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A COMPARATIVE STUDY OF THE EFFECTS OF DRUGS ON THE AROUSAL SYSTEM OF THE BRAIN

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The effects of twelve drugs, eleven of which are reported to be tranquillizers, have been studied in relation to thresholds for arousal produced both by direct stimulation of the brain stem reticular formation and also by afferent nerve stimulation. The drugs can be grouped according to whether (a) like chlorpromazine they produce a slight rise in thresholds for brain stem stimulation and block sensory-induced arousal, (b) cause dissociation between behaviour and electrical activity of the brain, (c) have no effects on thresholds for brain stem stimulation and only slight effects on afferent nerve-induced arousal or (d) have no effect on arousal responses at all.

The effects of twelve different drugs on arousal responses have been investigated. Most of the drugs chosen for the present study have been classed as tranquillizers on the basis of their clinical effects although there are differing reports regarding the clinical efficacy of some of them. It is, however, not our intention to discuss this question here.

Much of the early experimental work with chlorpromazine suggested an action at the level of the reticular activating system of the brain stem (Terzian, 1952; Longo, von Berger, and Bovet, 1954; Hiebel, Bonvallet, and Dell, 1954) and further investigations (Bradley and Hance, 1957) confirmed this. The drug blocked the behavioural and electroencephalographic effects of arousal stimuli, both in the conscious unrestrained animal and in acute preparations (*encéphale isolé*) and also blocked the excitant action of amphetamine, which is itself thought to act at the brain stem level (Bradley and Elkes, 1957). A more detailed study of the effects of drugs on arousal responses (Bradley and Key, 1958) demonstrated that chlorpromazine had little depressant action on the brain stem activating system itself but produced a selective blocking of the afferent influences on this system. This led to the hypothesis that chlorpromazine might act by depressing the collateral inflow into the reticular formation from the afferent pathways. So far no evidence has been produced which suggests that this hypothesis is wrong and further studies support it (Bradley, 1957; Key and Bradley, 1958). On the other hand we have not attempted to suggest that this particular central action of chlorpromazine is solely responsible for its clinical properties or that it is the only action of this drug on the central nervous system.

The tranquillizers form a group which, for almost all investigated properties, is so heterogeneous that we thought it might be worth while to examine the effects of a number of these compounds upon the arousal system. In this way it should be possible to see how far the hypothesis which has been elaborated to explain the action of chlorpromazine in neurophysiological terms might be extended to include other tranquillizers.

METHODS

Experiments were carried out on 56 adult cats, all of which were *encéphale isolé* preparations (Bremer, 1936). The animals were prepared under ether anaesthesia using a technique which has been described by Bradley and Key (1958). Twelve to sixteen cortical electrodes (Bradley and Elkes, 1953) were inserted through small burr holes in the skull over both cerebral hemispheres, and an earthing electrode was put in the midline over the frontal sinus.

A concentric, bipolar stimulating electrode, with the points separated by about 1 mm., was orientated in the brain stem reticular formation by means of a Horsley-Clarke stereotactic instrument, using co-ordinates obtained from an atlas of the cat brain. When the stimulating electrode was in the correct position it was fixed with dental cement and the animal was released from the stereotactic instrument. Local anaesthetic (procaine) was infiltrated in the skin and muscle over the top of the head and at least 1 hr. was allowed to elapse following the removal of ether before any measurements of thresholds were made.

The electrocorticogram was recorded on a 4-channel Ediswan portable pen recorder and stimuli were delivered from an electronic stimulator remotely controlled. The stimulus lasted for a period of 10 sec. and consisted of rectangular pulses of 1 msec. duration at a frequency of 300 cycles/sec. The thresholds were determined by gradually increasing the applied voltage in steps, each lasting for 10 sec., starting from 0.1 V. The electrocorticogram was recorded continuously and, when the threshold for a behavioural response was reached (recognized by opening of the eyes and contraction of the nictitating membrane), the record was examined to see at what voltage it had been activated. This was taken as the threshold for electrocortical arousal.

In some experiments the threshold for arousal produced by sensory stimulation was also determined. An auditory stimulus of constant frequency was used, obtained from a loudspeaker placed at a fixed distance from the head of the cat. The procedure for determining the arousal threshold was the same as that for stimulation of the brain stem; the voltage which was applied to the loudspeaker and which caused arousal was taken as a measure of the threshold. In these experiments the thresholds for click responses at the auditory cortex were also determined.

The drugs studied were: chlorpromazine, promazine, acepromazine, imipramine, hydroxyzine, benactyzine, hyoscine, meprobamate, azacyclonal, reserpine, deserpidine, and rescinnamine. With the exception of the last three, all the drugs were injected intravenously in incremental doses and the arousal thresholds checked twice after each injection, usually after 10 and 20 min. Reserpine, deserpidine, and rescinnamine appear to have a delayed action and a single dose of these drugs was injected and the thresholds checked at 30 min. intervals.

At the end of each experiment the animal was killed and the brain perfused with formal-saline with the stimulating electrode still in place. The position of the tip could be subsequently determined by histological examination.

RESULTS

The *encéphale isolé* preparation shows fluctuating states of wakefulness and sleep. These two states are easily distinguished by the appearance of the head and by the electrical activity of the brain. In the waking and apparently "alert" state, movements of the ears, eyes, and occasionally the jaws and vibrissae may be seen together with a pupillary response to light. The electrical activity of the cortex is fast and of low voltage, similar to that in the intact animal in the alert state (Rheinberger and Jasper, 1937; Bradley and Elkes, 1957). When the animal is in the drowsy or "sleeping" state the nictitating membrane is relaxed, the eyes are rotated upwards and the pupil is fixed and constricted, whilst the electrocorticogram is dominated by slow waves at 1 to 4 cycles/sec. and spindle activity at 6 to 12 cycles/sec. This correspondence between the electrical activity and the behavioural state in these preparations is always maintained, although individual variations in the pattern of "sleep activity" can often be recorded.

Activation of the electrocorticogram and behavioural arousal can still be evoked in *encéphale isolé* preparations by certain types of sensory stimuli, since the brain is capable of receiving afferent impulses from the intact cranial nerves. In the present series of experiments, electrical stimulation of the brain stem reticular formation produced almost identical responses, except that the period of activation following the stimulus was usually longer. The transition from the "sleeping" to the "alert" state was abrupt and well marked.

Control experiments were carried out to determine the viability of the preparations and the stability of the arousal thresholds. Thresholds for behavioural arousal produced by stimulation of the reticular formation, determined over periods of 2 to 4 hr., showed very little variation. The first sign of deterioration in the preparation was usually a slow falling of blood pressure. Following this, the behavioural response became less marked and artefacts appeared in the electrocorticogram which made the determination of the arousal threshold more difficult.

Experiments were also undertaken to determine the stability of arousal responses produced by an auditory stimulus of constant frequency. Thresholds were first determined and then checked every 20 min. over 3 to 4 hr. In all preparations habituation to the stimulus developed very rapidly. The duration of the period of activation in the electrocorticogram progressively decreased and at the fourth trial behavioural arousal was indicated by only a slight movement of the ears. By the fifth to sixth trial, stronger stimuli were needed to produce arousal, but even then the responses were weak. In the eighth and subsequent trials the stimulus failed to produce any alteration in the electrocorticogram or in the behaviour of the animal even when the intensity of the stimulus was increased considerably above control levels (Fig. 2*a*). If, at this stage, the frequency of the auditory stimulus was changed, or another of marked qualitative difference was presented, arousal was immediately obtained. Similarly, pinching the ear or blowing on the face produced arousal.

Chlorpromazine

The effects of this drug on the thresholds for arousal produced by stimulation of the brain stem

have already been reported (Bradley and Key, 1958). It was found in fifteen experiments that doses of 2.0 to 4.0 mg./kg. of body weight caused a slight rise in thresholds for both electrocortical and behavioural arousal (Fig. 1*a*). Larger doses caused no further change in thresholds and, above 10 mg./kg., artefacts appeared in the electrocorticogram. These were thought to be due to changes in blood pressure induced by such large doses of chlorpromazine. An interesting feature of about one-third of these experiments was that very small doses of chlorpromazine (0.1 to 0.5 mg./kg.) caused a slight fall in the behavioural and electrocorticographic thresholds, but with a total dose of 0.8 mg./kg. the thresholds returned to their original levels. This effect was not observed with any of the other drugs tested.

In contrast to these results, the effect of chlorpromazine on arousal responses produced by sensory stimulation was quite marked. Thresholds for behavioural arousal and electrocorticographic activation produced by auditory stimulation remained unaltered with doses of chlorpromazine between 0.1 and 0.3 mg./kg., but when the dose was increased from 0.5 to 1.8 mg./kg. both thresholds showed a progressive rise (Fig. 2*b*). Moreover, the arousal responses themselves became less marked. For example, after 0.8 mg./kg. the period of activation in the electrocorticogram was shorter and did not usually outlast the period of stimulation, whilst behavioural arousal was indicated by only a slight movement of the ears and a transient and partial opening of the eyes. When the total dose was increased to 2.0 to 4.0 mg./kg., auditory-evoked arousal was blocked completely, irrespective of the frequency or quality of the auditory stimulus. At the same time, the preparation became unresponsive to other types of sensory stimulation, although pain (caused by pinching the ears) still produced some response. The threshold for click responses recorded at the auditory cortex remained unchanged throughout the experiment.

In two experiments, (±)-amphetamine (4.0 mg./kg.) was given after chlorpromazine, but this drug was without effect on the electrical activity of the cortex or on the arousal thresholds, and the behavioural state induced by chlorpromazine was not modified. These observations confirm previous studies (Bradley and Hance, 1957).

Promazine

The effect of promazine on arousal responses produced by stimulation of the reticular formation was studied in three experiments. The drug was injected in doses of 0.1 to 20.0 mg./kg. No fall in threshold was observed with small doses (0.1 to 0.3 mg./kg.) although there was transient rise and fall in blood pressure similar to that seen with chlorpromazine. When the total dose was increased to 1 to 2 mg./kg., a slight rise in both behavioural and electrocortical arousal thresholds occurred (Fig. 1*b*). Increasing the dose further, however, did not produce any additional change although high doses (5 to 10 mg./kg.) caused large blood pressure fluctuations which affected the electrical activity of the cortex.

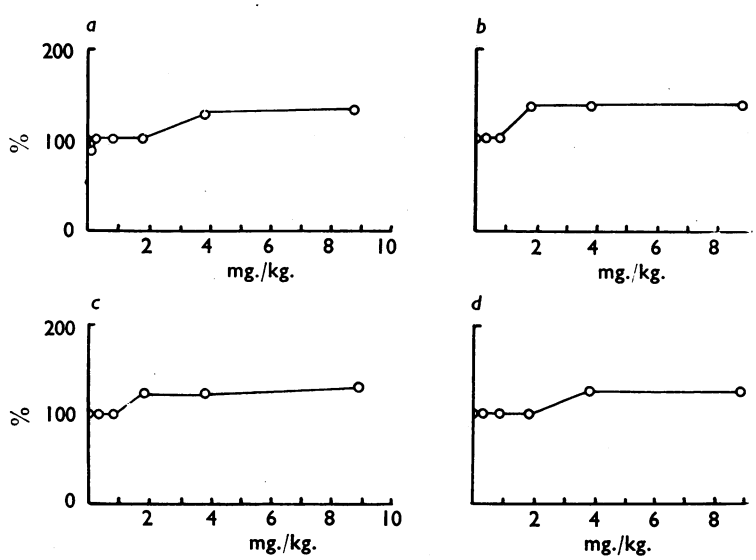

FIG. 1.—The effects of four drugs on thresholds for arousal produced by stimulation of the brain stem reticular formation. The mean % change in threshold for all experiments has been plotted against dose. *a*, Chlorpromazine; *b*, promazine; *c*, acepromazine; and *d*, hydroxyzine.

The effects of promazine on auditory-induced arousal were great and similar in many ways to those of chlorpromazine. With doses of 0.3 to 2.0 mg./kg., the preparations became less easy to disturb and, following 1.0 to 2.0 mg./kg., very intense auditory stimulation produced only a transient change in the electrocorticogram. Similarly, visual and tactile stimuli which had previously evoked arousal responses now failed to do so. Increasing the dose above 2.0 mg./kg. usually blocked arousal induced by afferent stimuli completely although pain stimuli sometimes still produced a

transient activation of the electrocorticogram and an alerting of behaviour.

Physostigmine and atropine given with promazine produced their characteristic effects on the electrical activity of the cortex without affecting the behaviour of the animal. Amphetamine, in large doses (4 to 6 mg./kg.), brought about a slight increase in the responsiveness of the preparation to afferent nerve stimulation, but the full effects of amphetamine on the electrical activity of the cortex and the behaviour of the animal were never observed. Thus, amphetamine appeared to block only partially the effects of promazine.

Acepromazine

Acepromazine was injected in three experiments in doses of 0.1 to 20.0 mg./kg. Qualitatively and quantitatively the effects on arousal responses alone or with atropine, physostigmine, and amphetamine did not appear to be different from those of promazine. After 1.0 to 2.0 mg./kg. of acepromazine there was a slight increase in the thresholds for arousal produced by stimulation of the brain stem reticular formation (Fig. 1*c*). Concomitantly, the preparations became unresponsive to visual, auditory, and tactile stimulation.

Hydroxyzine

Hydroxyzine (10 mg./kg.) had very little effect on the electrical activity of the brain in *encéphale isolé* preparations. There was usually an increase in the amount of slow activity, but this was always associated with increased drowsiness of the animal.

Although periods of spontaneous electrocorticographic activation and behavioural alertness were noted, they were less frequent than in the control period before the drug. Furthermore, physostigmine and atropine still produced their characteristic effects on electrical activity when given with hydroxyzine, but they did not change he behavioural state of the animal. These effects closely paralleled those when chlorpromazine was used with these two drugs. However, whereas chlorpromazine completely blocked the effects of amphetamine both on the electrical activity of the brain and on behaviour, the latter drug still produced some effect when it was given after hydroxyzine, although there was never complete alerting. Thus, hydroxyzine like promazine appeared to block only partially the effects of amphetamine.

The effect of hydroxyzine on arousal responses produced by stimulation of the reticular formation

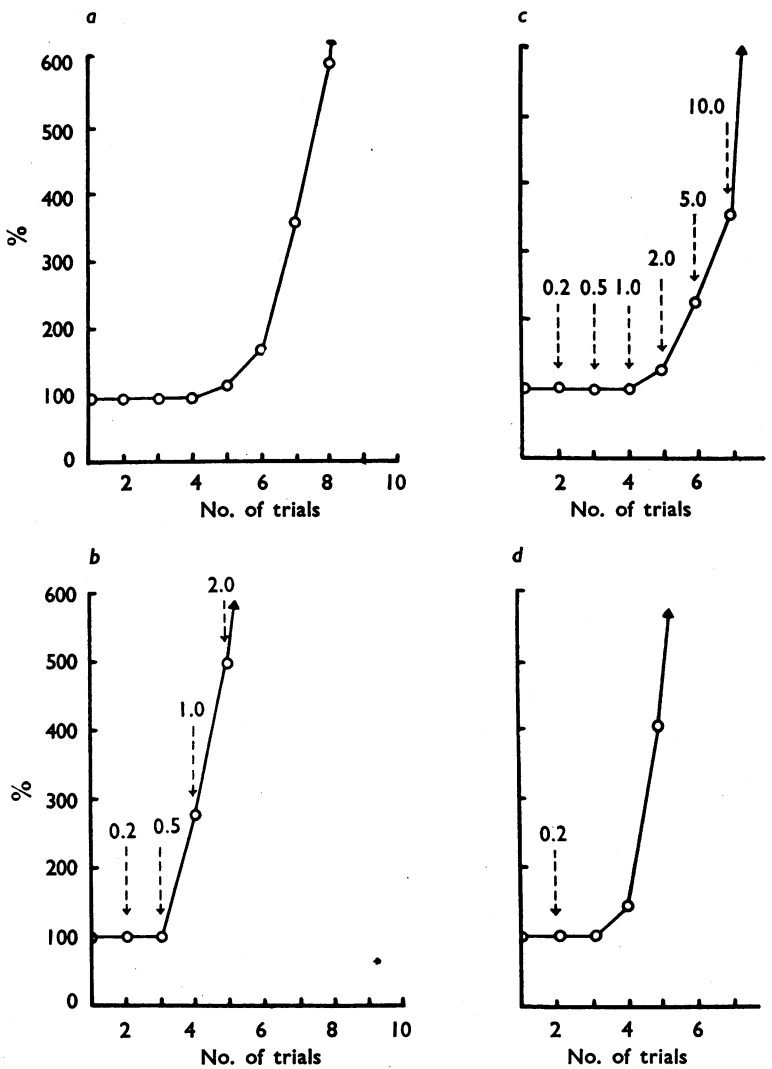

FIG. 2.—The effects of drugs and habituation on thresholds for arousal produced by afferent nerve (auditory) stimulation. The mean % change in threshold for all experiments has been plotted against the number of trials. The injections of drugs are indicated by the arrows, with doses in mg./kg. *a*, Habituation; *b*, chlorpromazine; *c*, azacyclonal; and *d*, reserpine.

was studied in four experiments. The drug was injected intravenously in incremental doses from 0.1 to 8.8 mg./kg. and thresholds for arousal were found to be unchanged until a total dose of 2 to 4 mg./kg. was reached. With this dose both electrocortical and behavioural arousal thresholds showed a slight but consistent rise (Fig. 1*d*). No further increase in the threshold was observed with larger doses, although it was sometimes difficult to establish the electrocorticographic threshold owing to the low blood pressure brought about by the large doses of this drug.

The effects of hydroxyzine on arousal responses induced by sensory stimuli were also studied and they were found to be similar in many ways to those observed with chlorpromazine. Doses of 0.8 to 1.8 mg./kg. caused a progressive rise in the thresholds for both the electrocorticographic and behavioural arousal, and when a total dose of 2 to 4 mg./kg. was reached arousal responses to auditory stimuli were completely blocked, irrespective of the frequency. Sometimes an intense auditory stimulus, qualitatively different from the previous one, would produce an effect when first applied, but this soon disappeared. Similarly, visual and tactile stimuli failed to evoke an arousal although the response to pain was little altered. The threshold for click responses recorded at the auditory cortex remained unchanged even when the sensory-induced arousal responses were blocked.

Benactyzine

Benactyzine was given in three experiments in doses of 0.1 to 4.0 mg./kg. With 0.2 mg./kg. or less, no effects on the thresholds for arousal produced by stimulation of the brain stem were seen, but as the dose was increased above 0.5 mg./kg., large amplitude slow waves, similar to those after atropine, appeared in the electrocorticogram. At the same time the threshold for electrocortical arousal was raised, reaching a maximum with doses between 3.0 and 4.0 mg./kg. (Fig. 3*a*). The effect of benactyzine on the threshold for electrocorticographic arousal was not associated with any corresponding change in that for behaviour and the threshold/dose curves for electrocorticogram and behavioural arousal showed a wide divergence. A similar dissociation was also noted in the threshold/dose curve for arousal produced by afferent (auditory) stimulation. After 0.8 to 2.0 mg./kg. of benactyzine activation of the electrocorticogram by auditory, visual or tactile stimuli was blocked completely but there was no observable effect on the behavioural alerting response. Moreover, amphetamine given in conjunction with benactyzine always caused

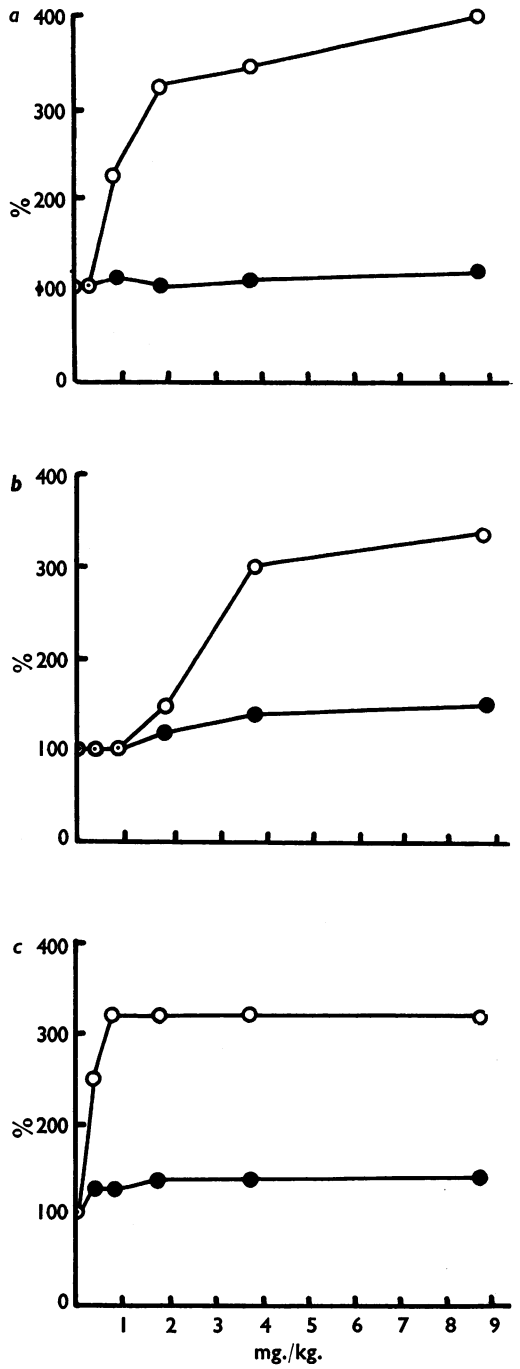

FIG. 3.—The effects of drugs on thresholds for behavioural arousal (solid circles) and electrocortical activation (open circles) produced by stimulation of the brain stem reticular formation. The mean % change in thresholds for all experiments has been plotted against dose. *a*, Benactyzine; *b*, imipramine; and *c*, hyoscine.

alerting of behaviour without affecting the electrical activity of the cortex. Physostigmine desynchronized the electrocorticogram without producing behavioural alerting.

Imipramine

Imipramine is an aminodibenzyl derivative which bears certain structural resemblances to chlorpromazine. For this reason and the fact that recent studies have shown that this drug may be of use in clinical psychiatry in the treatment of depression, it was included in the present study.

The drug was given in doses of 0.1 to 20 mg./kg. at intervals of 20 min. With 0.1 mg./kg. or less no effects on arousal thresholds were seen, but larger doses caused a progressive rise in the threshold for electrocortical arousal produced by stimulation of the reticular formation (Fig. 3*b*), and slow activity, similar to that seen after atropine, appeared in the electrocorticogram. There was also a slight rise in the behavioural threshold after 2.0 to 4.0 mg./kg., but the increase was never as great as that for electrocorticographic arousal and the threshold/dose response curves for this drug always showed a marked divergence.

Electrocortical activation produced by sensory stimulation was blocked completely after 0.1 to 2.0 mg./kg. Behaviourally, however, the animal still responded to afferent nerve stimulation, provided the intensity was above control levels.

When amphetamine was given after imipramine the sedation produced by the latter drug was completely antagonized. Increasing doses of amphetamine from 0.5 to 3.0 mg./kg. caused the behavioural arousal threshold to decrease until the animal became completely alert. At the same time the electrical activity of the cortex showed a decrease in the amount of slow activity, but a marked discrepancy between the thresholds for electrocortical and behavioural arousal still remained.

Physostigmine following imipramine abolished the slow waves induced in the electrical activity of the cortex by the latter drug but in no way affected the behavioural state of the preparation or modified the threshold for behavioural arousal.

Hyoscine

The effects of hyoscine on arousal responses have been reported in another paper (Bradley and Key, 1958). It was included here because the effects it produced were in many ways similar to those of benactyzine and imipramine. Less than 0.03 mg./kg. was without effect, but larger doses induced slow wave activity in the electrocorticogram and caused the threshold for electrocorticographic arousal produced by brain stem stimulation to increase progressively (Fig. 3*c*). The maximum effect appeared with doses of 0.9 to 1.0 mg./kg. and a further increase in the dose caused no additional change. There was a slight increase in the threshold for behavioural arousal with doses of 1.0 mg./kg. The threshold/dose response curves showed a marked divergence, however. If physostigmine was injected in doses from 0.1 to 1.0 mg./kg. the threshold for electrocorticographic arousal, which had been raised by hyoscine, was progressively lowered to zero as the slow activity was abolished and replaced by low amplitude, fast activity. The threshold for behavioural arousal usually fell to the control level or sometimes remained slightly raised. Amphetamine, however, always resulted in complete alerting of behaviour.

Reserpine

Since the thresholds for arousal responses of the *encéphale isolé* preparation are not stable for periods longer than 5 to 6 hr. and since reserpine appears to have a delayed action, incremental doses of this drug could not be given. Instead a large dose of 250 or 500 μg./kg. was injected intravenously and the arousal thresholds checked after each succeeding $\frac{1}{2}$ hr. over a $3\frac{1}{2}$ hr. period.

The effects of reserpine were slow to appear and no apparent change in the behavioural state of the animal was seen until 1 to $1\frac{1}{2}$ hr. after the injection. The threshold for arousal produced by stimulation of the reticular activating system remained unaltered but the preparation became less easy to disturb by sensory stimulation. Thresholds for behavioural and electrocortical arousal produced by the auditory stimulus rose progressively and after $2\frac{1}{2}$ to 3 hr. both responses were blocked completely (Fig. 2*d*). In contrast the threshold for click responses recorded at the auditory cortex remained unchanged and at this point stimulation of other sensory modalities still produced responses. In addition, arousal could be obtained if a different auditory stimulus was applied, but the intensity of the latter was sufficiently high to suggest that the threshold may have increased. The threshold/time response curves after the administration of reserpine may therefore represent the effect of the drug on sensory-induced arousal responses, together with the effect of habituation to the stimulus.

In some experiments following a dose of 500 μg./kg., the incidence of slow wave activity characteristic of the "drowsy" state decreased until the electrocorticogram consisted entirely of low amplitude, fast activity. This change was not reflected on the behavioural state of the animals and for most of the time they appeared to be asleep.

Rescinnamine and Deserpidine

These two drugs were given in six experiments in single doses of 250 μg./kg. or 500 μg./kg. Thresholds were determined every ½ hr. over a 3½ hr. period following the injection. Both deserpidine and rescinnamine appeared to exert effects similar to those of reserpine on responses tested in the *encéphale isolé*. Arousal responses produced by stimulation of the brain stem reticular formation remained unaltered throughout the experiment whilst there was a tendency with the higher dose (500 μg./kg.) for faster rhythms to appear in the electrocorticogram. This was not associated with any change in the behaviour of the animals, which continued to sleep for most of the time.

After 1 to 1½ hr. the preparations became a little less easy to disturb with auditory and visual stimuli although the response to pain remained unaltered. Thus, as long as the frequency of the stimulus was not constant, auditory stimulation continued to alert the animal behaviourally and produced activation of the electrocorticogram.

Meprobamate

In the present study three experiments were conducted with meprobamate in doses of 0.1 to 40 mg./kg. There was no change in the thresholds for arousal produced by stimulation of the reticular formation, and even with the highest doses no appreciable alterations were observed in the patterns of electrical activity of the cortex. In quantitative studies of the effects of meprobamate on sensory-induced arousal there was an increase in both the behavioural and electrocortical arousal thresholds until finally the animal failed to respond. This effect, however, was not dose-specific. Thus the blocking of sensory-induced arousal was probably due to habituation to the stimulus, since other stimuli (auditory, visual, or tactile) still produced arousal responses.

Azacyclonal

This drug was injected in doses of 0.1 to 25 mg./kg. in three experiments. As in the case of meprobamate no effect on the threshold for arousal produced by stimulation of the reticular formation was observed and the preparations continued to show periods of wakefulness and sleep. Even after 25 mg./kg. the patterns of electrical activity of the cortex remained unchanged, and behaviourally the animal showed no signs of sedation. Thresholds for arousal produced by auditory stimuli rose progressively during the experiment until eventually the animal failed to respond (Fig. 2*c*). This effect, as with meprobamate, was not dose-specific and, since the animal responded to other afferent stimuli, it could not be distinguished from habituation.

Discussion

The results show that the drugs studied can be classified into four groups according to their effect or lack of effect on arousal responses. *Group* 1: Drugs which caused slight depression of arousal responses produced by direct stimulation of the brain stem reticular formation and affected the electrocortical activity and behaviour in the same way; they also depressed thresholds for arousal produced by afferent nerve stimulation. *Group* 2: Drugs which increased arousal thresholds but at the same time produced a dissociation between behaviour and electrical activity. *Group* 3: Drugs which had no effect on arousal thresholds but which, in high doses, caused a change in the electrocorticogram towards the activation pattern. *Group* 4: Drugs with no detectable effects. The properties of the drugs in each of these groups will be discussed in more detail.

Group 1.—Chlorpromazine, promazine, acepromazine, and hydroxyzine all fall into this group. As can be seen in Fig. 1, they all increased the thresholds for arousal produced by stimulation of the brain stem reticular formation, but this rise was only slight and was never more than a 50% change. The rise was roughly the same for all four drugs but occurred with different doses. Thus with chlorpromazine and hydroxyzine the effect appeared with doses between 2.0 and 4.0 mg./kg. and with promazine and acepromazine between 1.0 and 2.0 mg./kg. With all four drugs the effect was not progressive and once it had occurred further increases in the dose caused no appreciable change. The threshold for behavioural arousal and electrocortical activation always changed together and there was no dissociation between the electrocortical activity and behaviour. No fall in the arousal threshold for brain stem stimulation, such as had often been seen with small doses of chlorpromazine, was observed with the other three drugs.

The effects of chlorpromazine on the threshold for arousal produced by sensory stimulation are shown in Fig. 2*b*. Promazine, acepromazine, and hydroxyzine caused similar effects on thresholds for sensory-induced arousal responses and doses which increased arousal thresholds for brain stem stimulation blocked arousal responses caused by visual, auditory, and tactile stimuli completely.

On the basis of evidence presented here, the mode of action of these drugs cannot be a direct depression of the arousal mechanism at the level of the

reticular activating system. On the other hand, the lack of effect of chlorpromazine on the electrical activity of the *cerveau isolé* preparation (Bradley and Hance, 1957) and the similarity between the effects exerted in the *encéphale isolé* and those seen in the intact animal led to the suggestion that chlorpromazine might exert its main action on some structure lying between the two planes of section in the acute preparations at a brain stem level. This suggestion is further supported by the results of experiments in which chlorpromazine was given with other drugs. Amphetamine, which is thought to act at the level of the brain stem (Bradley and Elkes, 1957), is antagonized by chlorpromazine (Bradley and Hance, 1957). This result has been confirmed in the present study, but whereas chlorpromazine completely blocked the effects of amphetamine the latter drug still produced some effect when it was given after promazine, acepromazine, or hydroxyzine, although there was never complete alerting. Thus, these drugs appeared to block the effects of amphetamine only partially. In the experiments in which the drugs of Group 1 were given with atropine and physostigmine, the latter drugs produced their usual effects on the electrical activity of the brain but did not modify the behavioural state of the animal. However, since it is unlikely that atropine and physostigmine act at the level of the brain stem (Bradley and Elkes, 1957), it might be expected that if the drugs of Group 1 do act at this level then the behavioural state of the animal would remain unaltered.

It has since been suggested, on the basis of the effect on afferent nerve-induced arousal responses, that chlorpromazine exerts an action which is related to the inflow of impulses from the ascending sensory pathways into the reticular formation (Bradley and Elkes, 1957; Bradley and Key, 1958). The similarity between the effects of chlorpromazine and those of promazine, acepromazine, and hydroxyzine on the thresholds for arousal produced by an auditory stimulus would indicate that the latter drugs act in a similar way to chlorpromazine. Thus the hypothesis put forward for the site of action of chlorpromazine can be extended to include the other three members of Group 1.

The pharmacological properties of the drugs in Group 1 lend further support to this suggestion. All of them exhibit some antispasmodic action against 5-hydroxytryptamine, histamine, and acetylcholine (Tripod, 1957), but probably more outstanding is the fact that, of all the drugs tested in the present series of experiments, chlorpromazine (Courvoisier, Fournel, Ducrot, Kolsky, and Koetschet, 1953), promazine (Tripod, 1957), acepromazine (Schmitt, Mercier, Aurousseau, Hallot, and Comoy, 1957) and hydroxyzine (Hutcheon, Scriabine, and Schrogie, 1957) are the ones which exhibit the most potent antiadrenaline properties, both at the periphery and centrally. Thus the hypothesis of a site of action in the brain stem reticular formation and the adrenergic properties of this system (Bonvallet, Dell, and Hiebel, 1954) would be consistent with current information on the properties of these drugs.

Group 2.—This group contains benactyzine and imipramine, for which a tranquillizing action has been reported, and we have also included hyoscine since its effects on arousal responses were similar. All caused a marked rise in the threshold for electrocortical activation produced by stimulation of the brain stem reticular formation and induced slow waves similar to those seen in sleep. Effects on the threshold for behavioural arousal were either absent (benactyzine) or only slight (imipramine and hyoscine) (Fig. 3). The drugs therefore caused a dissociation between the electrocorticogram and behaviour and in this respect they resembled atropine. None of these drugs antagonized the alerting effects of amphetamine as far as behaviour was concerned, nor did they block thresholds for behavioural arousal induced by afferent nerve stimulation.

It would appear that these drugs have little or no action on arousal mechanisms as far as behaviour is concerned and their effects on the electrical activity of the cortex are similar to those of atropine. It has been suggested that the action of atropine, unlike that of amphetamine, is not restricted to the brain stem reticular formation, but is probably exerted much more diffusely in producing changes in the electrocorticogram (Bradley and Elkes, 1957). The three drugs may have a similar action to atropine with which, in fact, they showed many other properties in common. For example, the administration of amphetamine after either benactyzine, imipramine, or hyoscine alerted the behaviour but in no way affected the electrical activity of the cortex, whereas physostigmine replaced the slow wave electrocortical pattern with low voltage fast activity but did not modify the behavioural state of the preparation. Moreover, in contrast to the drugs of Group 1, benactyzine, imipramine, and hyoscine are weakly anti-adrenaline (Tripod, 1957; Berger, 1957; and Lehmann, Cahn, and de Verteuil, 1958) and may, as benactyzine, even potentiate the pressor responses of adrenaline. However, all three drugs of this group show anti-acetylcholine properties and they appear

to be more effective in blocking the blood pressure and antispasmodic effects of acetylcholine than any of the drugs in the other groups.

Group 3.—This group includes reserpine and two related compounds, rescinnamine and deserpidine. The effects of all three drugs were the same. They caused no change in the threshold for arousal by stimulation of the brain stem although high doses produced a change in the electrocorticogram towards faster frequencies which was, however, unrelated to the behavioural state and usually the animals were asleep. The drugs appeared to block arousal by an auditory stimulus, but it was difficult to assess how much this was due to habituation to the stimulus (Fig. 2*a*) and how much it was due to the drug (Fig. 2*d*), since a novel stimulus usually had some effect. It is useful to compare this effect with that of chlorpromazine, which blocks the effects of all such stimuli, including those to which the animal had become conditioned (Key and Bradley, 1958). Thus reserpine, rescinnamine, and deserpidine, in contrast to chlorpromazine and other drugs of Group 1, appear to have no direct action on the reticular activating system of the brain stem nor do they block the effects of afferent nerve stimulation on this system but appear merely to modify these influences. The concept of an action at a cortical level, producing an increase in " cortical inhibition of diencephalic centres " (Schneider, Plummer, Earl, and Gaunt, 1955), would not conflict with this conclusion. In fact, it may offer a possible explanation of the slight increase in the threshold for sensory induced arousal seen in previous experiments (Key and Bradley, 1958) since the cortex is known to exert some influence upon conduction within the brain stem reticular formation (French, Hernandez-Peon and Livingston, 1955; Adey, Segundo, and Livingston, 1957). The precise pharmacological and biochemical mechanisms underlying such an inhibitory influence must at present remain an open question. 5-Hydroxytryptamine has been implicated in the central depressant action of reserpine (Woolley and Shaw, 1957), and it is interesting to note that, of all the rauwolfia alkaloids, only those which release 5-hydroxytryptamine are effective in psychiatric practice (Pletscher, Shore, and Brodie, 1956). The three compounds of Group 3 fall into the above category, but it must be remembered that these drugs also possess appreciable antispasmodic activity against adrenaline, acetylcholine, and histamine and have no effect against strychnine toxicity, while in contrast to Group 1 drugs they potentiate the pressor responses of adrenaline. It is likely, therefore, that reserpine, deserpidine, and rescinnamine have a common site and mode of action and that these are different from those of chlorpromazine and the other drugs in Group 1.

Group 4.—The two drugs of this group, meprobamate and azacyclonal, have been classified together for convenience. They are conspicuous in the present study not for what they did but for what they failed to do. Meprobamate, in doses (20 to 40 mg./kg.) which are said to produce tranquillization (Berger, 1954), had no effect on thresholds for arousal induced by direct stimulation of the reticular formation. The responses to afferent stimuli of all modalities were little changed, and even with the largest doses the patterns of electrical activity of the cortex remained unaltered. Similarly, azacyclonal produced no change in arousal thresholds nor did it alter the responses to visual, tactile or auditory stimuli unless the frequency of the latter was kept constant, resulting in habituation (Fig. 2*c*). It seems unlikely, therefore, that the site of action of these drugs is related to receptors situated at the level of the reticular activating system of the brain stem, unless the receptors are completely unrelated to the arousal system. The tranquillizing action of azacyclonal has been linked with inhibition of 5-hydroxytryptamine (Rinaldi, Rudy, and Himwich, 1955; Costa, 1956), whilst the central muscle relaxant properties of meprobamate may be implicated in the action of this drug. The pharmacological properties of meprobamate and azacyclonal show that Group 4 is a heterogeneous one. Meprobamate has little antispasmodic action against acetylcholine, histamine, 5-hydroxytryptamine, and adrenaline, whereas azacyclonal is said to inhibit contractions of isolated rat uteri induced by 5-hydroxytryptamine. However, neither drug releases 5-hydroxytryptamine and of the two only meprobamate is effective against strychnine convulsions.

All the drugs used in this study have been reported to be useful in the clinical treatment of psychiatric disturbances. It is therefore not possible to compare their effects on arousal responses with those of drugs having little or no clinical usefulness. Nor must we suggest at this stage that the electrophysiological effects produced by these drugs are related to the mode of action by which they change the mental states in man. We need to know much more about the mechanisms underlying mental disturbances before this can be attempted.

It is interesting to note that those drugs which have the most marked effect on arousal thresholds (Group 1) are used clinically to treat disturbed

psychiatric patients and therefore are presumably most effective in reducing psychomotor hyperactivity. It is for these drugs that the most satisfactory hypothesis regarding their mode of action has been produced, namely, that they depress the influence of afferent stimuli on the reticular formation at the brain stem level, and this hypothesis appears to cover all the drugs in this group. An inhibition of afferent stimuli could well account for some of the clinical effects of these drugs such as their tranquillizing action, potentiation of barbiturate anaesthesia, and anti-emetic effect. Azacyclonal and meprobamate showed no electrophysiological effects and there is some doubt or disagreement about their clinical effectiveness. It is difficult to account for the action of reserpine from its electrophysiological effects and we must be careful not to draw conclusions from observations of electrical activity unrelated to behaviour. Since the effects of this drug are so slow in appearing, we should perhaps look more closely at the biochemical mechanisms involved in its action.

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References

Adey, W. R., Segundo, J. P., and Livingston, R. B. (1957). *J. Neurophysiol.*, **20**, 1.
Berger, F. M. (1954). *J. Pharmacol. exp. Ther.*, **112**, 413.
—— (1957). *Ann. N.Y. Acad. Sci.*, **67**, 685.
Bonvallet, M., Dell, P., and Hiebel, G. (1954). *Electroenceph. clin. Neurophysiol.*, **6**, 119.
Bradley, P. B. (1957). *Psychotropic Drugs*, p. 207. Amsterdam: Elsevier.
—— and Elkes, J. (1953). *Electroenceph. clin. Neurophysiol.*, **5**, 451.
—— —— (1957). *Brain*, **80**, 77.
—— and Hance, A. J. (1957). *Electroenceph. clin. Neurophysiol.*, **9**, 191.
—— and Key, B. J. (1958). Ibid., **10**, 97.
Bremer, F. (1936). *C.R. Soc. Biol. (Paris)*, **122**, 460.
Costa, E. (1956). *Proc. Soc. exp. Biol. (N.Y.)*, **91**, 39.
Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P. (1953). *Arch. int. Pharmacodyn.*, **92**, 305.
French, J. D., Hernandez-Peon, R., and Livingston, R. B. (1955). *J. Neurophysiol.*, **18**, 74.
Hiebel, G., Bonvallet, M., and Dell, P. (1954). *Sem. Hôp. Paris*, **30**, 2346.
Hutcheon, D. E., Scriabine, A., and Schrogie, J. J. (1957). *J. Pharmacol. exp. Ther.*, **119**, 154.
Key, B. J., and Bradley, P. B. (1958). *Nature (Lond.)*, **182**, 1517.
Lehmann, H. E., Cahn, C. H., and de Verteuil, R. L. (1958). *Canad. psychiat. Ass. J.*, **3**, 155.
Longo, V. G., von Berger, G. P., and Bovet, D. (1954). *J. Pharmacol. exp. Ther.*, **111**, 349.
Pletscher, A., Shore, P. A., and Brodie, B. B. (1956). *Science*, **123**, 992.
Rheinberger, M. B., and Jasper, H. H. (1937). *Amer. J. Physiol.*, **119**, 186.
Rinaldi, F., Rudy, L. H., and Himwich, H. E. (1955). *Amer. J. Psychiat.*, **112**, 343.
Schmitt, J., Mercier, J., Aurousseau, M., Hallot, A., and Comoy, P. (1957). *C.R. Acad. Sci. (Paris)*, **244**, 255.
Schneider, J. A., Plummer, A. J., Earl, E. A., and Gaunt, R. (1955). *Ann. N.Y. Acad. Sci.*, **61**, 17.
Terzian, H. (1952). *Rass. Neurol. veg.*, **9**, 211.
Tripod, J. (1957). *Psychotropic Drugs*, p. 437. Amsterdam: Elsevier.
Woolley, D. W., and Shaw, E. N. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 649.