed that chlorpromazine somehow exerted a delaying action on both these effects. An increase in circulation time could account for such a delay and was a likely consequence of the marked fall in blood pressure caused by the drug. This hypothesis was tested in the following way (RC11):

The effects of adrenaline (7 µg./kg.) were determined during the control period. The rise in blood pressure occurred regularly within 7 seconds and EEG activation followed several seconds later, although it was difficult to time it since the background EEG was rapid (pattern "B"). Then the spinal cord was sectioned at C1-C2 causing a precipitous drop in blood pressure. The response to adrenaline was re-tested and the blood pressure rise this time occurred at 10 seconds - three seconds later than during the control period, the delay presumably being due to a slowing of the circulation since the animal remained in shock. Then 1.5 mg./kg. of chlorpromazine was given intravenously; there was no further fall in blood pressure. Testing again with adrenaline revealed that the rise in blood pressure had been considerably delayed, reaching its peak gradually by 36 seconds after injection, with EEG arousal beginning only slightly before.

The above experiment shows that the delay in the pressor effect of adrenaline attributable to slowed circulation time (produced by a total sympathetic paralysis after acute cord transection) was actually of a small order (the difference

between 7 and 10 seconds), while the delay exerted by chlorpromazine, even when it appeared to have no further effect on the blood pressure, was considerable (the difference between 10 and 36 seconds). It was concluded that the apparent delay caused by chlorpromazine was probably not due, except in small part, to any increase in circulation time. To avoid the deleterious effect of intravenous chlorpromazine upon blood pressure, the drug was subsequently administered intramuscularly or intraperitoneally, and in smaller doses.

In this series of experiments, once the control responses were established chlorpromazine was administered (usually 1 mg./kg., i.m.) and the responses to the same dose of adrenaline were observed at 10-15 minute intervals throughout the period of action of chlorpromazine (4-6 hours). Administering chlorpromazine in this fashion provided a stable preparation with normal blood pressure. The tissue content of chlorpromazine (as judged by its effects) rose and fell slowly over a long period of time, enabling one to examine the effects of different levels of the drug upon adrenaline EEG activation repeatedly. Excerpts from the protocol of RC27 are presented in tabular form below:

Time Interval after Chlorpromazine Administration (in minutes)	Effects of Adrenaline Injection		Effects of Sciatic Stimulation	
	EEG Activation (in seconds)	B.P. Change (in seconds)	EEG Activation (in seconds)	B.P. Change (in seconds)
control	9-?	rise 7-60	0-80	rise 2.5-60
25	10-50	fall at 17 rise 20-55	0-30	rise 2.5-60
70	17-36	fall at 17 rise 20-55	0-30	rise 2.5-60 (diminished rise)
130	20-47	fall at 16 rise 26-60	0-30	"
150	no effect	fall at 17	0-30	"
195	phase 1:15-25 " 2:72-118	slight rise at 8 slight fall at 18 marked rise 24-68 marked rise 90-125	phase 1:0-47 phase 2:62-93	rise 1:2-56 rise 2:71-110
240	phase 1: - 2: 25-90 3:115-140 4:162-184	rise 1: 9-20 2: 31-100 3:121-145 4:168-190	4 separate phases of EEG activation and B.P. rise, with the B.P. peaks occurring at 65 sec. intervals. EEG activation occurred with the onset of the stimulus and a fresh burst of activation preceded each B.P. rise by 6-8 seconds	
360	10-42	8-50	0-60	2.5-47

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Although at the height of action of chlorpromazine, inversion of the adrenaline pressor response and absence of EEG activation were not surprising, the patterns of response before and after this peak was reached were characterized by a bewildering series of delayed and multiple responses, both pressor and EEG activation. Interpretation of these phenomena is still difficult. However after examining this and similar protocols certain features common to all have become apparent. The following is an attempt to summarize and schematize our results with this particular preparation.

Stage I (first 15-20 minutes after intramuscular injection of chlorpromazine)

There is already some degree of peripheral adrenergic blockade; the pressor response to adrenaline is diminished, absent or inverted, depending on the exact degree of block.

The adrenaline EEG activation pattern is as yet unaltered. Under these circumstances, one may see EEG activation in the absence of any blood pressure rise or, as illustrated in the above protocol, the blood pressure rise may occur late, after the first fall, and well after EEG activation has begun. As will be explained later, it is not believed that this late rise in blood pressure is due to any peripheral vascular effect of adrenaline.

Stage II (occurs some time within the first 60-90 minutes)

Chlorpromazine continues to produce a peripheral ad-

renergic blockade and the initial blood pressure response (onset usually 18-20 seconds after injection) is always a fall, although whether or not it will be succeeded by a rise will be shown to depend upon the EEG response.

EEG activation from adrenaline begins to occur later and later although the duration of the response is often not much affected. The latest that a clear-cut EEG activation followed adrenaline was 96 seconds - a much greater delay in onset than was ever observed merely by lowering the adrenaline dose towards threshold.

Stage III (between the second and third hour)

The peripheral vascular effects of chlorpromazine remain the same or the degree of blockade lessens somewhat so that there is often a small pressor response at the usual time (7-8 seconds) followed by a fall a little later (17-20 seconds).

The central effects are maximal. In some animals the EEG activation from adrenaline is abolished altogether. In others it is only delayed. Sometimes, as the adrenaline activation response passes through the period of maximum delay, a very attenuated activation appears again at the normal time (9-11 seconds); then, as time passes, the early response becomes more prominent and the late one vanishes.

Stage IV (2½-4 hours)

The peripheral vascular effects gradually wear off,

so that the initial pressor phase of adrenaline becomes increasingly prominent while the subsequent depressor phase becomes less so.

The central blocking effect upon adrenaline EEG activation lessens very slowly and the delay or latent period before activation shortens and returns towards normal.

It is during this stage that the period of multiple phases of EEG activation and blood pressure rise have invariably been seen, although they occasionally appear briefly in stage II also. After an injection of adrenaline, there is first seen a small blood pressure rise (at 7-9 seconds); sometime afterwards EEG activation appears (usually from 20-60 seconds after the injection, depending on dose and time elapsed). Shortly after EEG activation (6-8 seconds later) a second blood pressure rise appears which is much greater and more sustained than the first, and may be more prominent than the pressor response to adrenaline during the control period (Fig. 9). In the case of multiple phases, the EEG activation gradually dies down only to recur again rather abruptly usually 40-60 seconds after the onset of the first period of activation. It, too, is followed in 6-8 seconds by another pressor response. Phases of EEG activation followed shortly by pressor responses may continue to recur repeatedly. The highest number observed in succession was 6.

Very much the same sort of phasic pattern follows sciatic stimulation. There is a period of EEG activation immediately following the noxious stimulus and a pressor response at the usual time (2.5 seconds; the pressor response is usually less than normal but never completely abolished or reversed - illustrating the fact that a given dose of adrenergic blocking agent produces a higher degree of block to injected adrenaline than to discharge of the sympathetic nervous system). Then at varying intervals a second period of EEG activation appears; it too is followed in 6-8 seconds by a second period of increased blood pressure. During stage IV, sciatic stimulation uniformly produces multiphasic EEG and pressor responses; it is more consistent in this respect than intravenous adrenaline, and the number of phases is apt to be greater. At any given time, the interval between EEG and pressor phases, whether produced by sciatic stimulation or intravenous adrenaline, is about the same and is roughly equal to the latent period between the injection of adrenaline and the first marked EEG activation.

Stage V (4-6 hours)

During this period the animal gradually returns to its control state. The multiphasic responses disappear, and the latent period between adrenaline injection and EEG activation approaches normal.

b. In the Adrenalectomized Animal

Two totally adrenalectomized but otherwise intact animals were studied in the same fashion following intramuscular chlorpromazine (1 mg./kg.) after we had begun to suspect that the animal's own adrenals might be participating in the aforementioned multiphasic responses.

Stages I, II, and III followed each other in regular succession. During stage IV, multiphasic EEG and pressor responses from sciatic stimulation were never observed; only a single phase of each occurred. After intravenous adrenaline, EEG activation appeared either once late (more than 20 seconds after injection), or twice, the first occurring at the normal time (9 seconds), the second considerably later (the latest observed was 96 seconds after injection). The initial pressor response showed its usual diminution due to chlorpromazine; a second and greater pressor response sometimes occurred following the delayed EEG activation period. A third EEG or pressor phase was never seen.

c. In the Animal with Mid-reticular Coagulation

It was previously mentioned that it was difficult to time or measure EEG activation in the intact animal because of the relatively rapid asynchronous background. Although this was more easily accomplished after the animal had

received chlorpromazine, since this drug tended to induce EEG slowing, the preparation with artificial EEG deactivation from mid-reticular coagulation was employed as well. This permitted more accurate timing of events and at the same time revealed certain important differences from the intact animal.

The following is extracted from the protocol of RC15. Before coagulation it was difficult to determine adrenaline EEG activation because of the fast background activity. Coagulation was carried out, with the installation of constant spindles and slow waves.

Time after Chlorpromazine Administration	EEG Activation Left Hemisphere	Rt. Hemisphere
control	11-34 sec.	11-34 sec.
20 minutes	47-77 "	40-100 "
40 "	19-25;91-103 "	17-26; 90-103 sec.
70 "	80-90 "	11-28; 70-103 "
120 "	no effect	no effect
180 "	40-90 "	40-100 sec.
240 "	15-59 "	10-60 "

The pressor response behaved in parallel fashion, gradually lessening, then returning. The EEG activation behaves in the same fashion here as in the intact animal, i.e., as time passes the response appears later and later after injection and sometimes splits into a very brief activation occurring early and a more prominent one occurring very much later. The brain stem coagulation was not quite symmetrical; the right hemisphere showed less frequent spontaneous spindles and underwent EEG activation for longer periods after sciatic stimulation. This is reflected in the

slight quantitative difference in the adrenaline EEG activation response after chlorpromazine.

In certain respects, however, the preparation with brain stem coagulation behaved differently from the intact or adrenalectomized animal. Multiphasic pressor responses were never seen, nor were more than two phases of EEG activation. Unlike the response in the adrenalectomized cat, the second EEG activation was never followed by a pressor response. In addition, this cat showed no pressor response to sciatic stimulation.

d. The Multiphasic EEG and Pressor Responses

In order to arrive at some understanding of the repeated bouts of EEG activation and increase in blood pressure seen in stage IV of chlorpromazine action, a list has been drawn up of the observed properties of this multiphasic response under a variety of circumstances.

1. The multiphasic response is seen when the effects of chlorpromazine are intermediate in magnitude, not at the peak of action.
2. In the intact animal, multiphasic responses occur after both adrenaline administration and sciatic stimulation, but the latter is usually more effective.
3. After adrenaline injection, the initial pressor response occurs at the usual time (7-8 seconds), but is diminished and sometimes reversed. The initial EEG

activation (at 9-15 seconds) is either attenuated or absent. The succeeding EEG activation (often the only one) is late (20-90 seconds), often quite intense, and is followed in 6-8 seconds by the second blood pressure response, which is much more marked than the first and always purely pressor. Succeeding phases, if present, always begin with EEG activation, then the pressor response, and the interval between the phases is roughly equal to the interval between the injection of adrenaline and the first major EEG activation.

4. Multiphasic EEG or pressor responses to adrenaline or sciatic stimulation are not seen in the animal with mid-reticular coagulation; this same preparation shows EEG activation in response to sciatic stimulation, but no pressor effect.
5. Multiphasic responses to sciatic stimulation are not seen in the adrenalectomized animal, but after adrenaline, a second pressor response (a high rise) occurs after the delayed EEG activation. Successive EEG or pressor responses are not seen however.
6. During stage IV, the multiphasic responses to sciatic stimulation and adrenaline administration can be temporarily abolished by giving small amounts of thiopental. Doses too small (1 mg./kg.) to produce any

noticeable effect upon the EEG but capable of completely abolishing adrenaline EEG activation eliminated the multiphasic response after both adrenaline and sciatic stimulation for the duration of action of the anaesthetic drug. When the EEG activation response to adrenaline reappeared (1 hour later), the multiphasic responses to sciatic stimulation and adrenaline reappeared also.

2. Dibenzyline

Dibenzyline was selected as an example of a potent adrenergic blocking agent which lacks the prominent central effects of drugs like chlorpromazine. Experience with it has been confined to experiments in the intact animal. Under these circumstances it may be stated that doses of Dibenzyline (1 mg./kg., i.v.) sufficient to cause reversal of the adrenaline pressor response do not affect the EEG activation in any noticeable way. In this respect it resembles the stage of chlorpromazine shortly after intramuscular administration, when the pressor response is reversed but the nervous system is still apparently unaffected.

IV. DISCUSSION

A. EEG Activation by Adrenaline; Site and Manner of Action

EEG activation is not only a concomitant of behavioral 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 (Moruzzi 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 for adrenaline action. If such should prove to be the case, it would be important to distinguish an action of the drug directly upon this neural substrate from any indirect effect mediated through one or more of the many sensory pathways that are known to play upon it. By progressively sectioning the brain stem in a rostral direction, Bonvallet, Dell, and Hiebel (1954) were able to show that adrenaline EEG activation persists until intercollicular section is performed, proving that the medulla and pons were not indispensible for this response, but that some structure at the collicular level was.

We attempted to localize this responsive area as accurately as possible by making restricted lesions in the midbrain and diencephalon, or by injecting very small

quantities of adrenaline directly into this same structure (the results of these investigations are to be reported in detail subsequently). Coagulation of the tegmentum at the ponto-mesencephalic junction did not abolish the response to adrenaline; it lowered the threshold if anything. Other animals with similar or larger lesions, whose adrenaline threshold was not necessarily low, nonetheless showed an unusually intense activation from adrenaline (pattern "A"). This seems to correspond to the findings of Bonvallet, Dell, and Hiebel (1954) who noted a particularly marked adrenaline EEG activation after pre-bulbar brain stem section.

Sufficient destruction of the mesencephalic tegmentum in a rostral direction was capable of abolishing the EEG response to adrenaline altogether, showing that the integrity of this region is necessary for the adrenaline action, and demonstrating that adrenaline acts upon it, or at least through it. Partial destruction of this region simply raises the threshold to adrenaline in a manner rather proportional to the extent of the lesion, suggesting that each portion of this region makes a contribution to the total response. Unilateral coagulation of the mesencephalic tegmentum is capable of abolishing the response to adrenaline strictly ipsilaterally, the other hemisphere serving as an excellent control. The system is apparently uncrossed between the mid-brain and cortex.

The above evidence shows that the mechanism of adrenaline EEG activation is not to be sought in any diffuse cortical effect, either upon the blood vessels or the nervous elements, but rather is dependent upon a fairly circumscribed portion of the brain stem. The ways in which adrenaline might affect this region will be considered.

Electrographic activation by adrenaline is not the consequence of a rise in blood pressure. The EEG changes sometimes precede by several seconds the pressor response; activation often disappears during the phase of rapid blood pressure increase and returns as the pressure is gradually falling. When using very small doses of adrenaline or when Dibenzyline has produced some degree of peripheral adrenergic blockade, EEG activation may be accompanied by no discernible alteration in blood pressure or a fall rather than a rise. When the animal is under the influence of chlorpromazine, adrenaline EEG activation may be delayed long after the blood pressure has returned to normal.

The EEG response to adrenaline is unlikely to be due to effects upon cerebral blood vessels or blood flow. Adrenaline and noradrenaline generally have the opposite effect upon blood vessels, the former being predominantly vasodilator, the latter vasoconstrictor (Goldenberg et al., 1948); at those sites where their actions are similar (coronary vasodilatation, and skin vasoconstriction), their

effects are not quantitatively the same. Adrenaline produces an increase in total cerebral blood flow, whereas noradrenaline causes a decrease (King, Sokoloff, and Wechsler, 1952). Despite all these important differences, these two agents have, on a weight basis, indistinguishably similar effects upon the EEG. Moreover, a drug which blocks the excitatory effects of adrenaline upon blood vessels (Dibenzyline) does not alter EEG activation, while very small doses of barbiturates, which have no perceptible effect upon the vascular responses, block EEG activation altogether. Although a restricted local vascular action cannot be ruled out until it is possible to study the effects of all these drugs specifically upon brain stem blood flow, such a mechanism does not at present account satisfactorily for the central effects of adrenaline.

The stimulating effect which adrenaline has upon the metabolism of tissues in general and upon cerebral oxygen consumption in particular also fails to account for the EEG activation, for while noradrenaline is just as effective as adrenaline in producing EEG activation, it is much less active metabolically (Reale et al., 1950; King et al., 1952).

It can be argued that adrenaline might produce some peripheral sensation which, firing into the reticular formation, would in turn arouse the animal. However, the pre-

paration with mid-reticular coagulation is unresponsive to ordinary stimuli such as light, touch, or sound, and much less sensitive to pain than the intact cat. Nevertheless, such preparations are often more sensitive than the intact cat to the EEG effects of adrenaline. Moreover, Bonvallet, Dell and Hiebel (1954) have demonstrated EEG activation from adrenaline in the cerveau isolé preparation.

By its action, adrenaline sets in motion a number of reflex readjustments, principal among which is stimulation of the carotid and aortic pressoreceptors. However the effect upon the EEG of stimulating these receptors has been shown to be inhibitory, i.e., deactivation (Bonvallet, Dell, and Hiebel, 1954), thus scarcely accounting for adrenaline's predominantly activating effect.

There is every reason to believe then that adrenaline exerts its effects directly upon the neural elements themselves, rather than indirectly through vascular, reflex, or sensory mechanisms. Furthermore, the only neurones which appear indispensable are those located primarily in the mesencephalic tegmentum, a region where electrical stimulation will produce precisely the same effect, EEG activation. All the evidence suggests that noradrenaline acts in the same manner and is about equally effective.

B. Participation of the Adrenal Glands; Delayed Arousal

Bonvallet, Dell, and Hiebel (1954) have shown quite clearly that EEG activation may result not only from injections of adrenaline but also from endogenous adrenaline released by splanchnic nerve stimulation. Furthermore they have shown a late phase of EEG activation which appears following sciatic stimulation when the immediate activation has been abolished by pre-bulbar section; it has about the same delay (10-15 seconds) as that following adrenaline injection or splanchnic stimulation. Since sciatic stimulation is known to produce discharge of adrenal medullary hormones (principally adrenaline rather than noradrenaline - von Euler and Folkow, 1953) it is likely that this late phase is due to endogenous adrenaline release. A similar pattern has been seen after brain stem coagulation (Fig. 3) when the immediate response to sciatic stimulation has been sufficiently reduced to allow the late phase to stand out clearly (compare with Fig. 4). It is not seen more often after mid-reticular coagulation most probably because the lesion frequently involves the rostral pons where the adrenaline-release reflex center has been localized by Cannon and Rapport (1921); thus after mid-reticular coagulation both the pressor and adrenaline-release reflex responses to sciatic stimulation are often absent.

An attempt was made to assess the contribution of the adrenals to the EEG response which follows sciatic stimulation by studying the same response in adrenalectomized animals (two intact and one with mid-reticular coagulation). After sciatic stimulation EEG activation occurred promptly and lasted as long as it did in their non-adrenalectomized counterparts (0-60 seconds); EEG activation to injected adrenaline occurred as in the non-adrenalectomized animals (10-40 seconds). It is apparent that the purely neural phase of EEG activation completely overlaps and may outlast the "humoral" phase due to adrenaline. This accounts for the fact that two distinct phases of EEG activation or increase in blood pressure are so rarely seen in the intact animal. The contribution of the adrenal glands to EEG activation after a brief noxious stimulus is small and ordinarily obscured by the neural phase, but it does appear under certain experimental conditions in which case it is important to recognize it. There is nothing to suggest from observations of adrenalectomized animals or man that the adrenal medulla plays any role in maintaining alert wakefulness.

It must be mentioned however that not all delayed EEG activation is necessarily due to a humoral mechanism involving the blood stream or adrenal glands. A 2-3 second delay in EEG activation after a noxious stimulus was observed

by Lindsley et al. (1950), in chronic cats with lesions of the direct sensory pathways. A delay as long as 30 seconds between a very mild tactile stimulus and EEG activation has been observed repeatedly in a cat with peripherally placed mesencephalic lesions (Sharpless and Rothballer, 1955) and in intact monkeys (Morrell, 1955). A similar sort of delay is observed to follow gentle rousing of someone deeply asleep.

The adrenal glands were not responsible for the delayed response because in the first instance the interval was too short (less than the minimum latent period ever observed between injection of adrenaline directly into the inferior vena cava and subsequent EEG activation); in the second instance, the delayed response persisted after bilateral adrenalectomy; in the last instance, the stimuli were mild and quite unlike those strong enough to induce sympatho-adrenal discharge. It should be recognized then that EEG activation may appear after an appreciable latent period following the initiating stimulus. This is most likely to appear when the stimulus is very mild or after peripherally-placed mesencephalic lesions (involving direct sensory pathways), and it is unrelated to adrenal medullary discharge.

The reverse situation is seen after centrally-placed mesencephalic lesions, in which case the EEG activation fol-

lowing a noxious stimulus is immediate but does not outlast the stimulus. This has been observed by Lindsley et al. (1950) in chronic preparations, and we have made the same observation in some of our animals with mid-reticular coagulation. Taken together, the evidence suggests that peripherally-placed mesencephalic pathways mediate a very prompt EEG activation in response to noxious stimuli which is poorly sustained when working alone. On the other hand, centrally-located mesencephalic structures mediate a sustained EEG activating effect which is apt to show a long latent period when acting alone or when the stimulus is very mild.

C. The Adrenergic Component of the Reticular Activating System

It might be asked why any circumscribed portion of the brain should show such marked and specific sensitivity to adrenaline and noradrenaline. Of probable pertinence to the question is the observation that this same region contains both these substances (7% adrenaline and 93% noradrenaline in the cat); the content of these substances is labile and falls after noxious stimuli such as anoxia or ether anaesthesia, suggesting that they participate actively in the function of these regions (Vogt, 1954).

All of the above mentioned facts plus additional observations that will be mentioned in the discussion of sensitizing and blocking agents have suggested the possibility

that at least some of the reticular activating system is actually adrenergic, i.e., that adrenergic substances (probably principally noradrenaline) participate in synaptic transmission. Since there are reasons to believe that the reticular activating system is not entirely homogenous functionally or neurohumorally, such a system will be referred to tentatively as the adrenergic component of the reticular activating system. A similar proposal has been made by Dell, Bonvallet, and Hugelin (1954) on the basis of observations on the adrenaline-sensitivity of the descending bulbar fascilitatory system. Up to the present time, studies of snyapses have been made on those which are presumably cholinergic. Compared to acetyl choline, however, adrenergic transmitter substances occurring peripherally (principally noradrenaline) are destroyed much more slowly; excitation of the peripheral sympathetic system produces effects which are generalized, sustained and which fluctuate comparatively slowly, over seconds or minutes, rather than milliseconds.

Such properties as these are not dissimilar to those just attributed to the centrally-placed EEG activating mechanisms - those which produced EEG activation of a sustained sort well outlasting the stimulus, but which as mentioned may show a certain amount of delay. It is suggested

then that the mechanism subserving sustained EEG activation, and its behavioral counterpart, consciousness or wakefulness, is at least partly the adrenergic component of the reticular activating system. The properties of such a system may account for some of the characteristics of the conscious state, including its prolonged, tonic nature, the slowness with which changes in it normally take place and possibly the long latent period between a gentle stimulus and complete arousal from deep sleep.

D. Adrenaline EEG Deactivation

It has been stressed that although EEG activation is the most prominent effect of adrenaline, it also has the opposite effect, deactivation. The first phase of deactivation usually occurs just as the blood pressure starts to rise and is rather brief. The second phase comes later as the blood pressure is falling or has returned to normal, when phases of activation and deactivation sometimes alternate with one another. Although only activation from adrenaline was mentioned by Bonvallet, Dell, and Hiebel (1954), their record of adrenaline EEG action (Fig. 8, page 127) also shows this early deactivation phase quite clearly. Thus there is reason to conclude that the duration of EEG activation does not correspond simply with the presence of adrenaline in the brain but is the resultant of two simul-

taneous and opposing actions, activation and deactivation. At the peak of action of the drug, activation usually predominates, but when levels are rising or falling the phases alternate or deactivation predominates. After preparation of the animal with thiopental, the activation phase is permanently blocked, but the deactivation phase often appears alone.

The deactivation phase may be at least partly due to stimulation of the pressor receptors by the blood pressure rise and consequent inhibition via the bulb (Bonvallet, Dell, and Hiebel, 1954). This may explain why the animal with mid-reticular coagulation or pre-bulbar section is so unusually sensitive to adrenaline, or responds at times with such an intense activation, but it does not account for the late phase when the blood pressure has often fallen to normal. Adrenaline does seem to have intrinsic inhibitory properties, however. Electrical changes interpreted as showing inhibition of cortical synapses have been reported after intracarotid adrenaline by Marazzi (1950). Intravenous adrenaline tends to inhibit the release of vasopressin by the supraoptico-hypophysial system in response to noxious stimuli (O'Connor and Verney, 1945), while acetyl choline stimulates this same system (Pickford, 1947). Intraventricular injection of adrenaline or noradrenaline in cats produces a state resembling light anaesthesia (Feldberg and Sherwood, 1954). Lastly,

it is a common pharmacological observation that a drug may stimulate or depress depending on concentration. It seems clear then that there are both excitatory and inhibitory elements to the central effects of adrenaline (much as there is to its peripheral effects) and that in the final analysis its influence upon the EEG is the resultant of these two opposing actions.

E. Action of Sympathomimetic Amines and Related Compounds; Correlation with Clinical Properties

Examination of the sympathomimetic amines shows that their actions fall into two general categories. (a) To a certain extent, they duplicate the EEG activation of adrenaline and in this sense they are adrenaline analogues. (b) Some however seem to increase the effectiveness of adrenaline itself, in which case they act as adrenaline sensitizers.

Those acting as analogues are noradrenaline and phenylephrine. Compared to adrenaline noradrenaline is equally effective; phenylephrine is less so but its central effects resemble its peripheral effects in that intensity suffers by comparison more than duration of action. Neither of these drugs changed the threshold to adrenaline or to themselves. Clinically, adrenaline tends to produce anxiety and irritability; such effects are produced by noradrenaline to a much lesser extent and by phemylephrine hardly at all.

To produce EEG activation experimentally requires the intravenous injection of amounts of adrenaline which ordinarily have marked pressor effects - something not usually done clinically. Furthermore, irritability or excitement is not necessarily the psychological counterpart of arousal or even an excess of it. Since the EEG activating effect and the production of irritability do not run parallel the two actions are apparently not directly related, and it must be concluded that that capacity of adrenaline to produce irritability or anxiety represents a separate action from its EEG activating effect, one possessed to a much lesser extent by noradrenaline and phenylephrine. That adrenaline and noradrenaline should be different in this regard is not surprising when one recalls that they are called forth from the adrenal medulla under quite different circumstances (von Euler and Folkow, 1953), and that they even seem to have a separate central representation (Folkow and von Euler, 1954).

On the other hand the two drugs which have very conspicuous central stimulating effects clinically - cocaine and methamphetamine - could scarcely have accomplished this through their adrenaline-analogue activity, since methamphetamine is very feeble in this respect and rapidly exhibits

tachyphylaxis, while cocaine lacks such properties altogether. The key to their action seems to lie in their ability to sensitize the organism to adrenaline or noradrenaline, an effect apparent both peripherally and centrally. They lower the adrenaline threshold, and in large enough doses produce a sustained EEG activation in all respects similar to that of normal wakefulness. It may be asked whence the adrenaline comes to which the body is sensitized by these drugs when they produce EEG activation acting alone. For reasons already mentioned, the organisms' own adrenals are an unlikely source. However, an adrenergic component within the reticular activating system, in which variable amounts of adrenergic substances are constantly being produced, might well be the source of such agents. If one were to accept this mechanism of action for methamphetamine and cocaine, it would be further evidence in favor of actual adrenergic transmission within the brain stem, especially since in the absense of adrenergic nerves or adrenaline, cocaine lacks sympathomimetic effects altogether.

In summary, adrenaline and its analogues do not produce sustained arousal clinically, but produce anxiety and irritability to differing degrees. This is looked upon as an effect unrelated to EEG activation. On the other hand, cocaine and methamphetamine, adrenergic sensitizers, produce a clinical state of prolonged wakefulness, buoyancy, and

freedom from fatigue which is more likely the true behavioral counterpart of EEG activation. Thus it is seen that EEG activation and behavioral arousal are attained clinically not by trying to supplement adrenaline systemically (which results in a variety of unpleasant circulatory and central side-effects) but by sensitizing the brain to the adrenergic substances which it itself produces, in which case the effects are confined largely to a single set of systems within the central nervous system.

Serotonin is found within the nervous system in the same regions where adrenaline and noradrenaline are encountered (Amin, Crawford, and Gaddum, 1954). It was administered to intact cats and those with mid-reticular coagulation to see whether it showed the similarity shown by adrenaline and noradrenaline in their EEG effects. Although serotonin did affect the EEG, there were important differences both quantitatively and qualitatively between it and adrenaline. It required larger doses (comparing adrenaline HCl and serotonin creatinine sulfate on a weight basis) to produce any change in the EEG, and the change was different from that produced by adrenaline; in fact the responses were something like mirror images of one another: serotonin produced a brief EEG activation, then a longer predominating period of deactivation, sometimes with abnormal slow waves, then followed, after high doses, by a second brief period of activation.

F. Action of Anaesthetics

As might be expected, anaesthetic agents tend to lessen, then abolish, the EEG activating effect of adrenaline just as they do the effects of natural sensory stimulation. Bonvallet, Dell, and Hiebel (1954) have already remarked on the unusual sensitivity to anaesthetics of the adrenaline EEG response. A variety of agents were similar in this respect (ether, thiopental, pentobarbital, chloralosane, alcohol). As the amount of anaesthetic agent was increased, the activation response to adrenaline gradually became shorter, then vanished. It did so after amounts of these agents which themselves often had no particular influence upon the EEG. When the anaesthetic agent did affect the EEG, this wore off long before the response to adrenaline returned. Animals prepared under thiopental which had been allowed to recover completely showed a perfectly typical waking EEG yet were completely unresponsive to adrenaline.

The dose of barbiturate which abolished the EEG response to intravenous adrenaline appears to be in the sedative, rather than in the hypnotic or anaesthetic range, and this may bear some relation to the calming effect so useful clinically obtained from small doses of barbiturates (particularly phenobarbital). It should be remembered that even though the response to intravenous adrenaline is completely abolished, this does not indicate that the adrenergic

component itself is completely "blocked". Intravenous administration of adrenaline is probably an inefficient and unphysiological way of stimulating this system, and it is not surprising that it should prove so vulnerable.

G. Chlorpromazine

The effect of chlorpromazine itself upon the human EEG has already been the subject of considerable study (Benassi and Cenacchi, 1953; Monroe et al., 1955; Turner and Berard, 1954; Shagass, 1955; Terzian, 1952). In general the drug produces no change or produces what in the human EEG would probably be the counterpart of "deactivation" - a disappearance of the "arousal" pattern, with increase in alpha and theta rhythms, occasional slow waves and even a normal sleep record. Behaviorally the patients were characterized as being tranquil, indifferent, drowsy, or apathetic. With larger doses they fell into a sleep from which they were easily aroused.

We have made comparable observations on the cat, in which deactivation and shift to pattern "C" is the usual effect. The animals were always responsive to noxious stimuli, but EEG activation to milder stimuli was less prominent and lasted a shorter time than normally. With comparable doses some cats were apparently unaffected; this was especially true of those mounted in the stereotaxic

apparatus. Hiebel, Bonvallet, and Dell (1954) noted the appearance of irregular high voltage slow waves (without spindles) in the cat after 0.5-1.0 mg./kg. of chlorpromazine. We never observed such an effect except immediately after intravenous doses of chlorpromazine large enough to bring about a marked fall in blood pressure. In summary, chlorpromazine in doses of 0.25-2.0 mg./kg. in both man and the cat produces a gradual deactivation of the EEG resembling in all respects spontaneous relaxation and drowsiness. In the absence of hypotension, abnormal rhythms are not seen. Responsiveness to mild arousing stimuli is diminished but EEG activation still takes place readily. The EEG and behavioral changes parallel each other closely.

Chlorpromazine has a rather definite influence upon adrenaline EEG activation and differs in a rather interesting way from the barbiturates. With very low doses or early during its period of action, chlorpromazine may exert only peripheral adrenergic blockade. Intermediate dose levels have the curious effect of delaying the EEG activation long after its customary time of appearance, an action shown not to be due to circulatory changes alone. Still higher doses abolish EEG activation to adrenaline altogether, an observation in agreement with that of Hiebel, Bonvallet, and Dell (1954).

Chlorpromazine may delay EEG activation much more than does simply reducing the dose of adrenaline towards threshold; thus chlorpromazine does not act merely by raising the threshold to adrenaline. Furthermore once the delayed EEG activation occurs it may be surprisingly intense, whereas it becomes progressively more feeble when the dose of adrenaline is progressively lowered. The conclusion is inescapable that chlorpromazine delays the adrenaline response much more than it diminishes it; the reverse is true of barbiturates.

It is difficult to offer a satisfactory explanation for this primarily delaying effect of chlorpromazine. However it should be recalled that adrenaline EEG activation is only the resultant of two simultaneous and opposing processes, activation and deactivation, and that the period of activation usually seen is by no means co-extensive with the total period of action of adrenaline. It is conceivable that chlorpromazine acts differently upon the central excitatory and inhibitory components of adrenaline action, and that altering the relationship between the two produces the apparent delay in activation. Further elucidation of this action will have to await techniques which can study and measure the central inhibitory and excitatory components separately.

Pharmacologically, chlorpromazine has many actions: it is anti-adrenergic, anti-cholinergic, and anti-histaminic;

it may exert a direct effect upon blood vessels as well (Foster et al., 1954). By far the most prominent action in the concentrations used (dosage of 1 mg./kg.) is the anti-adrenergic one and it is only natural to suggest that it is this action which accounts as well for its ability to delay or block the central (EEG) response to adrenaline. Centrally, chlorpromazine partly paralyses the sympathetic component of the temperature-regulating mechanism - the heat conserving mechanism - with the result that the body temperature falls when that of the environment is low; it protects decerebrate animals against rage responses (Dasgupta, Mukherjee, and Werner, 1954); in many species it exerts a unique tranquilizing effect, which in larger doses goes on to light sleep (Das, Dasgupta, and Werner, 1954). The drug seems to have selected out a number of functions which are ordinarily regarded as "sympathetic" or "ergotrope" and as the dose is increased, its blocking effect remains confined to these functions but increases; in this way it is quite unlike increasing doses of barbiturates which cause ataxia, slurred speech, deep sleep and finally coma. Many of the above functions can be localized anatomically to the posterior hypothalamus and mesencephalon where they seem to form a unit of interrelated mechanisms. A depressing effect of chlorpromazine upon the auditory evoked potentials of the brain stem reticular formation (but not the cortex) as well as its spon-

taneous activity has been reported (Angeleri, Carreras, and Urbani, 1954). This same region is rich in "sympathin" - noradrenaline and adrenaline - (Vogt, 1954). The other most conspicuous clinical effect of chlorpromazine is its antiemetic action; in the dog it is capable of protecting against emetic agents which are believed to act through the medullary chemoreceptor trigger zone (Brand et al., 1954). It is interesting that the areas postrema, which is intimately associated with the trigger zone (Borison and Wang, 1953), has the highest noradrenaline-adrenaline content of the entire central nervous system (Vogt, 1954).

The fact that chlorpromazine blocks all these functions so selectively in a wide range of dosage suggests that the whole group are adrenergic and are thus singled out by a central adrenergic blocking agent such as chlorpromazine. This might illustrate the manner in which a large number of drugs seem to exert their characteristic and patterned effects upon the central nervous system - by selecting out a system or set of systems which possess some biochemical or neurohumoral factor in common vulnerable to the drug action; it also illustrates the futility of trying to localize the site of drug action to some arbitrarily defined anatomical region such as the "hypothalamus", a region which is so heterogeneous functionally and biochemically.

It may be asked why if chlorpromazine acts as a central adrenergic blocking agent all other such blocking agents do not show the same central effects. That they do not is illustrated by the absence of any EEG effect from doses of Dibenzyline which reversed the adrenaline pressor response and the known fact that this and many other similar blocking agents have no particular central side effects in ordinary dosage. This question cannot be answered definitively, but the observation has been made that the presence or absence of central activity seems in some instances to be related to the nitrogen valence (Funderburk and Case, 1951). Using the EEG as their criterion, they noted that the tertiary amines eserine and atropine had central (though opposing) effects, while the quaternary amines neostigmine and eucatropine had none, and suggested that this valence difference may influence their ability to pass the blood-brain barrier. It is worthwhile noting that chlorpromazine is a tertiary amine while Dibenzyline is believed to be converted in the body to a quaternary amine (by becoming a cyclic ethylenimonium derivative).

H. Multiphasic Adrenaline Responses; Adrenaline Positive Feed-Back

It remains to be explained why during a certain phase of chlorpromazine action (usually as the drug is slowly wearing off) one is apt to see multiple phases of EEG action

and blood pressure rise both after adrenaline and sciatic stimulation. Again it is impossible to provide a completely satisfactory explanation, but a great deal of the evidence points to the active participation of the animal's own adrenals in propagating the recurrent phases.

It has been noted that the late phase of adrenaline EEG activation seen under the influence of chlorpromazine is not only quite intense but is often followed in a few seconds by a prominent rise in blood pressure (Fig. 9). It is very unlikely that this late pressor response is due to any peripheral action of adrenaline since it occurs during the period when chlorpromazine has produced varying degrees of peripheral adrenergic blockage, often reversal. Actually the peripheral vascular response to adrenaline still persists and occurs at the usual time (7-8 seconds after the injection); it may consist of a small rise, no change or a fall, depending upon the completeness of the adrenergic blockade at the moment. The late pressor response is quite unlike this early peripheral response in that the blood pressure always rises and never falls, the rise is always quite high - much more so than the early response, and it always follows EEG activation, never appearing without it. A small dose of barbiturate which will abolish the EEG activation also abolishes the late pressor response while not affecting the early one.

Because the late pressor response always follows EEG activation, never appears without it, and is very unlikely to be due to the peripheral effects of adrenaline, it has been necessary to postulate that the central excitatory actions of adrenaline not only cause EEG activation but may also cause sympatho-adrenal discharge. A very close parallelism between the central mechanisms responsible for EEG activation and those which determine the level of activity of the peripheral sympathetic system (as evidenced by blood pressure and pupil size) has been emphasized by Bonvallet, Dell, and Hiebel (1954) who suggested that the cortical "tonus" and the peripheral sympathetic tonus are controlled by a single common or two closely interrelated brain stem mechanisms. Further evidence that this late pressor phase results from central excitation is derived from the fact that it is never seen in animals with midreticular coagulation, where the adrenaline-secretion and vasomotor center of Cannon has been injured or destroyed. It requires the unique circumstances brought about by chlorpromazine to reveal this central-pressor activity of adrenaline; the central activation period must be maintained in intensity but displaced in time so that it no longer occurs at the same instant when the peripheral vascular effects are predominant since the latter will obscure the former. At these levels, chlorpromazine exerts con-

siderable blocking effect upon the actions of injected adrenaline but has very little effect upon direct sympathetic discharges, as elicited by sciatic stimulation.

If one is willing to admit that under the above conditions adrenaline can produce combined EEG activation and sympathetic discharge (resulting in a late but quite prominent pressor response), it follows that the sympathetic discharge might be accompanied simultaneously by discharge of adrenaline from the animals' own adrenals, since discharge of the sympathetics and adrenal medulla commonly occur together. If this were the case, we might expect to see a second period of EEG activation produced by this endogenous adrenaline release occurring with about the same delay after the first EEG activation and sympatho-adrenal discharge as occurred after the initial adrenaline injection and the first phase. This actually seems to be the case, for during the period when late EEG and pressor responses to adrenaline occur to adrenaline, multiple responses (as many as 6 in succession) at the same interval are often seen.

This constitutes a positive feed-back mechanism, for the delayed EEG activating and sympathoadrenal discharge inducing effects of adrenaline are sufficient to cause the release of more adrenaline and the process repeats itself. Since sciatic nerve stimulation also induces adrenaline release, it is also capable of starting this series of events,

the only difference being that there is an initial immediate period of EEG activation not seen after adrenaline administration. Further support for this explanation comes from the observations that multiple phases are never seen in the animal with mid-reticular coagulation (the central adrenaline-sensitive vasomotor and adrenomotor center has been destroyed), they are reversibly abolished by small doses of anaesthetics (just big enough to abolish the central excitatory effects of adrenaline) and are never seen in the adrenalectomized animal. (In the adrenalectomized animal, two phases are seen after adrenaline - the first direct vascular response and the second delayed EEG and pressor response; never more than this; in the same preparation after sciatic stimulation only one phase is seen, the immediate EEG and pressor response).

It may be asked why the positive feed-back mechanism does not function normally. After an injection of adrenaline in the normal intact animal the EEG activation lasts roughly from the 10th to the 40th second; if the central excitatory effect of adrenaline were to produce immediate adrenaline release along with EEG activation, allowing an additional 7-10 seconds latency, the central effects of the endogenous adrenaline would begin at the 20th second and would largely overlap the initial activation response and last a little into the inhibitory phase of the injected adrenaline,

the two cancelling each other out. The essential features of chlorpromazine action which permit the propagation of multiple phases seems to be the marked delay (so that activation occurs well after the usual peripheral and central effects are over) and the fact that activation, when it does occur, is quite intense.

I. Cholinergic EEG Activation

The conclusion has been drawn that the reticular activating system is cholinergic, on the basis of experiments which have demonstrated EEG activation in the curarized rabbit from intracarotid acetyl choline or cholinergic potentiators such as DFP, and its abolition with atropine (Rinaldi and Himwich, 1955 a and b). Others have made similar observations (Bradley, 1953; Longo, 1955). Working with freely moving intact cats from which EEG records were made simultaneously, Bradley (1953) has made important observations on the correlation between EEG activity and behavior. In such preparations, amphetamine produced both EEG and behavioral arousal, while physostigmine produced EEG "arousal" with no concomitant behavioral change. This important dissociation between the EEG and behavior was first noted by Wikler (1952) in atropinized dogs, which showed EEG sleep patterns when they were awake or even excited. This was confirmed in the cat (Bradley, 1953) and

we have observed it ourselves. Furthermore, Bradley (1953) noted that mesencephalic transection abolished the EEG effectiveness of amphetamine but did not eliminate the EEG activating effects of physostigmine, suggesting a different site of action. Rinaldi and Himwich (1955, b) found that the EEG effects of acetyl choline were not abolished by lower mesencephalic transection but were absent in the isolated hemisphere.

All of this evidence suggests certain fundamental differences between the adrenergic and the cholinergic EEG activating mechanisms. The former is closely associated with the mesencephalic reticular formation while the latter may be somewhat more rostrally situated (between the mesencephalon and the centrum ovale). The EEG effects produced by adrenergic sensitizing and blocking agents (methamphetamine, chlorpromazine) run parallel to behavior. On the other hand cholinergic potentiating and blocking agents (physostigmine, atropine) produce EEG effects with the conspicuous absence of any behavioral counterpart, i.e., dissociation. At present, the most that one can say is that it appears that the reticular activating system (in its EEG sense only) contains both adrenergic and cholinergic components; the brain stem arousal mechanism (in its behavioral sense) appears to be adrenergic; it remains to be shown that it is cholinergic as well.

V. SUMMARY AND CONCLUSIONS

Experiments were carried out to determine the effect of adrenaline, other sympathomimetic amines and related compounds, and adrenergic blocking agents upon the EEG of the intact unanaesthetized cat, the adrenalectomized cat and the cat prepared with a lesion in the tegmentum at the pontomesencephalic junction (mid-reticular coagulation).

Adrenaline in doses of 2-8 µg./kg. produced a series of alternating phases of EEG activation and deactivation in the period between 6-60 seconds after the beginning of the intravenous injection. Of these, the phase of activation was the most conspicuous, and dominated the middle portion of the response. The deactivation phases came at the beginning and end of the response, and whether or not they were detectable depended primarily upon the background activity.

By coagulating the pontomesencephalic tegmentum an apparent increase in sensitivity to adrenaline could be produced, with thresholds encountered as low as 0.05 µg./kg. Reducing the adrenaline dose towards threshold produced a gradual shortening of the activation phase, then a delay in its onset just before the response disappeared altogether. Further coagulation in this same region gradually raised the threshold as the lesions were made more and more an-

teriorly, although sometimes the intensity of the activation response actually increased. By carrying the coagulation up to the meso-diencephalic border the response to intravenous adrenaline was abolished altogether. Unilateral coagulation abolished the response only over the ipsilateral cortex.

On a weight basis, noradrenaline was just as effective as adrenaline. Phenylephrine was much less potent in producing EEG activation but intensity was more diminished than duration. Methamphetamine possesses EEG activating effects to only a small degree and rapidly showed tachyphylaxis.

Methamphetamine and cocaine, in amounts that had no EEG effects in themselves, lowered the threshold to adrenaline markedly both with respect to its EEG and vascular effects. Larger doses of both these drugs produced sustained EEG activation even in the animals with mid-reticular coagulation.

Ether anaesthesia and very small doses of other anaesthetics (thiopental, pentobarbital, chloralosane, alcohol) falling within the "sedative" range completely abolished adrenaline EEG activation. This effect was reversible.

Examination of the adrenergic blocking agents, chlorpromazine and Dibenzyline, showed that in doses sufficient to

lessen or reverse the adrenaline pressor response, chlorpromazine had definite effects upon the adrenaline EEG activation, while Dibenzyline did not. In high doses or at the peak of its action chlorpromazine abolished adrenaline EEG activation in a manner similar to barbiturates. In smaller concentrations, it exerted a delaying effect upon the activation phase which was otherwise quite intense; with the same concentration of chlorpromazine, multiple and repeated phases of blood pressure increase and EEG activation (as many as six in a row) were observed after both adrenaline and sciatic stimulation. Reasons are given for believing that the recurrent phases depend upon the animals' own adrenal glands and constitute a positive feed-back mechanism.

Evidence in favor of the existence of an adrenergic component within the reticular activating system is given and some of the possible properties of such a system are discussed, including an explanation of the clinical effects of such drugs as methamphetamine, cocaine, and chlorpromazine.

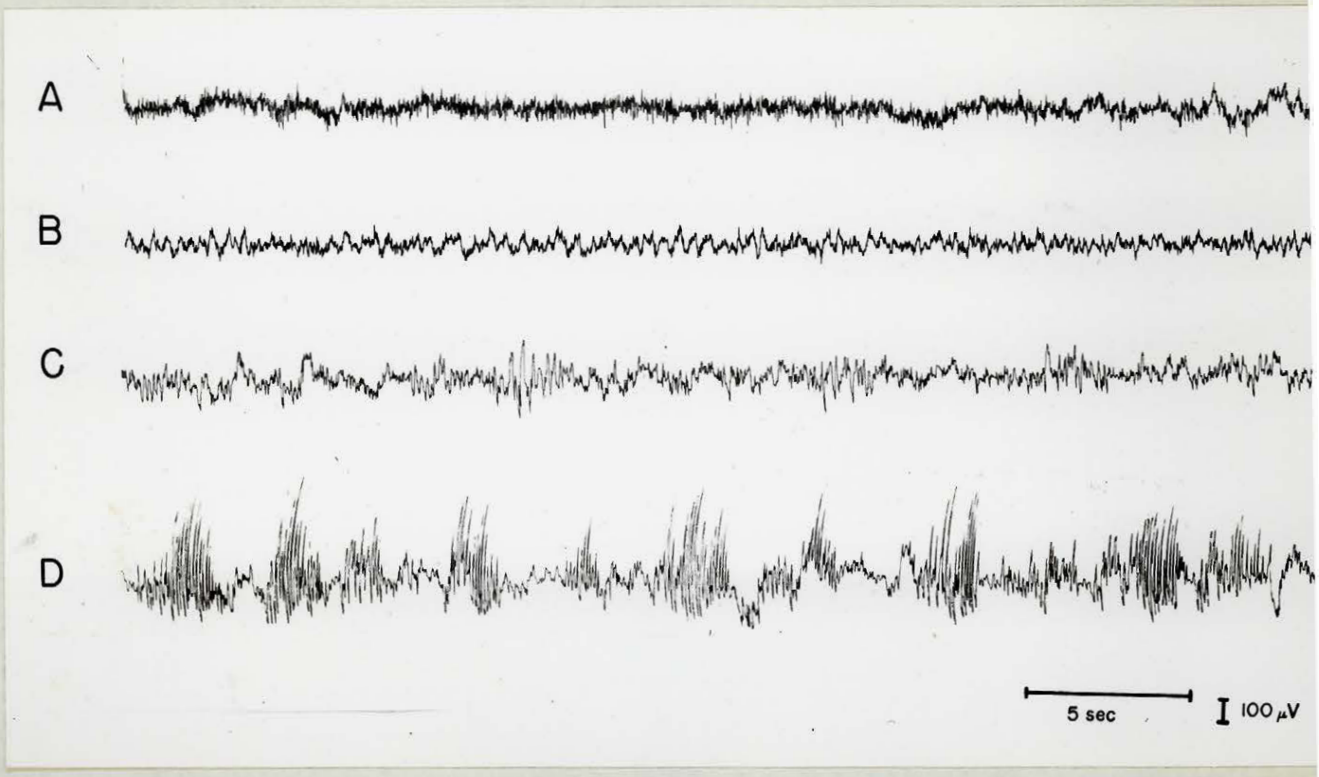


Fig. 1

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.

Figs. 2-9

Certain conventions have been observed in the following records. Channels A through D are bipolar recordings of the cat EEG, the electrodes having been placed on the dura: A, right frontal-right central; B, right central-right posterior; C, left frontal-left central; D, left central-left posterior. Channel E is a recording of the blood pressure; the height of the calibrating pulse ("Cal."), when present, is equivalent to 55 mm. of Hg. The continuous base-line below the blood pressure record is not at 0 but has been raised to between 60 and 90 mm. of Hg. to serve as a more convenient reference line.

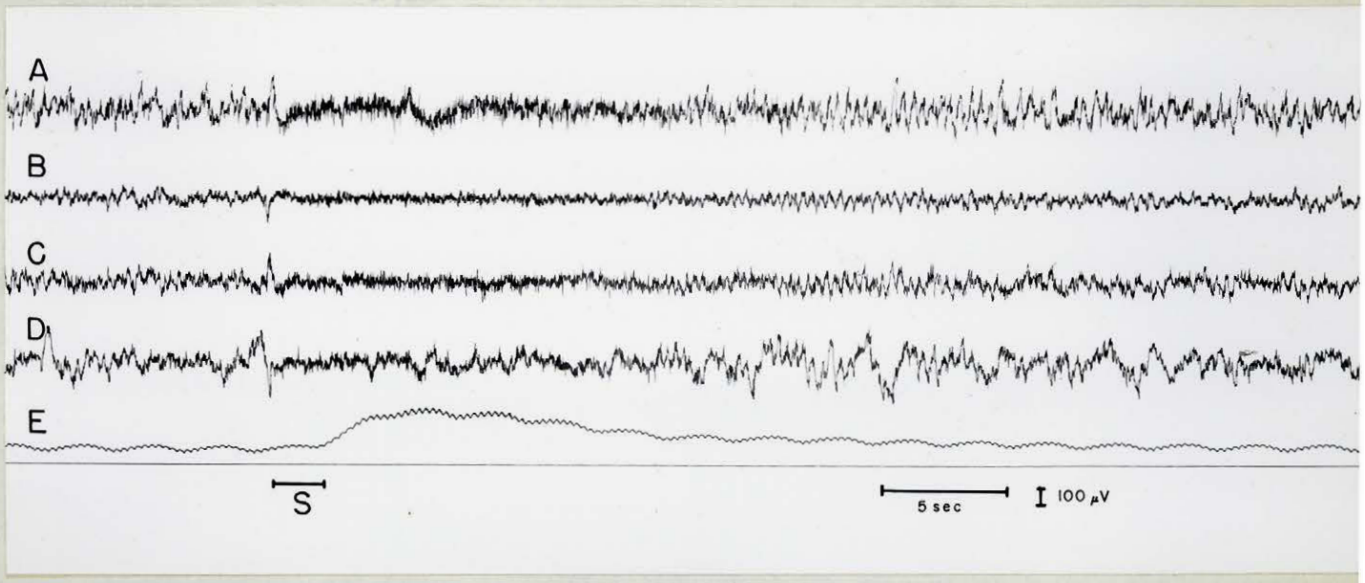


Fig. 2

This record shows the effects of a brief and relatively mild electrical stimulus ("S") applied to the sciatic nerve upon the EEG and blood pressure of an intact, curarized cat. Note the immediate EEG activation, and the pressor response within 2.5 seconds.

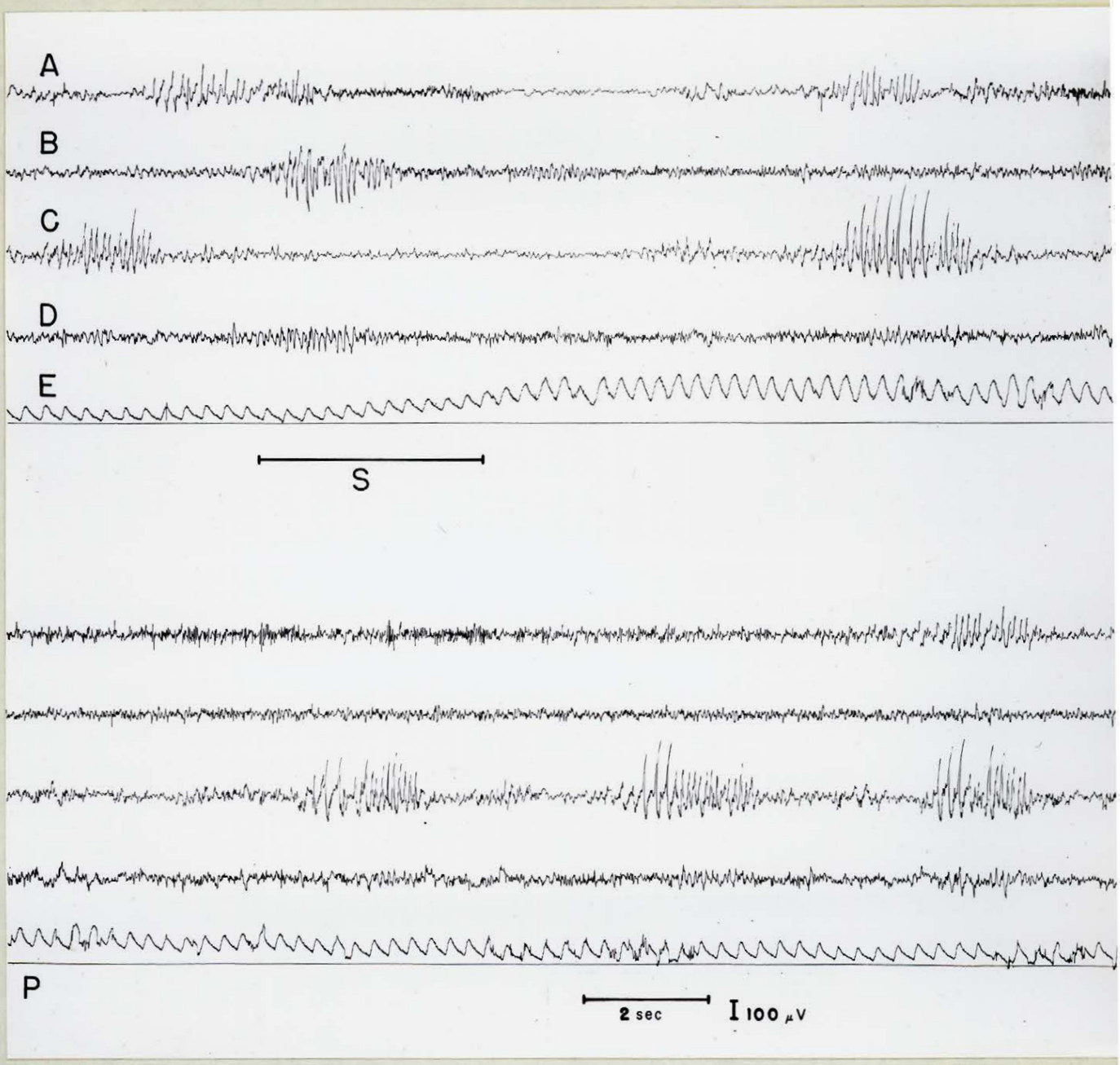


Fig. 3

The effect of sciatic stimulation after midreticular coagulation. EEG activation does not outlast the stimulus ("S") and is observed only in channel A. Approximately 12-13 seconds after the beginning of the stimulus (shortly before point "P"), a second period of activation appears in channels A and B and lasts for 16 seconds. The pressor response is unchanged.

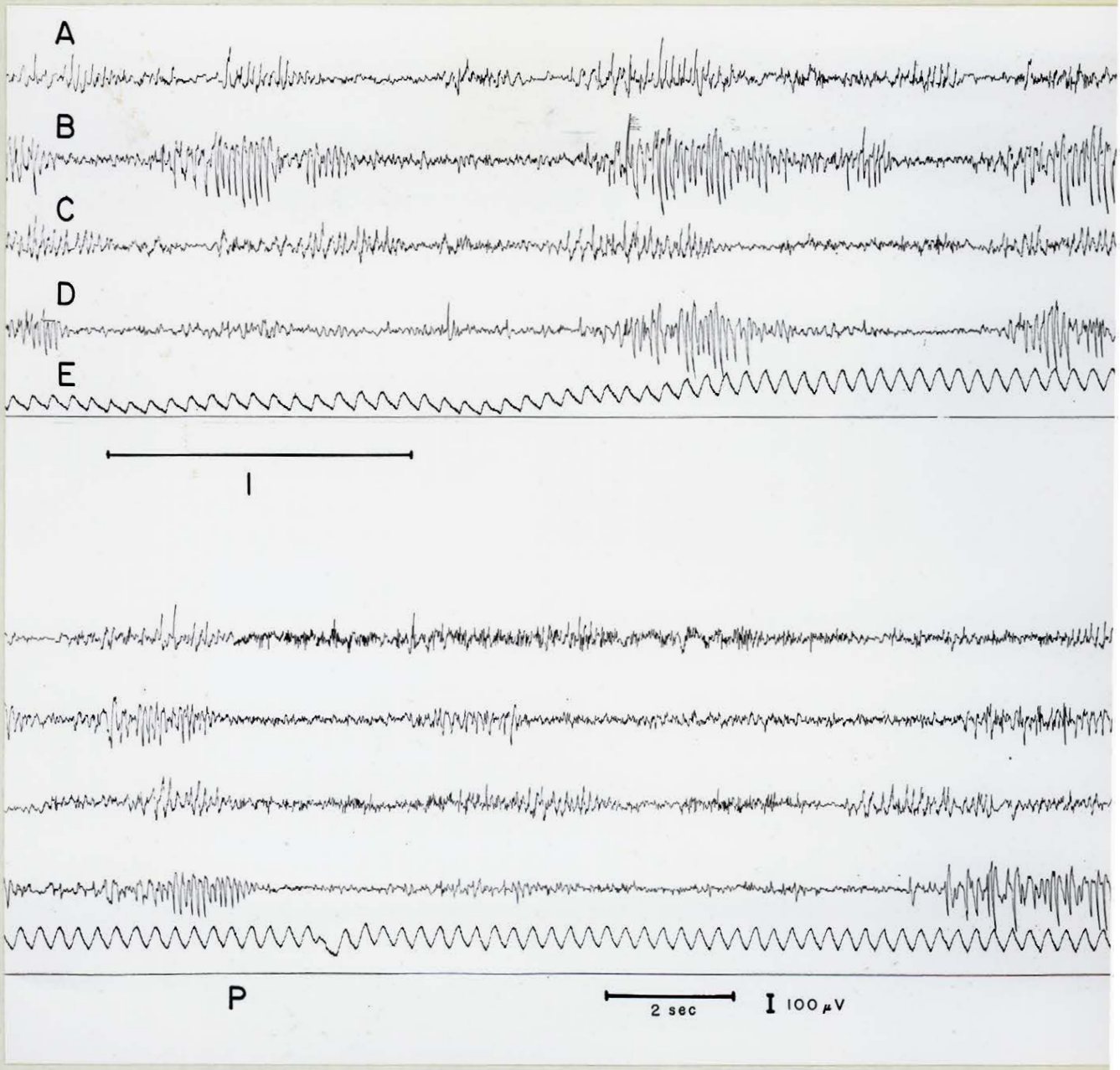
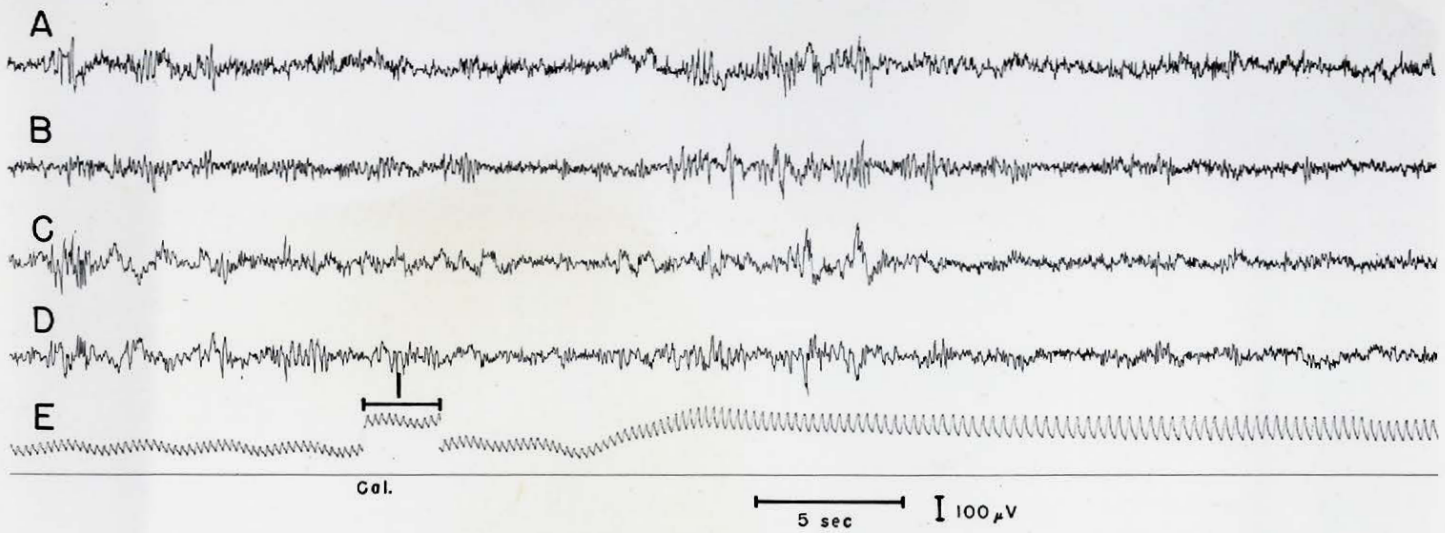


Fig. 4

Effect of the intravenous injection ("I") of 8 microg./kg. of adrenaline in the same preparation illustrated in Fig. 3. This brings about EEG activation (at point "P") similar in appearance and timing to the second period of EEG activation shown in Fig. 3, indicating that the endogenous release of adrenaline could have been responsible for the late phase in Fig. 3.

Fig. 5

Effect of the intravenous injection ("I") of 5 microg./kg. of adrenaline upon the EEG of an intact, curarized cat. Alternating phases of EEG activation and deactivation are seen. The pressor response begins at about 7.5 seconds.


A
B
C
D
E
Cal.
5 sec
100 μV

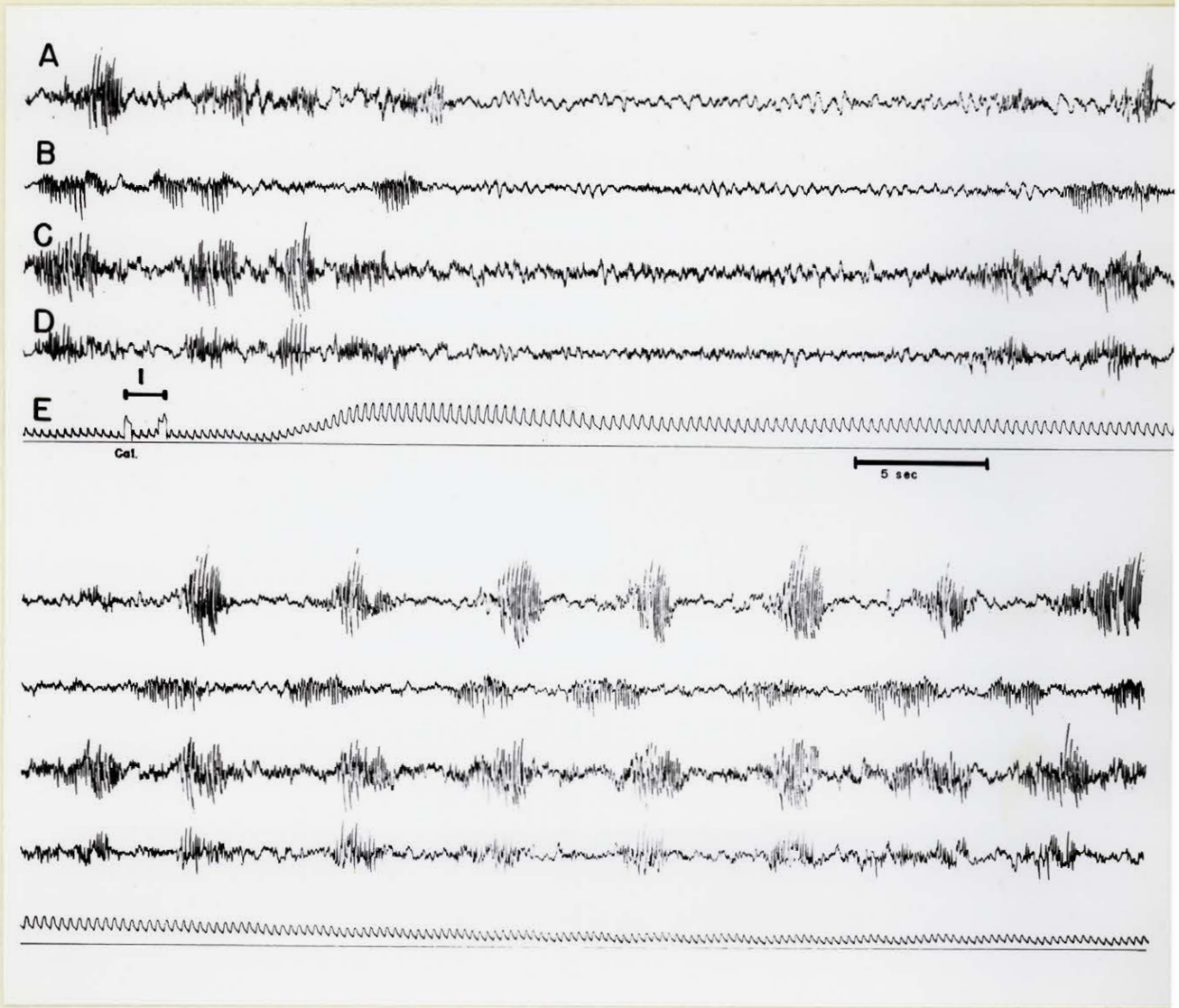
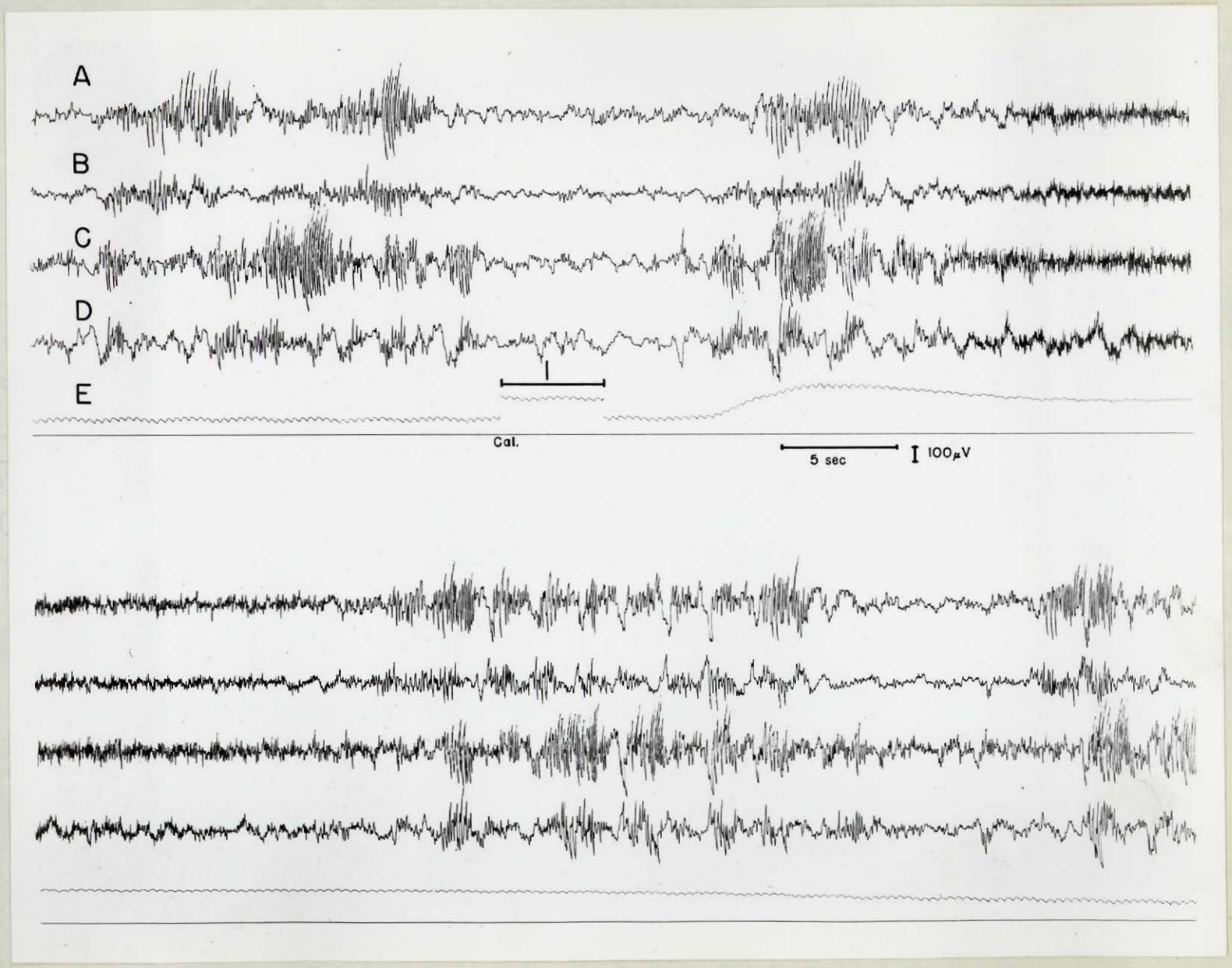


Fig. 6

Effect of the intravenous injection ("I") of 5 microg./kg. of adrenaline upon the EEG of a cat with mid-reticular coagulation. Only the activation phase is seen and it lasts from 10-32 seconds after the beginning of the injection. The EEG activation consists of a shift from pattern "D" to "B". The pressor response is similar to that seen in Fig. 4.


A
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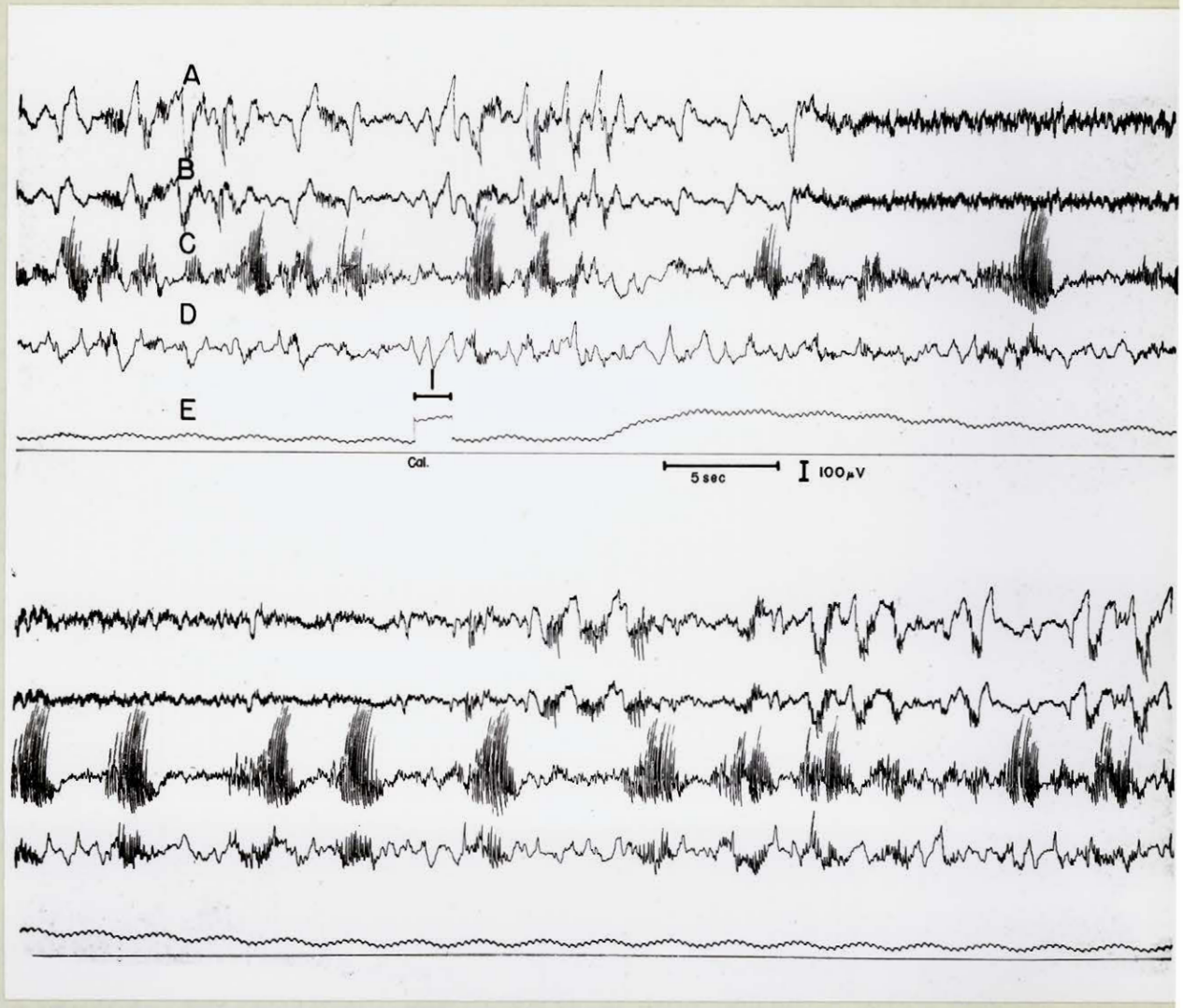
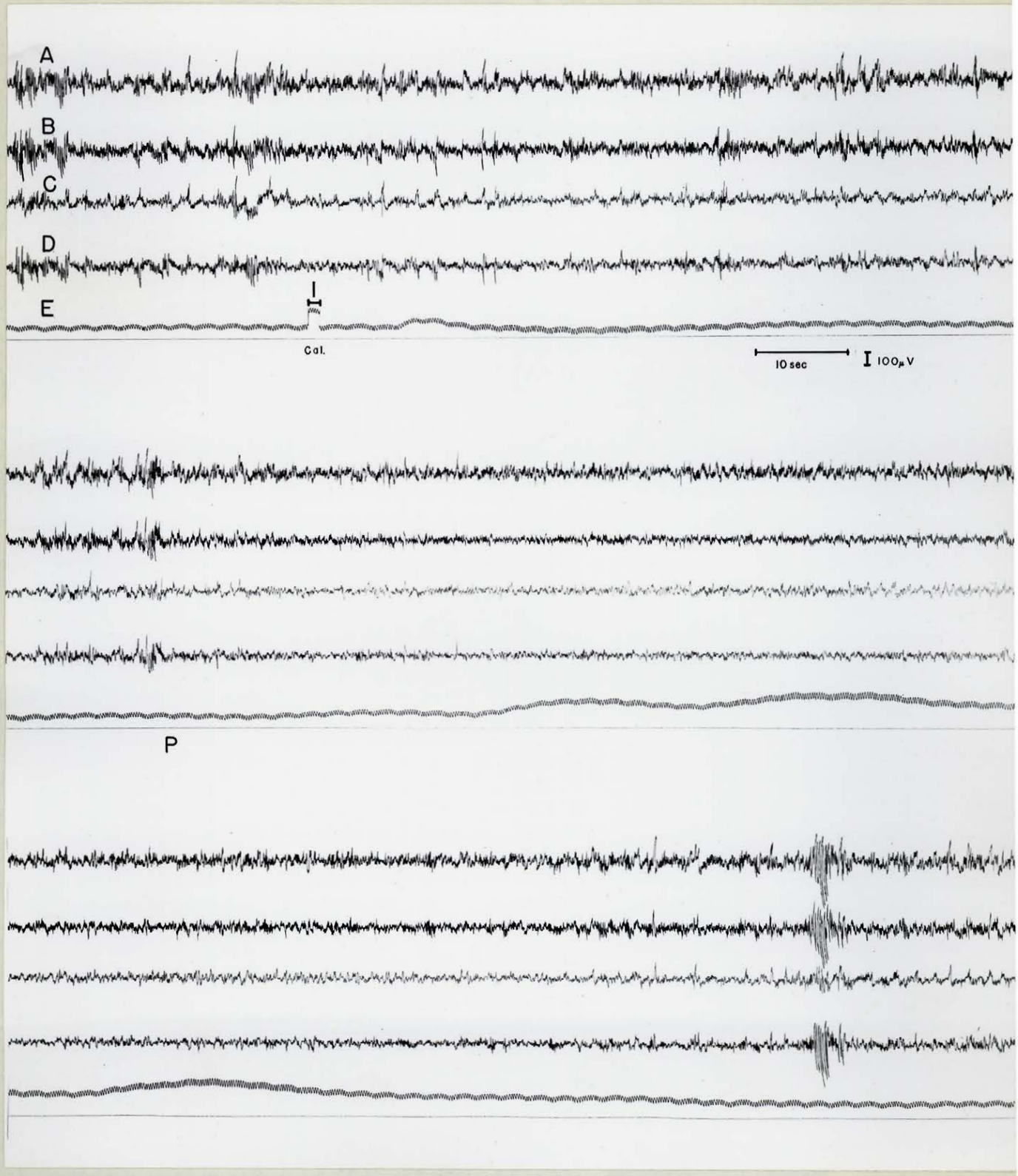


Fig. 8

Effect of the intravenous injection ("I") of 5 microg./kg. of adrenaline upon the EEG of a cat in which coagulation of the left half of the mesencephalic tegmentum had been performed. Intense EEG activation is noted on the EEG recorded from the right cerebral hemisphere (channels A and B) but no change is apparent in the recordings from the left hemisphere (channels C and D).

Fig. 9

Effect of the intravenous injection ("I") of 4 microg./kg. of adrenaline upon the EEG of a cat which had received an intramuscular injection of chlorpromazine (1 mg./kg.) 3 hours previously. Nine seconds after the beginning of the injection there occurs a mild rise in blood pressure followed by a longer very slight fall; by 50 seconds, the blood pressure has reached the control level again. At point "P", 96 seconds after the injection, activation of the EEG occurs (conversion from patterns "C" to "B"). About 10 seconds after "P", the blood pressure begins to rise again and does so in progressive waves, ultimately reaching levels much higher than the initial pressor response. Both the late EEG activation and pressor response then gradually decrease but do not reach control levels until 268 seconds after the injection.


A
B
C
D
E
Cal.
10 sec
100μV
P

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