THE EFFECT OF DRUGS ON AROUSAL RESPONSES PRODUCED BY ELECTRICAL STIMULATION OF THE RETICULAR FORMATION OF THE BRAIN

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INTRODUCTION

Studies on the effects of drugs on the electrical activity of the brain and on behaviour in the conscious, unrestrained animal have shown that the drugs studied fell into two distinct groups, according to whether or not the effects which they produced on the EEG correlated with the observed changes in behaviour. Thus, certain parasympathomimetic and parasympatholytic drugs (physostigmine, atropine and *l*-hyoscyamine) produced patterns of electrical activity which did not correlate with the behavioural state of the animal (Bradley and Elkes 1953a, 1957). Sympathomimetic drugs and others such as *d*-lysergic acid diethylamide, on the other hand produced parallel effects on the EEG and on behaviour (Bradley and Elkes 1953b, 1957). The differences between these two groups of drugs were further emphasized by the level of the lesion in the central nervous system which blocked their effects in acute experiments. For example, physostigmine, atropine and *l*-hyoscyamine still produced effects on electrical activity irrespective of whether the lesion was made at a spinal or mesencephalic level, but the effects of amphetamine were abolished by a transection at the intercollicular level, and those of *d*-lysergic acid diethylamide (LSD 25) by high spinal section. These observations led to the suggestion (Bradley and Elkes 1953b, 1957) that amphetamine might act on receptors situated at the level of the brain stem reticular formation, and that LSD 25 may also have an action at this level, though not necessarily on the same receptors. It has also been suggested (Bradley and Elkes 1956, 1957) that it is unlikely that the arousal system at the brain stem level is activated by a predominantly cholinergic mechanism.

Recent experimental studies with chlorpromazine (Longo, Berger and Bovet 1954; Bradley and Hance 1955, 1957) have provided evidence which indicates that this drug may exert its central depressant action at the level of the brain stem reticular formation.

Moruzzi and Magoun (1949) in their original studies on the activation of the electrocorticogram by electrical stimulation of the brain stem, obtained consistently low thresholds for points within the reticular formation. If, as has been suggested, certain drugs act at this level, then they might be expected to modify these thresholds, whilst those drugs which are thought to act more diffusely in the central nervous system might be expected to have little effect on thresholds for stimulation of the reticular formation.

This study represents an attempt to determine quantitatively the effects of different concentrations of drugs on arousal responses produced both by direct stimulation of the reticular formation of the brain stem, and also by afferent stimulation. Some of the results which follow were presented at the 20th International Physiological Congress, Brussels, 1956 (Bradley and Key 1956).

METHODS

Experiments were carried out on 35 cats, all of which were *encéphale isolé* preparations. The animals were prepared under ether anaesthesia administered through an intratracheal cannula, and, after careful dissection of the muscles at the back of the head and neck, the spinal cord was sectioned at the C_1 level. Respiration was then maintained artificially by means of a pump. Cortical recording electrodes of the same type as those de-

scribed previously (Bradley and Elkes 1953c) were inserted through bur holes drilled in the skull over both cerebral hemispheres, together with an earthing electrode in the midline, over the frontal sinus. The number of cortical electrodes used varied in different experiments, but it was usually between 12 and 16. The stimulating electrode consisted of a bipolar, stainless steel, concentric needle, insulated with varnish except at the tip. It was made by inserting a varnished .021″ diameter stainless steel needle through the bore of a No. 20, three inch long serum needle, the end of which had been cut off and ground square. The inner needle was sealed into place with "Araldite" cement (Ciba) and the whole assembly varnished. The tips of both inner and outer conductors were carefully bared so that the distance between the stimulating points was approximately 1 mm. Care was also taken to ensure that the tips were smooth and that "shoulders" were avoided as far as possible. The electrode was oriented in the brain stem by means of a Horsley-Clarke stereotactic instrument and co-ordinates obtained from a previously prepared atlas of the cat brain. The hole in the skull through which the electrode was inserted was made as small as possible (about 3 mm. in diameter), to avoid undue exposure of the surface of the cortex, and the electrode was passed through a small slit in the dura mater. When the correct position for the electrode had been reached, the skull defect was packed first with gelatine sponge and then with small pledgets of cotton wool. Dental wax, which had been softened by warming, was moulded into a plug around the electrode and when the wax had set hard, the electrode was released from the carrier of the stereotactic instrument. The animal's head could then be removed from the instrument with the stimulating electrode still in place. In some experiments two stimulating electrodes were used, one being placed at the level of the mesencephalon (the normal position) and the second approximately 18 mm. more posterior, with its tip in the medulla.

The femoral vein, which was used for the injection of the drugs, was cannulated routinely, and in many experiments the femoral artery was used for the recording of blood pressure level, simultaneously with the electrical activity of the brain.

When all the operative procedures had been completed, the wound edges were infiltrated with a local anaesthetic (procaine) and the administration of ether stopped. The head of the animal was placed on a soft pad or cushion and the laboratory kept as quiet as possible. At least one hour was allowed to elapse following the removal of the anaesthetic, before the experiment was started. With these precautions (removing the animal from the stereotactic instrument, placing its head on a cushion, local infiltration of anaesthetic and reduction of extraneous noise to a minimum), sleep activity was observed in 28 of the 35 preparations. There appeared to be no relation between the blood pressure level and the presence of sleep activity in these preparations.

The electrocorticogram was recorded on a 4-channel Edison Swan portable recorder and stimuli were delivered from a remotely controlled electronic stimulator. The stimuli consisted of 0.1 msec. square waves at a frequency of 300 c/sec. and the period of stimulation (10 seconds) was determined by an automatic timer once the stimulus had been switched on.

Determination of the arousal threshold. This threshold was determined by gradually increasing the applied voltage in steps, starting at 0.1 V. and stimulating for one 10 sec. period at each step. The stimulation periods were spaced as widely as possible to avoid any possibility of accommodation to the stimulus. Exactly the same sequence was followed each time the threshold was checked and in all cases the threshold for behavioural and EEG arousal were determined independantly. The operator watched the animal's face as the stimulus was applied by remote control, whilst another operator set the level of the stimulator. The EEG record was taken continuously whilst this was being done and the stimulus indicated on the record by a marker pen. The signs of behavioural arousal varied in different preparations, but the criteria chosen were: the opening of the eyes and contraction of the nictitating membrane immediately the stimulus was applied, followed in most cases by movements of the ears, jaws and vibrissae.

These effects usually persisted beyond the period of stimulation and were therefore not considered to be due to motor effects caused by a spread of the stimulus. Motor effects were sometimes observed when high stimulating voltages were used and these could be clearly distinguished from the behavioural signs of arousal. When the threshold for behavioural arousal had been established, the EEG record was examined to see if at any point an activation of the electrocorticogram had occurred. If not, the stimulation sequence was continued until a voltage level was reached where an EEG arousal was obtained which lasted for the full 10 seconds of stimulation. This voltage was taken to be the EEG threshold. In the few preparations which were "awake" and in which no sleep activity could be recorded, the control thresholds for both behavioural and EEG arousal were taken to be zero.

The drugs studied were: pentobarbitone, chlorpromazine, atropine, hyoscine, physostigmine, *dl*-amphetamine and *d*-lysergic acid diethylamide (LSD 25). They were injected intravenously in incremental doses and the arousal thresholds checked twice after each injection, usually at 10 and 20 minute intervals. In the case of physostigmine, these intervals were 5 and 10 minutes.

In some experiments the threshold for arousal produced by afferent stimulation was also determined. This was done with an auditory stimulus consisting of a continuous note, obtained from a loudspeaker placed at a fixed distance from the head of the cat. The voltage applied to the loudspeaker was gradually increased until an arousal was obtained when the stimulus was switched on. This voltage was taken as a measure of the threshold. In these experiments, the threshold for click responses, recorded from electrodes placed over the auditory cortex, was determined in the same way.

At the end of each experiment the animal was killed, the brain fixed in formol saline with the stimulating electrode *in situ*, and the position of the tip subsequently determined by histological examination. Some of the stimulation points used in these experiments are shown in figure 1.

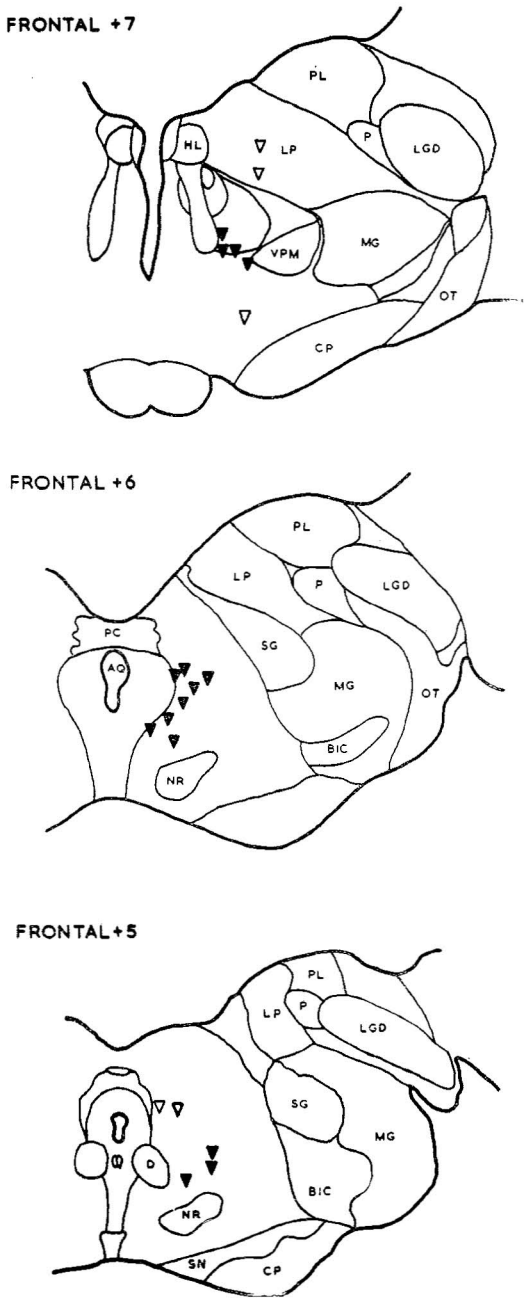

Fig. 1

Sections of cat brain in Horsley-Clarke plane showing some stimulation points in these experiments. *Black triangles*: points at which the control threshold was between 0.40 and 1.20 V. *White triangles*: points at which the control threshold was between 1.20 and 1.50 V.

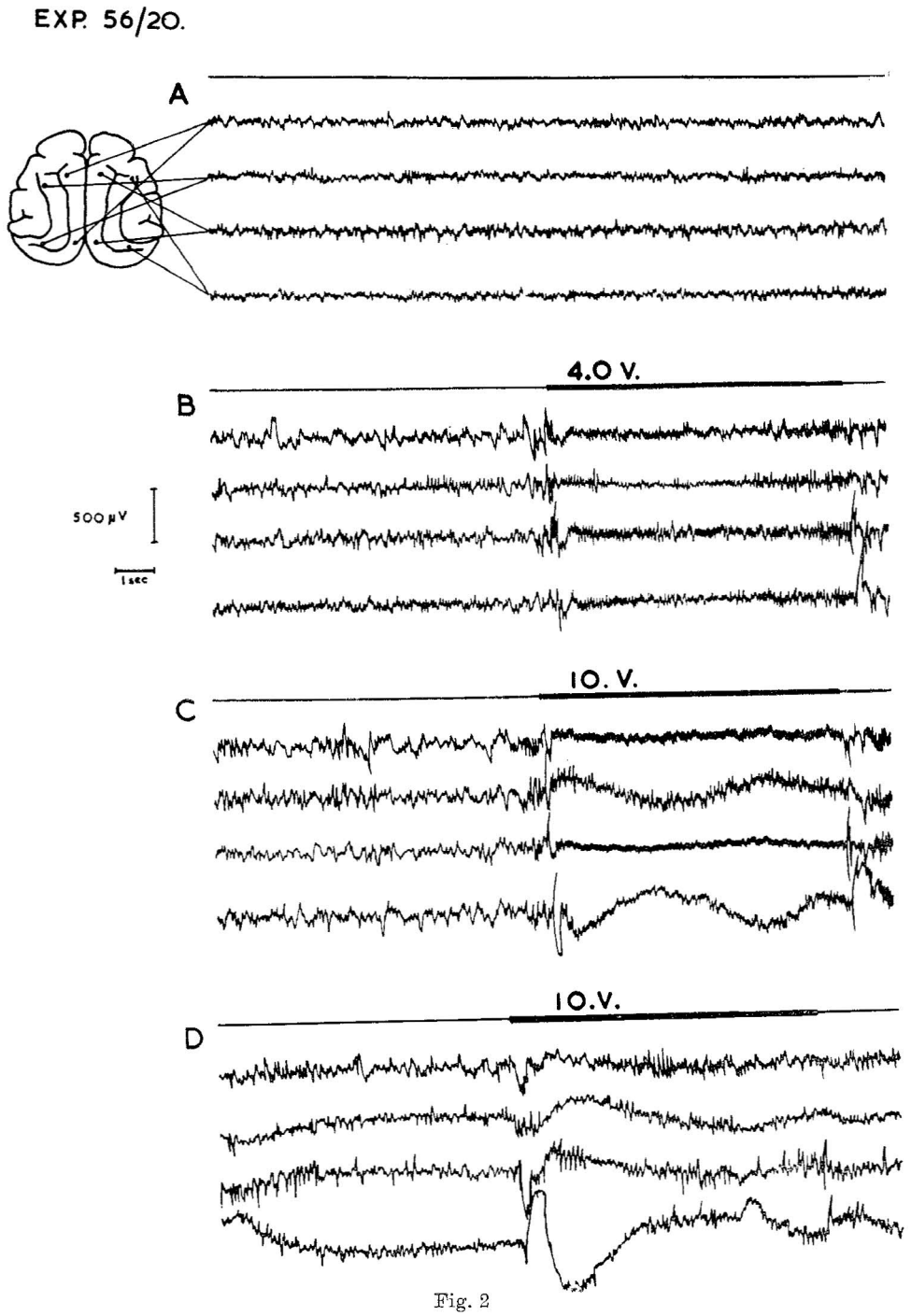

Fig. 2

Effect of pentobarbitone on EEG arousal produced by stimulation of the reticular formation. A: control record with the preparation "alert", B: after 3.5 mg/kg. of pentobarbitone (intravenously), stimulation of 4.0 V.; C: after 8.5 mg/kg., stimulation at 10 V.; D: after 18.5 mg/kg., stimulation at 10 V. ineffective.

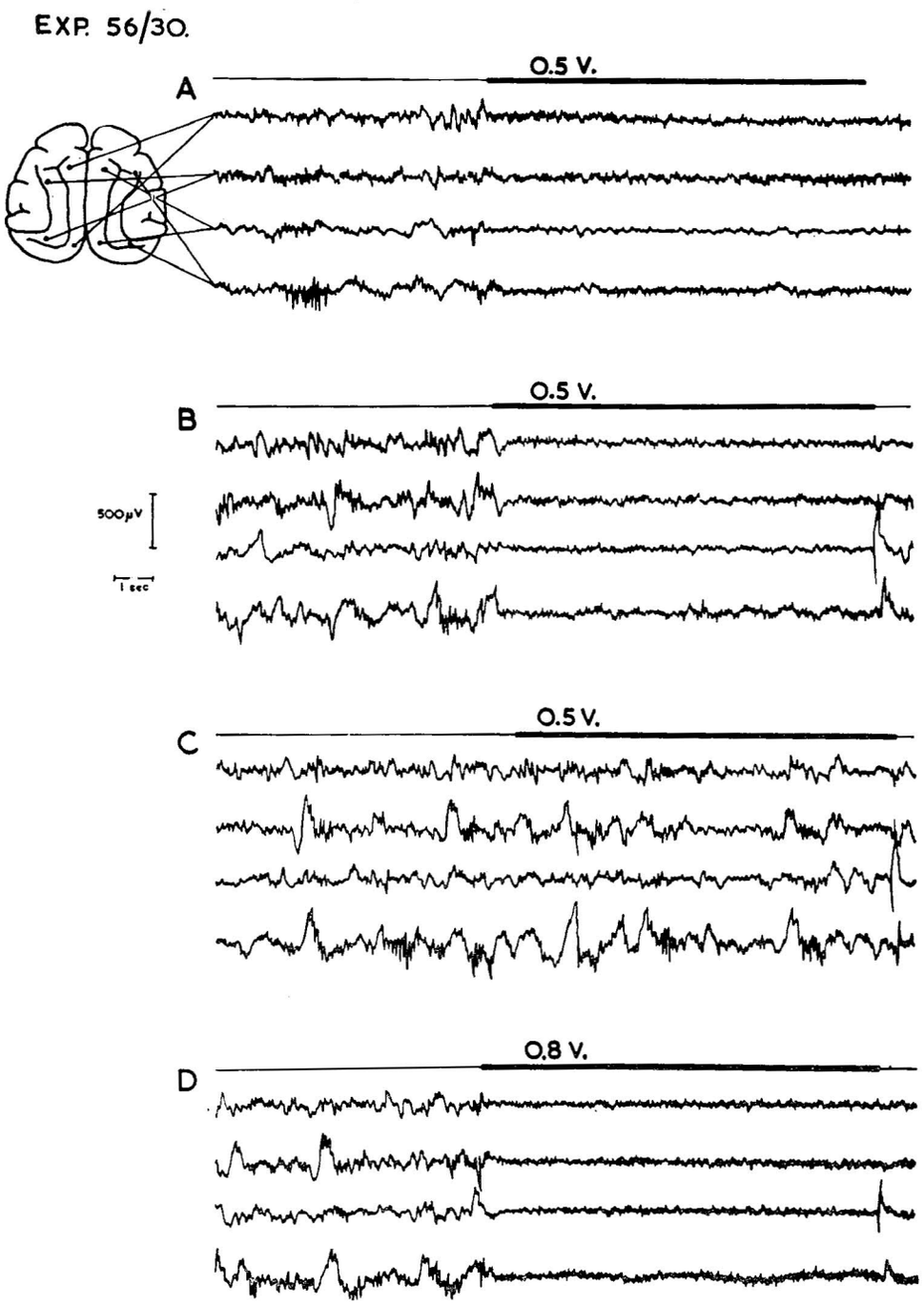

Fig. 3

Effect of chlorpromazine on EEG arousal produced by stimulation of the reticular formation. A: control record, stimulation at 0.5 V.; B: after 1.88 mg/kg., stimulation at 0.5 V.; C: after 3.88 mg/kg., stimulation at 0.5 V. is no longer effective; D: after the same dose (3.88 mg/kg.), stimulation at 0.8 V.

RESULTS

I. *The arousal threshold in the normal encéphale isolé preparation.*

In 7 preparations no sleep activity was observed and they appeared to be permanently "awake". In the other 28 it was possible to establish control thresholds and this was done at least twice before any drugs were given. In 24 experiments the control thresholds for both behavioural and EEG arousal were between 0.4 and 1.5 V. In the other 4 experiments the thresholds were between 2.0 and 4.0 V. and in these, it was found on subsequent histological examination of the brain, that the tip of the stimulating electrode was not in the reticular formation, as far as the limits of the latter could be determined. The results obtained with drugs in these experiments were in close agreement with those obtained in experiments where the control threshold was lower, and they have therefore been included in the relevant sections.

In establishing the control thresholds it was found that in many cases there was a slight difference between the threshold for behavioural arousal and that for EEG arousal. The difference was usually small, but it was consistent in that the EEG threshold was always the lower one.

II. *The effect of drugs on arousal thresholds.*

(a) *Pentobarbitone.* This drug, as the sodium salt, was injected intravenously in doses of 0.5 to 20 mg/kg. at intervals of approximately 20 min. A total dose of 1.5 mg/kg. was found to have no effect on arousal responses, but when this was increased to 3.5 mg/kg., the thresholds for both behavioural and EEG arousal showed a slight rise (fig. 2 B). At the same time it was found that arousal produced by afferent stimulation, such as clapping, blowing on the face or pinching the ears, was completely blocked and that these stimuli were no longer effective. With a total dose of 8-10 mg/kg., there was a further rise in the thresholds (fig. 2 C) and when the dose was increased above this level, arousal was in all cases completely abolished, even when stimulating voltages of up to 15 V. were used.

In these experiments, graphs have been plotted of the threshold (expressed as a percentage of the normal, i.e. that at the beginning of the experiment) against the total quantity of the drug. These threshold/dose response curves have been found to be consistent for the same drug in different experiments. The graph in figure 4 B represents the mean of five experiments with this drug.

(b) *Chlorpromazine.* The dose range used in 15 experiments with this drug was 0.05 to 20 mg/kg., and injections were made at intervals of 20 min. Very small doses were used

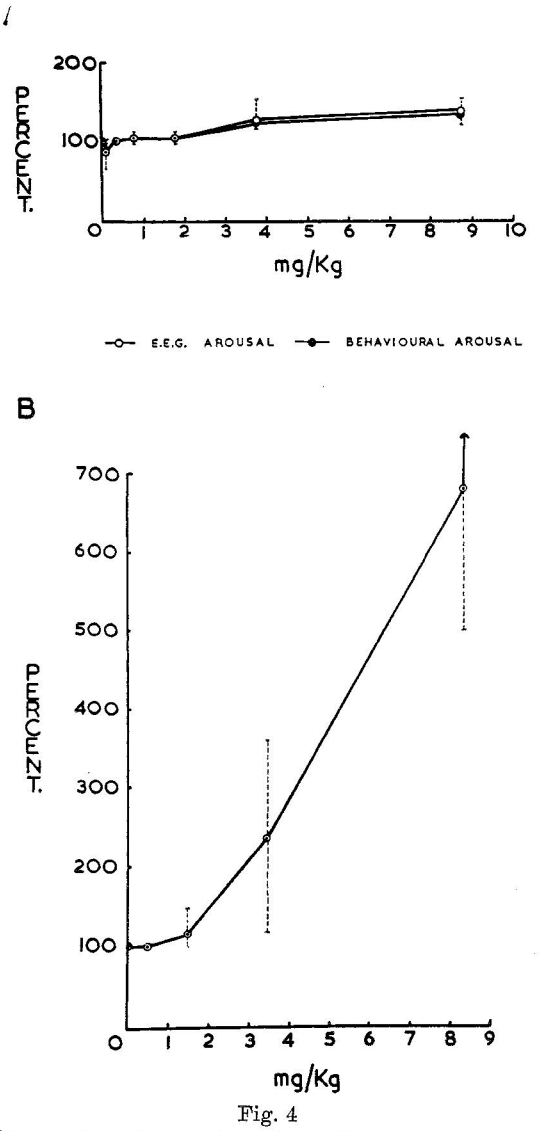

Fig. 4

Mean values of percentage change in arousal threshold plotted against dose. A: Chlorpromazine, B: Pentobarbitone. The dotted lines indicate the limits of experimental variation.

to start with because it had been observed in previous studies with this drug, that intravenous injections of 0.5 mg/kg. or less often caused a transient activation of the spontaneous electrical activity of the cortex in *encéphale isolé* preparations (Bradley and Hance 1955, 1957). In the present series of experiments it was found that doses of chlorpromazine between 0.1 and 0.3 mg/kg. often caused a slight fall in both the behavioural and EEG thresholds (fig. 4 A). This effect was observed in about one-third of the experiments with this drug. When the total dose was increased to 0.8 mg/kg., the thresholds returned to their original level. With doses between 2.0 and 4.0 mg/kg. it was seen that a slight rise in the thresholds for behavioural and EEG arousal occurred (fig. 3 C and D, fig. 4 A), and at the same time the preparation became unresponsive to afferent stimuli which would normally produce arousal. No further increase in the thresholds was observed with larger doses, though it was sometimes found difficult to establish the EEG threshold when the total dose exceeded 10 mg/kg. owing to the presence of artefacts in the record. These were thought to be due to the low or fluctuating blood pressure level which was observed following the injection of large doses of chlorpromazine.

In some experiments with this drug, two stimulating electrodes were utilized, one in the normal position in the mesencephalon, and the other in the medulla. The results obtained were the same for both positions of the stimulating electrode, however.

(c) *Atropine.* Atropine sulphate was given in 7 experiments in doses of 0.1 to 4.0 mg/kg. With 0.2 mg/kg. or less, no effects on the thresholds were seen, but as the dose was increased, large amplitude slow waves, such as have been observed in previous experiments with this drug, appeared in the electrocorticogram. These slow waves, which appeared singly at first, and then in groups, became more persistent as the dose level was increased. At the same time, the threshold for EEG arousal was raised (fig. 5), reaching a maximum at a dose level between 2.0 and 4.0 mg/kg. There was no change in the threshold for behavioural arousal, however, and the threshold/dose curves for EEG and behavioural arousal showed a wide divergence when this drug was used (fig. 5). In one experiment, atropine was administered to a preparation which was permanently alert, and it was seen that even after the largest doses of the drug (4.0 mg/kg.), the preparation remained behaviourally alert. In all experiments with this drug, after high amplitude, slow waves had been induced in the electrocorticogram by the injection of atropine, auditory or tactile stimuli caused behavioural alerting but did not in any way modify the EEG pattern of activity.

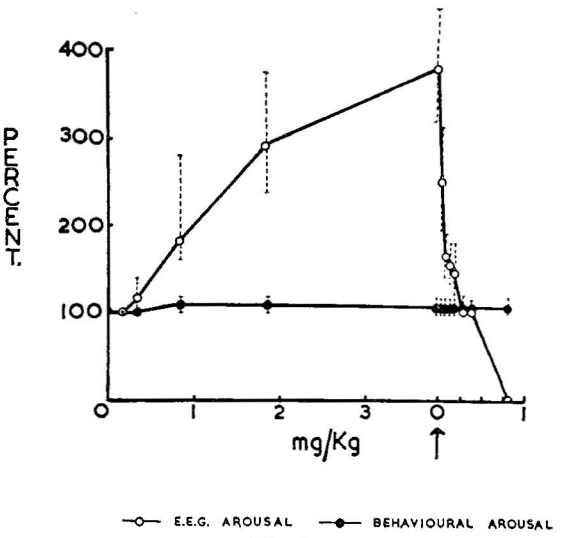

Fig. 5

Graph of mean values of percentage change in threshold for 5 experiments in which atropine was followed by physostigmine. The arrow indicates the point at which injections of physostigmine were started and the dotted lines the limits of experimental variation.

In one experiment atropine was injected after physostigmine had been administered in a dose sufficient to change the EEG pattern to one similar to that seen in the alert animal, but without change in the behaviour threshold, or alerting of behaviour. In this case the dose of atropine required to induce slow waves was slightly larger, but the effects on the arousal thresholds were identical to those observed in other experiments with this drug.

(d) *Hyoscine.* This drug, in the form of the sulphate, was injected in doses of 0.01 to 9.0 mg/kg. in three experiments and was found to produce effects similar to those ob-

served with atropine. With 0.03 mg/kg. or less, no effects were seen, but larger doses caused a progressive rise in the threshold for EEG arousal as slow activity appeared in the electrocorticogram. The maximum rise in the threshold appeared at a dose level of the order of 0.9 to 1.0 mg/kg. and a further increase in the dose after this produced no further change in threshold. There was, in the case of this drug, a slight rise in the threshold for behavioural arousal, following a total dose of about 1.0 mg/kg. but the graphs for EEG and behavioural arousal still showed a very marked divergence.

(e) *Physostigmine.* Physostigmine sulphate was used in 6 experiments in doses of 0.005 to 0.055 mg/kg. except where the drug was given after either atropine or hyoscine, when doses of up to 1.0 mg/kg. were used. In the normal *encéphale isolé* preparation, 0.005 to 0.020 mg/kg. had no effect on arousal responses, but 0.035 mg/kg. or more caused a desynchronization of the electrocorticogram. Thus the threshold for EEG arousal fell to zero, but at the same time the threshold for behavioural arousal did not change provided the doses used were minimal. It was found necessary to keep the dose as small as possible in order to avoid peripheral parasympathomimetic effects, since if these occurred, the behaviour of the preparation was affected.

The most striking effects with physostigmine were observed when it was injected following atropine. With doses of 0.01 to 0.8 mg/kg. the threshold for EEG arousal, which had been raised by atropine (fig. 5) was progressively lowered, as the slow activity was abolished by increasing amounts of physostigmine. The discrepancy between the thresholds for EEG and behavioural arousal was therefore diminished and the EEG threshold could be reduced to zero, but that for behavioural arousal remained unchanged, even when the EEG pattern was "alert". In figure 5 the point at which the administration of physostigmine was started is indicated by the arrow. Similar effects were observed when physostigmine was injected following hyoscine.

(f) *Amphetamine.* The racemic form of this compound, *dl*-amphetamine sulphate, was used in 6 experiments. It was injected in doses of 0.01 to 1.0 mg/kg. and the effects on arousal produced by afferent (auditory) stimulation, as well as on arousal produced by stimulation of the reticular formation were studied. The thresholds for behavioural and EEG arousal produced by direct stimulation of the reticular formation decreased steadily as the total quantity of amphetamine injected was increased (fig. 6 A). When a total dose of the order of

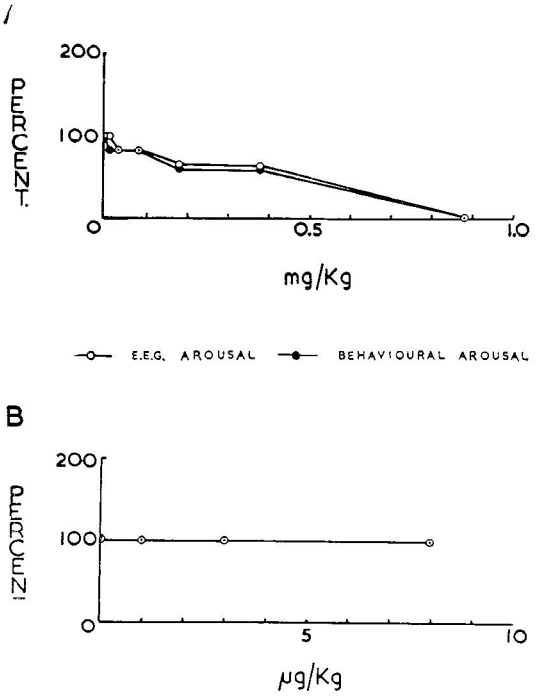

Fig. 6

Typical threshold/dose response curves for arousal produced by stimulation of the reticular formation, A: with *dl*-amphetamine and B: with LSD 25.

0.8 to 1.0 mg/kg. had been reached, the preparation remained alert, both in terms of behaviour and the electrical activity of the cortex. The threshold for arousal produced by auditory stimulation decreased in a similar manner (fig. 7 A), whilst that for click responses recorded from the auditory cortex showed little change. In the experiment from which the graph shown in figure 7 A was derived, there was a slight increase in the click threshold with the smallest dose of amphetamine.

(g) *d-Lysergic acid diethylamide (LSD 25).* This drug was injected in doses of 1.0

to 20 μg/kg. in 4 experiments and, as with amphetamine, the effects on arousal to afferent stimulation were also examined. Both stimulation positions in the reticular formation were used in most experiments. Doses of up to 20 μg/kg. were found to have no effect on arousal thresholds for direct stimulation of the reticular formation, irrespective of the position of the stimulating electrode (fig. 6 B). There was however, in the case of this drug, a marked change in the arousal threshold for auditory stimulation. Extremely small doses, of the order of 1.0 to 2.0 μg/kg., produced a marked fall in this threshold, sometimes as much as tenfold (fig. 7 B). This effect was

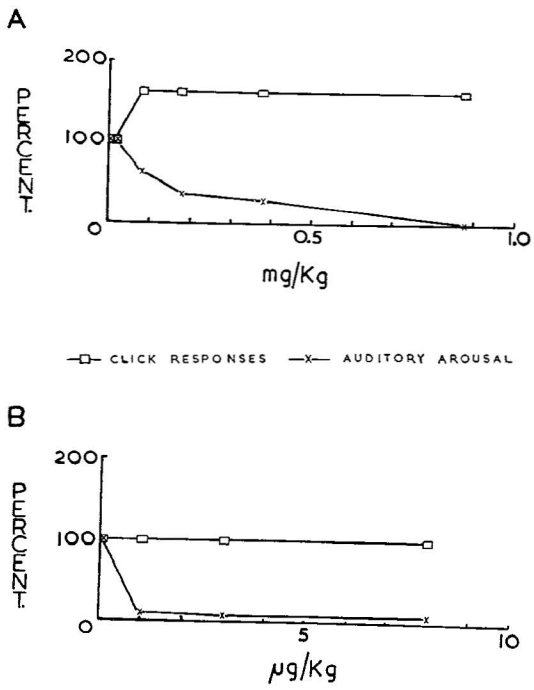

Fig. 7

Threshold/dose response curves for arousal produced by auditory stimulation and for click responses, obtained in the same experiments as those in figure 6. A: *dl*-amphetamine, B: LSD 25.

so striking that it was difficult to get the preparations to show sleep activity after these doses of the drug, so sensitive did they appear to be to the slightest noise. For example, the sound of the pencil with which the protocol was being written would often cause arousal. However, if all extraneous noise was rigidly excluded, the preparation would still sleep, and the arousal threshold for stimulation of the reticular formation was found to be unchanged. The threshold for click responses, recorded from the auditory cortex, was also unaffected (fig. 7 B).

DISCUSSION

The striking differences between the threshold/dose response curves obtained with the different drugs used in these experiments, together with the fact that similar curves were obtained in all experiments with the same drug, indicates that the method may be a useful one for quantitative studies of the action of drugs in relation to the reticular formation of the brain.

The results obtained with pentobarbitone confirm the findings of other workers that barbiturates, in quite small doses, block arousal responses. Arduini and Arduini (1954) found that 10 mg/kg. of pentobarbitone was sufficient to block arousal produced either by stimulation of the reticular formation or by peripheral (olfactory) stimulation. Killam and Killam (1956) and King (1956) observed a similar blocking of EEG arousal with doses of 5 to 20 mg/kg. of the same drug. In the experiments described here, not only EEG arousal but behavioural arousal produced by reticular stimulation was also blocked by doses of pentobarbitone which were small when compared to the normal anaesthetic dose in the cat (30-35 mg/kg.). These results further support the suggestion by French *et al.* (1953) that barbiturates depress conduction in the medial pathways at the brain stem level.

The results obtained with chlorpromazine are in complete contrast to those obtained with pentobarbitone, since even with the largest doses of chlorpromazine (20 mg/kg.), the increase in the arousal threshold was relatively small and complete blocking of arousal was never observed with this drug. This finding is in agreement with the observations of Killam and Killam (1956). The only similarity between the effects of chlorpromazine and those of pentobarbitone is that in both cases, the effects on the EEG threshold paralleled those on behaviour. The fact that, in many experiments the arousal thresholds showed a slight fall when small doses of chlorpromazine were injected confirms the previous observation that this drug, in small doses, has an

excitant action when injected intravenously (Bradley and Hance 1955, 1957).

The differences between the effects of pentobarbitone and chlorpromazine as demonstrated by their threshold/dose response curves (fig. 4), point to differences in the modes of action of these two drugs. Thus, the central effects of chlorpromazine may not be due to a direct depression of the reticular activating system, as would appear to be the case with barbiturates, although it has been claimed (Longo *et al.* 1954) that arousal produced by stimulation of the rostral part of the reticular formation in the rabbit was blocked by this drug. Hiebel, Bonvallet and Dell (1954), working with curarized cat preparations, also found that EEG arousal produced by stimulation of the reticular formation was blocked by chlorpromazine unless the stimulating voltage was considerably increased. In our experiments, the rise in the threshold, even with the largest doses of chlorpromazine, was never more than 50 per cent. If in their experiments, Longo and his co-workers did not alter the strength of the stimulus, then it is possible that what they interpreted as a blocking of the response was, in fact, a rise in the threshold.

The blocking of arousal responses to afferent stimulation following chlorpromazine has been reported by numerous workers (Longo *et al.* 1954; Hiebel *et al.* 1954; Bradley and Hance 1955, 1957). This effect is also observed with barbiturates but here the effect can be explained by the blocking of the arousal response within the reticular formation itself. This explanation will not hold in the case of chlorpromazine however, and it appears that the blocking of afferent arousal is a more specific effect in the case of this drug. One possible explanation (Bradley and Elkes 1956) is that chlorpromazine might produce its effect by an action more closely related to the afferent collaterals entering the reticular formation at the brain stem level.

Atropine and physostigmine affected only the threshold for EEG arousal produced by stimulation of the reticular formation and not the threshold for behavioural arousal, so that the threshold/dose response curves for these drugs show a wide divergence. This is perhaps not entirely unexpected, since these two drugs are known to cause a dissociation between EEG and behaviour in the conscious animal; atropine inducing a pattern of high amplitude slow waves without inducing sleep, and physostigmine causing the appearance of low amplitude, fast activity in the EEG, but without the animal becoming alert (Wikler 1952; Bradley and Elkes 1953a, 1957). Moreover, since it had been found that both drugs still produced their characteristic changes in the electrocorticogram after transection of the mid-brain, it was suggested (Bradley and Elkes 1956, 1957) that it is unlikely that these drugs produce their effects by an action only at the brain stem level and that the arousal system is probably not activated by a predominantly cholinergic mechanism. The results of the experiments described here support this view, since the blocking of the slow waves by reticular stimulation cannot be regarded as arousal unless it is accompanied by behavioural changes.

Rinaldi and Himwich (1955 a and b) in studies in the rabbit, claim that atropine is "a universal inhibitor of alerting reactions". They suggest that atropine acts on the "mesodiencephalic activating system" and that this system is cholinergic in nature. Since they studied only alerting of the electrocorticogram, they failed to observe that alerting of behaviour was unaffected by atropine. Furthermore, the mesodiencephalic activating system has not been defined either in functional or anatomical terms, but it would appear to include both the mid-brain reticular formation (Moruzzi and Magoun 1949) and the diffuse thalamic projection system (Jasper 1949). Our reasons for suggesting that atropine and physostigmine do not act at the brain stem level have already been stated. In the present series of experiments both drugs produced graded changes in the threshold for EEG arousal, and even with the largest doses of atropine, EEG activation could still be produced by stimulation of the reticular formation. It is therefore possible that these drugs may be acting on a system which is affected by the ascending influence of the reticular activating system, but which is not directly involved in neurological mechanisms governing

wakefulness and sleep. This system might well be the diffuse thalamic projection system, but it is unlikely to include the mid-brain reticular formation as it is well known that lesions at this level can lead to somnolence (Lindsley *et al.* 1950).

In a study of the effects of a series of atropine derivatives on the electrical activity of the brain in the conscious animal it had been observed that hyoscine induced slow waves in the electrocorticogram in smaller doses than atropine, but that it also produced slight sedation (Hance 1956). This drug was therefore included in the present series of experiments for comparison with atropine. The findings that the effects of hyoscine on EEG arousal were similar to those of atropine but that smaller doses were effective, and that the threshold for behavioural arousal also showed a slight rise, are consistent with previous observations with this drug.

The threshold/dose response curves obtained with *dl*-amphetamine showed a progressive fall in both EEG and behaviour thresholds for arousal with increasing doses until the preparations eventually became completely alert. The dose level at which this effect was obtained was the same as that which had been found to produce alerting of the spontaneous electrical activity and behaviour in previous experiments in the *encéphale isolé*. Since this drug had been observed to cause alerting of the EEG and behaviour in the conscious animal, but not in the *cerveau isolé*, it has been suggested that its site of action might be on receptors situated at the level of the brain stem reticular formation (Bradley and Elkes 1953b, 1957). The results obtained in the present series of experiments serve to confirm the close correlation between the EEG and behavioural effects of amphetamine and support the hypothesis that the site of action of this drug is closely related to the arousal system at the brain stem level.

Previous investigations with LSD 25 have shown that this drug also causes alerting of electrical activity and behaviour in the conscious animal, but that its effects appear to be dependant upon the precise environmental conditions, whereas this was not the case with amphetamine. Further, LSD 25 was found to have no effect upon the spontaneous electrical activity of the *encéphale isolé* preparation. The results of the experiments described here emphasize further the differences between the effects of amphetamine and LSD 25. LSD 25 caused no change in the threshold for direct stimulation of the reticular formation in the doses used, but quite small doses caused a marked fall in the threshold for arousal produced by auditory stimulation and the preparations became noticeably more sensitive to external stimuli after this drug had been given. With amphetamine the change in this threshold was relatively slight. These observations are consistent with the results of previous experiments (Bradley and Elkes 1957) in which it had been observed that if all extraneous sources of external stimulation (such as noise) were cut off, the alerting effect of LSD 25 in the conscious animal was greatly reduced.

It therefore seems possible that whilst amphetamine may act directly on the reticular formation and so produce alerting which is independant of the external environment, the action of LSD 25 might be more closely related to incoming influences from the environment. The absence of an effect on the threshold for click responses recorded from the auditory cortex suggests that the action of the drug is not on the afferent pathway itself. The effects of LSD 25 can be explained however, by an action at the level of the reticular formation, related to the influence of incoming impulses via the collaterals from the afferent pathways. Thus the action of this drug might be to sensitize the reticular activating system to these influences, rather than to excite it directly. This hypothesis might explain the lack of effect of LSD 25 on the spontaneous activity of the *encéphale isolé* preparation, since many afferents which normally feed into the reticular formation (Amassian and Devite 1954; Schiebel *et al.* 1955) are cut off in this preparation. It is also in keeping with the findings that in normal human subjects LSD 25 increased the responsiveness of the alpha rhythm to visual stimuli (Bradley, Elkes and Elkes 1953). In the intact conscious animal, in an environment providing a moderate level of excitation, the

effects of LSD 25 might appear to resemble more closely those of amphetamine.

These investigations into the effects of drugs on arousal responses, the results of which are summarized in table I, lead to the hypothesis of three different sites of action within the central nervous system for the drugs studied. Firstly, some drugs may act directly on the reticular activating system, either with a depressant action (eg. pentobarbitone) or an excitant action (eg. amphetamine). Secondly, certain drugs may have an action at this level which is less directly concerned with the arousal system, but which appears to be more closely related to the

TABLE I

	E.E.G. AROUSAL		BEHAVIOURAL AROUSAL	
	RET. STIM.	AFF. STIM.	RET. STIM.	AFF. STIM.
PENTOBARBITONE	↑	↑	↑	↑
AMPHETAMINE	↓	↓	↓	↓
CHLORPROMAZINE	↗	↑	↗	↑
L.S.D. 25	O	↓	O	↓
ATROPINE	↑	↑	O	O
PHYSOSTIGMINE	↓	—	O	O

↑ rise in threshold. ↓ fall in threshold. O no effect. ↗ a slight rise in threshold.

influence of afferent collaterals entering the reticular formation. This too may be depressant (chlorpromazine) or excitant (LSD 25). Finally, we have the cholinergic and cholinolytic drugs such as physostigmine and atropine, whose site of action does not appear to be primarily at the brain stem level, but which may be more diffuse or perhaps related to thalamocortical projections. Further studies will be necessary in order to prove or disprove the validity of this hypothesis and to determine more precisely the sites of action of these drugs. Some of these studies are at present in progress.

SUMMARY

Thresholds for behavioural and EEG arousal produced both by electrical stimulation of the reticular formation and by afferent (auditory) stimulation have been determined in *encéphale isolé* cat preparations. The effect of graded doses of pentobarbitone, chlorpromazine, atropine, hyoscine, physostigmine, amphetamine and *d*-lysergic acid diethylamide (LSD 25) on these thresholds were studied.

Pentobarbitone, in doses considerably smaller than the anaesthetic dose, blocked arousal responses completely, but chlorpromazine, even in large doses, caused only a slight rise in the thresholds for direct stimulation of the reticular formation. It blocked arousal produced by afferent stimulation.

Atropine, hyoscine and physostigmine produced a marked divergence between behavioural and EEG thresholds; atropine caused a rise in the EEG threshold and physostigmine a fall, whilst the behavioural threshold remained unchanged. The effects of hyoscine were similar to those of atropine except that it caused a slight rise in the threshold for behavioural arousal, consistent with a slight sedative effect.

Amphetamine caused the thresholds to fall until a dose was reached at which the preparation was fully alert, both behaviourally and in terms of the EEG activity. LSD 25 had no effect on the arousal threshold for stimulation of the reticular formation but produced a marked lowering of the threshold for arousal to afferent (auditory) stimulation.

The relation between these findings and the results of previous investigations is discussed, together with the possible sites of action of the drugs studied. Three different sites of action are postulated: (*a*) a direct action on the reticular activating system (pentobarbitone and amphetamine); (*b*) an action at the brain stem level but related to afferent collaterals entering the reticular formation (chlorpromazine and LSD 25), and (*c*) an action not directly related to the reticular formation of the brain stem but possibly to diffuse thalamocortical projections (atropine and physostigmine).

RÉSUMÉ

Les seuils d'excitabilité pour le déclenchement d'une réaction d'éveil par stimulation électrique de la formation réticulaire du tronc cérébral et par des stimuli afférents (auditifs), telle qu'elle se manifeste dans le comportement de l'animal et dans l'EEG, ont été déterminés chez des chats avec encéphale isolé. Les effets de doses graduées de pentobarbitone, chlorpromazine, atropine, hyoscine, physostigmine, amphétamine et du diéthylamide de l'acide lysergique (LSD 25) sur ces seuils d'excitabilité ont été étudiés.

Des doses subnarcotiques de pentobarbitone ont produit un bloquage complet des réactions d'éveil. La chlorpromazine d'autre part même à des doses considérables n'a élevé le seuil d'excitabilité pour la stimulation électrique de la formation réticulée que d'une façon légère, tandis que les réactions d'éveil par des stimuli afférents sensoriels ont été bloqués par cette substance.

Les réactions d'éveil déterminées sur la base du comportement de l'animal et celles déterminées sur la base des réactions électroencéphalographiques ont montré une divergence marquée de leurs seuils d'excitabilité respectifs sous l'influence de l'atropine, de l'hyoscine et de la physostigmine. L'atropine a produit une élévation du seuil de la réaction d'éveil dans l'EEG et la physostigmine a produit un abaissement de ce seuil, tandis que les seuils de la réaction d'éveil basés sur le comportement n'ont accusé aucun changement. L'effet de l'hyoscine a été semblable à celui de l'atropine.

L'amphétamine a produit un abaissement des seuils de la réaction d'éveil augmentant avec la dose jusqu'à un point où un état de vigilance complète a été obtenu aussi bien dans le comportement que dans le tracé électroencéphalographique. Le diéxylamide de l'acide lysergique n'a exercé aucun effet sur les seuils des réactions d'éveil produit par stimulation de la formation réticulée, mais a produit une diminution marquée du seuil de la réaction d'éveil produite par des stimuli afférents sensoriels. Pour expliquer les effets des substances étudiées trois différents lieux d'action doivent être considérés: (*a*) une action directe sur le système activateur de la formation réticulée du tronc cérébral (pentobarbitone et amphétamine); (*b*) un effet au niveau du tronc cérébral sur les collatérales des systèmes afférents au lieu de leur entrée dans la substance réticulée (chlorpromazine et diéthylamide de l'acide lysergique), et finalement (*c*) une action possible sur les systèmes de projection diffuse thalamo-corticale (atropine et physostigmine).

ZUSAMMENFASSUNG

Reizschwellen für Weckreaktionen ausgelöst durch elektrische Reizung der retikulären Formation des Hirnstammes und durch afferente (akustische) Reizung, wie sie sich im Verhalten oder im EEG manifestieren wurden in Katzen mit *encéphale isolé* bestimmt. Die Wirkung abgestufter Dosen von Pentobarbiton, Chlorpromazin, Atropin, Hyoscin, Physostigmin, Amphetamin und d-Lysergsäure-Diaethylamid (LSD 25) auf diese Reizschwellen wurde untersucht.

Pentobarbiton in Dosen, welche viel kleiner waren als die welche notwendig sind Narkose herbeizuführen, verursachte eine komplette Blockierung der Weckreaktionen, während Chlorpromazin, selbst in grossen Dosen, die Reizschwelle für elektrische Reizung der retikulären Formation nur leicht erhöhte, jedoch Weckreaktionen durch afferente sensorische Reize blockierte.

Weckreaktionen bestimmt auf Grund des Verhaltens des Versuchstiers und solche bestimmt auf Grund des Elektroenzephalogramms zeigten eine starke Divergenz ihrer respektiven Reizschwellen unter dem Einfluss von Atropin, Hyoscin and Physostigmin. Atropin erzeugte eine Erhöhung der EEG-Weckreizschwelle und Physostigmin eine Erniedrigung derselben, während die Reizschwellen, bestimmt auf Grund des Verhaltens, unverändert blieben. Die Wirkung von Hyoscin war ähnlich derjenigen von Atropin.

Amphetamin produzierte eine Erniedrigung der Reizschwellen solange bis eine Dosis erreicht war bei welcher das Tier vollständig wach war sowohl in seiner Verhaltensweise als auch in seinem EEG. LSD 25 hatte keinen Effekt auf die Reizschwelle der

Weckreaktion durch elektrische Reizung der Retikulärformation, produzierte jedoch eine markante Erniedrigung der Reizschwelle für afferente, sensorische Reize.

Drei verschiedene Angriffsspunkte der Pharmaka werden in Betracht gezogen: (*a*) direkter Effekt auf das aktivierende retikuläre System (Pentobarbiton und Amphetamin); (*b*) eine Wirkung im Hirnstammgebiet auf die Kollateralen afferenter Fasersysteme, welche in die Substantia reticularis einmünden (Chlorpromazin und LSD 25), und schliesslich (*c*) möglicherweise eine Wirkung auf diffuse thalamo-kortikale Projektionssysteme (Atropin und Physostigmin).

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