

THE SITE OF ORIGIN OF THE SEIZURE DISCHARGE  
PRODUCED BY TUBOCURARINE ACTING FROM  
THE CEREBRAL VENTRICLES

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Several years ago Samson Wright (1955) showed that intraventricular and intracisternal injections of tubocurarine into anaesthetized cats produced an abnormal discharge in the electroencephalogram (e.e.g.) resembling the seizure discharge of epilepsy. The discharge consisted of large surface-negative spikes or sharp waves, at first single but later becoming multiple, and of bursts of fast synchronous activity of large voltage. Samson Wright pointed out that the discharge tended to appear first in the posterior half of the brain. However, he gave no indication of the site where tubocurarine acts when evoking this discharge. More recently profound changes in the electrical activity of the brain have been recorded with micro-electrodes from various cortical and subcortical regions after intraventricular injections of tubocurarine (Feldberg, Malcolm & Sherwood, 1956; Feldberg, Malcolm & Darian Smith, 1957; Feldberg & Malcolm, 1959), but attempts to locate the site of this action of tubocurarine failed. Thus Feldberg & Malcolm (1959) were unable 'to decide the pertinent question of where the tubocurarine acts when, on intraventricular injection, it produces the profound changes in the electrical activity of the brain which we presume to be associated with the convulsive activity'.

In the present experiments a definite pattern in the development of the seizure discharge has been found, and it is concluded that the discharge originates in structures reached by the tubocurarine from the posterior half of the lateral ventricle. This conclusion is derived from experiments in which either the whole or parts of the ventricular system were perfused with tubocurarine. In the course of these experiments a number of effects mediated via the autonomic nervous system were observed, i.e. rises in arterial blood pressure, mydriasis, retraction of the nictitating membranes and pilo-erection. Some of these autonomic effects were correlated with the abnormal discharge in the e.e.g.

Finally, it is shown that perfusion of bromophenol blue through

the ventricular system results in an abnormal discharge in the e.e.g., similar to that seen after tubocurarine; but bromophenol blue does not produce any of the autonomic effects.

#### METHODS

The experiments were carried out in cats anaesthetized with pentobarbitone sodium, immobilized with Flaxedil (gallamine triethiodide; May and Baker), and artificially ventilated. The pentobarbitone sodium (36 mg/kg) was injected intraperitoneally and supplementary doses were given when required. The trachea and right femoral vein were cannulated and the left femoral artery was freed so that later a fine polythene tube could be introduced for registering the arterial blood pressure by means of a Statham strain gauge. With the cat lying on its belly, the head was fixed to the ear bars and mouth-piece of a head holder similar to the Horsley Clark stereotaxic instrument.

For intracisternal injections the muscles covering the atlanto-occipital membrane were dissected away at the beginning of the experiment and the injections were made through the exposed membrane.

The methods of intraventricular injection through an implanted Collison cannula and of perfusing the ventricular system from the lateral ventricle to cisterna or aqueduct have been described elsewhere (Feldberg & Sherwood, 1953; Bhattacharya & Feldberg, 1956).

The following modification in the perfusion of the ventricular system was adopted in order to perfuse either one lateral ventricle or both with tubocurarine or bromophenol blue. Both lateral ventricles were cannulated and perfusion to either aqueduct or cisterna was begun simultaneously from both lateral ventricles, but with separate slow injectors. For bilateral perfusion with tubocurarine both injectors were filled with tubocurarine solution, but when it was intended to prevent the tubocurarine from entering one lateral ventricle, tubocurarine was perfused from one side only, and artificial cerebrospinal fluid (c.s.f.) from the other, the rate of perfusion through both lateral ventricles being the same (0.1 ml./min).

The fluid used for perfusion of the cerebral ventricles was the artificial c.s.f. described by Merlis (1940). Its composition is (g/l.): NaCl 8.1; KCl 0.25; CaCl<sub>2</sub> 0.14; MgCl<sub>2</sub> 0.11; NaHCO<sub>3</sub> 1.78; NaH<sub>2</sub>PO<sub>4</sub> 0.07; CO(NH<sub>2</sub>)<sub>2</sub> 0.13; and glucose 0.61. The tubocurarine used was crystalline D-tubocurarine (Burroughs Wellcome); it was dissolved in artificial c.s.f. and all values refer to the salt. The bromophenol blue (British Drug Houses) was obtained in the form of the acid and prepared as described by Feldberg & Fleischhauer (1960).

The e.e.g. was recorded with frontal and occipital monopolar leads on both sides. Epidural electrodes were inserted through burr holes over the right and left posterior cruciate gyri and over the dorsal parts of the right and left middle supra-sylvian gyri, i.e. over the somatosensory and visual areas. The electrodes consisted of platinum wire held in a nylon screw. An earthed steel needle placed in the skin of the forehead served as indifferent electrode. The time constant of the amplifier of the 4-channel Ediswan pen recorder was set at 0.3 sec. Before the records were taken artificial respiration was begun and the animal was immobilized by an intravenous injection of Flaxedil 4 mg/kg repeated from time to time as required. The Flaxedil injections did not affect the e.e.g.

#### RESULTS

##### PART I. EFFECTS OF TUBOCURARINE ON THE E.E.G.

*Intraventricular injections* of 200–400 µg tubocurarine result in the disappearance of the barbiturate spindles and in the appearance of an abnormal discharge of large surface-negative waves in the e.e.g. The dis-

appearance of barbiturate spindles often precedes the onset of the abnormal discharge, which is more pronounced on the side of injection and greater in the occipital than in the frontal leads.

The onset and development of the abnormal discharge follows a characteristic pattern which varies little from experiment to experiment. After a latency of 2-5 min, surface-negative high-voltage spikes occur in the e.e.g. following each other at intervals of 3-6 sec. Later the spikes become broader and multiple owing to the appearance of small waves on their descending limbs. This change often occurs suddenly. It is associated with a decrease in the frequency of the spikes, but later, as the action of tubocurarine continues, the frequency gradually increases again. At times the rhythmic discharge is interrupted by short periods of fast high-voltage activity, which we have termed 'episodes'. The episodes usually begin and end with a few multiple spikes. After an episode there is often a period during which no abnormal spikes are recorded. This absence of abnormal spikes is referred to as electrical silence. The number of episodes that occur after a single injection of tubocurarine is small; often there was only one, usually occurring a few minutes after the onset of the abnormal discharge.

A typical experiment is illustrated in Fig. 1. About 3 min after the injection of 400  $\mu$ g tubocurarine into the left lateral ventricle, large surface-negative spikes appear. *A* shows the first abnormal negative spike recorded in the left occipital lead. During the next minute similar spikes of increasing amplitude occur at intervals of 2-8 sec. Synchronously with these, smaller spikes appear, first in the contralateral occipital lead and later also in both frontal leads (*B*). Records *C-F* show the first episode. Two large surface-negative spikes occur in quick succession, with small waves on the descending limb of the second spike, and are followed by a period of fast activity (*C*), which after 7 sec suddenly slows down to a pattern of synchronized high-voltage activity (*D*). At *E*, 10 sec later, the pattern changes to multiple spikes and after eight such spikes, the last of which is seen at *F*, there is a 10 sec period of electrical silence. Then the abnormal discharge begins again and spikes of increasing size (*G* and *H*) occur at intervals of 3-8 sec. After the 22nd the spikes become broader, have small waves on the descending limb and are followed by a surface-positive wave (*L*). Such multiple spikes recur every 8-10 sec and are shown on a slow paper, at *J*. Similar changes, but of lower voltage, occur synchronously in the right occipital lead, and in the frontal leads there is a small reflexion of these changes.

About an hour after the injection the abnormal spike activity declines. The multiple spikes become first smaller and then the pattern reverts suddenly to that of single spikes (*K*). The details of this change are illustrated on a fast paper in *L-N*. Record *L* is taken before the abnormal

discharge declines, and shows that every small wave in the left occipital lead finds its counterpart at a lower amplitude in the right occipital lead. *M* shows the first phase of the decline and is taken about 8 min before the change from multiple to single spikes. There is some attenuation of the discharge in the left occipital lead, but pronounced attenuation in the right, where it is characterized by extreme reduction of the initial large spike, yet without apparent reduction of the subsequent small waves which no longer seem to originate from the descending limb of an initial spike, but to arise from the background activity. Record *N* is taken

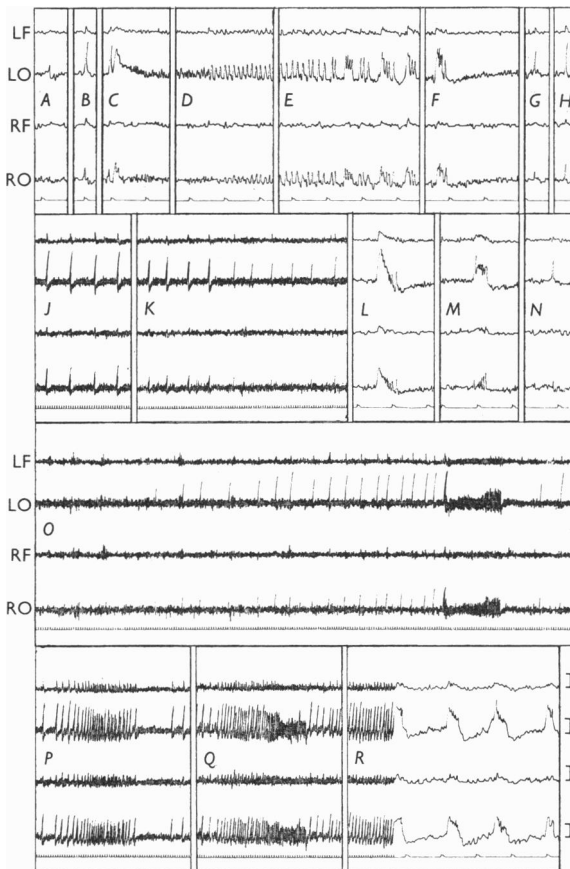


Fig. 1. E.g. of a cat anaesthetized with pentobarbitone sodium, immobilized with Flaxedil and artificially ventilated. Effects of three injections, 2 and 2½ hr apart, of 400 µg tubocurarine into the left lateral ventricle. Records *A-H* taken 3-6 min, and records *J-N* 25, 67, 12, 60 and 69 min, after the first injection. Record *O* begins 70 sec after the second injection and *P-R* are taken 3, 20 and 30 min after the third injection. LF, LO, RF and RO refer to left and right frontal and occipital leads. Calibration 600 µV; negativity upwards; time marker, seconds.

shortly after the change in character of the spikes has occurred: a single high-voltage spike is now recorded in the left occipital lead, and a minute replica of it in the right but none in the frontal leads. The spikes in the occipital leads become gradually smaller and disappear within the next 45 min.

When a second injection of 400  $\mu\text{g}$  tubocurarine is given the abnormal discharge reappears and develops in the same manner as after the first injection (*O*). After a further intraventricular injection of tubocurarine the following additional features are observed (*P*, *Q* and *R*):

(1) The abnormal discharge becomes more accentuated in the contralateral occipital lead, and its various features now become discernible in both frontal leads. Yet the striking difference in amplitude between occipital and frontal leads remains. (2) The frequency of the rhythmic discharge of multiple spikes becomes increasingly greater. (3) Episodes occur more frequently, and (4) their pattern changes. A much greater part or the whole of the episode is taken up by multiple spikes which are similar to those of the rhythmic discharge, but follow each other in quick succession. (5) Finally, with increasing frequency of the rhythmic discharge, the episodes become less distinct.

At *P* and *Q* two episodes are shown, recorded 3 and 20 min respectively after the third intraventricular injection of 400  $\mu\text{g}$  tubocurarine. At *P* the episode consists from beginning to end of multiple spikes at a frequency of about 1/sec; at *Q* this kind of discharge is recorded only during the first half of the episode, the second half consisting of fast synchronous activity. The episode at *P* is followed by a period of electrical silence, whereas that at *Q* illustrates the less common feature, an immediate resumption of the rhythmic discharge of abnormal spikes. Record *R* is taken 10 min after *Q* and shows the greatly increased frequency of the rhythmic discharge. At this stage the episodes become less distinct.

#### *The site of action of tubocurarine*

The abnormal discharge must be attributed to the action of tubocurarine on structures reached from the ventricular cavities, i.e. from the inside of the brain, and not from the subarachnoid space, i.e. from the outside. This conclusion is based on the findings that tubocurarine does not produce the abnormal discharge when injected into the cisterna magna, but does so when it is perfused from the lateral ventricles to the aqueduct, i.e. when it is prevented from entering the subarachnoid space.

It can be shown that the abnormal discharge results from an action on structures reached from the lateral ventricles, because it is confined for long periods to one hemisphere when the tubocurarine is perfusing through one lateral ventricle only, and fails to occur when tubocurarine does not

enter either lateral ventricle, its perfusion being restricted to the third ventricle. Finally, it has been possible to locate the site within the lateral ventricle. The tubocurarine must act on structures reached from the posterior half, because no discharge is obtained when this part of the ventricular system is excluded from the perfusion.

*Injections into the subarachnoid space*

The finding of Samson Wright (1955) that tubocurarine elicits an abnormal discharge in the e.e.g. on intracisternal injection could not be confirmed. An injection of 200–400  $\mu\text{g}$  tubocurarine into the cisterna magna produced either no change in the e.e.g. or only a slight accentuation of the background spindle activity. As the accentuation developed gradually in the course of 1 hr or more it was not even certain whether it was brought about by the action of tubocurarine. It may have been due in fact to a change in the depth of anaesthesia.

Further evidence for the view that the abnormal discharge following intraventricular injection of tubocurarine did not result from an action on structures reached from the subarachnoid space was obtained in one experiment in which the cannula inserted for intraventricular injections had traversed the left lateral ventricle. Its opening was in the subarachnoid space between the hippocampus and the thalamus, so that the injected tubocurarine (400  $\mu\text{g}$  in 0.2 ml.) reached the outer surface of the mid-brain and of the occipital lobes. The position of the cannula is illustrated in Fig. 2. The regions of the brain reached by the injection were ascertained at the end of the experiment by an injection through this cannula of 0.2 ml. of bromophenol blue. There was deep staining of the superior and inferior colliculi and of the outer surface of the thalamus. The lateral and ventral surfaces of the brain stem and the surface of both occipital lobes were stained less deeply, and there was faint staining of the parietal and frontal lobes.

The injection of tubocurarine into this region of the subarachnoid space was not as ineffective as the intracisternal injections. It produced an abnormal discharge, more pronounced in the occipital than in the frontal leads, but different from the discharge obtained on intraventricular injections. The discharge consisted of surface-positive spikes, which were of equal size in both occipital leads. They did not occur synchronously in the two leads and were smaller and more frequent than the surface-negative spikes produced by intraventricular tubocurarine. Further, the onset of the positive spikes occurred simultaneously in both occipital leads and no episodes of synchronous fast activity were recorded. The abnormal discharge is illustrated in Fig. 3. Record *A* shows the e.e.g. before, and *B* and *C* the positive spikes after, a first and a second injection of 400  $\mu\text{g}$

tubocurarine; the second injection merely increased the size of the positive spikes. The asynchronism of the spikes in the occipital leads is shown on fast paper at *D*. When the right lateral ventricle was then cannulated, an intraventricular injection of 400  $\mu\text{g}$  through this cannula produced the typical large surface-negative spikes appearing synchronously in both occipital leads, superimposed on the previous asynchronous discharge of small positive spikes which continued (*E*).

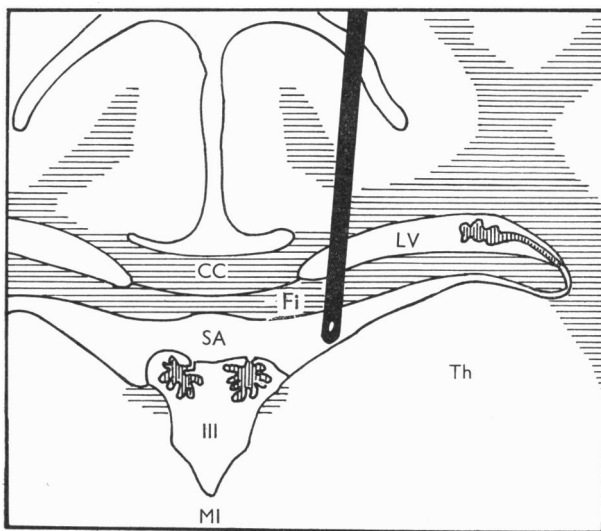


Fig. 2. Drawing of the upper part of a coronal section through a cat's brain illustrating the position of the cannula in an experiment in which the tip had traversed the fimbria, and was located in the subarachnoid space. CC, corpus callosum; Fi, fimbria; LV, lateral ventricle; MI, massa intermedia; SA, subarachnoid space; Th, thalamus; III, third ventricle.

#### *Perfusion of the cerebral ventricles*

Perfusions of tubocurarine 1/5000–1/500 at a rate of 0.1 ml./min from lateral ventricle to aqueduct or cisterna produce the same abnormal discharge in the e.e.g. as intraventricular injections. On perfusion with the different concentrations of tubocurarine the following differences were observed. Whereas with tubocurarine 1/5000 the latency of the abnormal discharge is 30–60 min, with 1/1000 or 1/500 it is a few minutes only. Further, on perfusion with the weak concentration every phase of the abnormal discharge lasts longer. This difference is particularly striking with regard to the episodes. On perfusion with 1/5000 there is usually a long period with many episodes, whereas on perfusion with 1/1000, and particularly with 1/500, episodes occur during a short period only and are

few in number. This is because the quick succession of multiple spikes is reached early in the perfusion.

*Perfusion with tubocurarine from both lateral ventricles.* When tubocurarine is perfused from both lateral ventricles to aqueduct, there is practically no difference in the abnormal discharge recorded from both hemispheres.

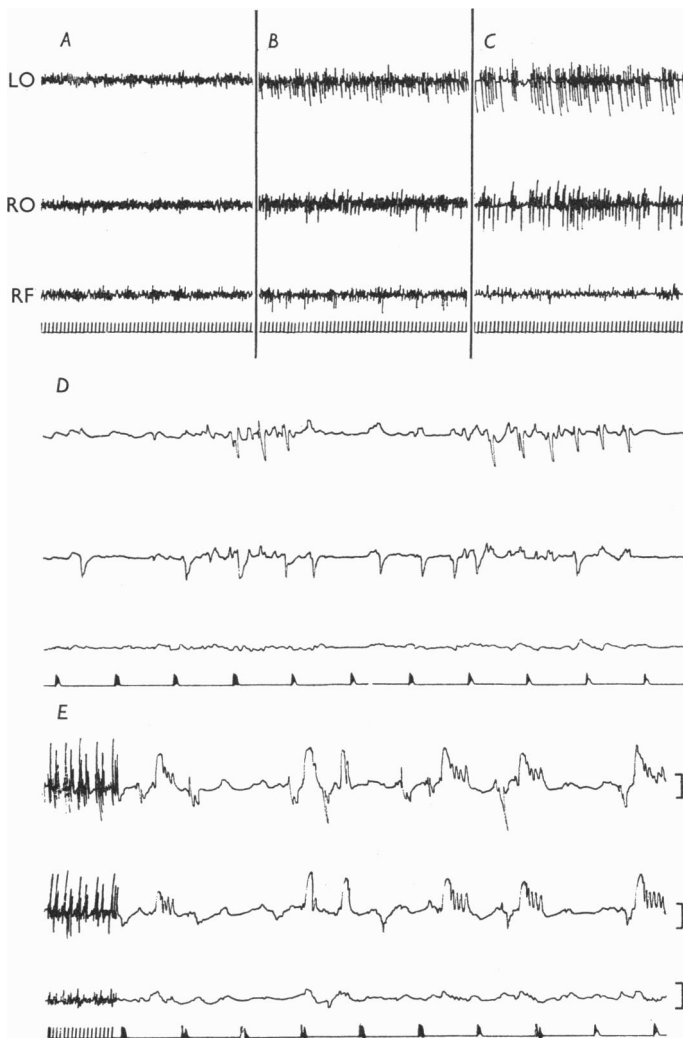


Fig. 3. E.e.g. of a cat prepared as for Fig. 1. Two injections of  $400\ \mu\text{g}$  tubocurarine were made at 1 hr interval into the subarachnoid space through a cannula implanted in the left side of the skull. Tip of cannula in subarachnoid space, as shown in Fig. 2. Record *A* before, *B* 30 min after, the first injection, and *C* and *D* 3 and 21 min after the second injection of tubocurarine. Five minutes after *D*  $400\ \mu\text{g}$  tubocurarine was injected into the correctly cannulated right lateral ventricle; *E* taken 10 min later. Lettering and calibration as in Fig. 1.



This is different from the effect produced by intraventricular injection of tubocurarine where the abnormal discharge is dominant on the injected side.

Figure 4 illustrates an experiment in which tubocurarine 1/5000 was perfused from both lateral ventricles to the aqueduct. Record *A* shows the onset of the abnormal discharge on both occipital leads. In record *B* taken

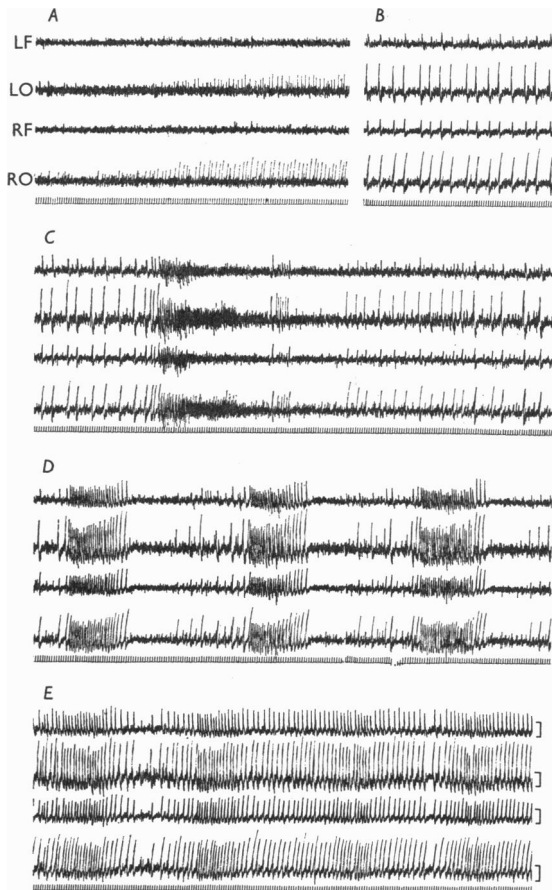


Fig. 4. E.e.g. of a cat prepared as for Fig. 1. Perfusion with tubocurarine 1/5000 from both lateral ventricles to aqueduct. Records *A-E* taken 60, 73, 122, 148 and 152 min after onset of perfusion. Lettering and calibration as in Fig. 1.

7 min later, the spikes have grown and become multiple; they are now also recorded in the frontal leads. Record *C* is taken 50 min after *B*. The episode is the fifth which in the main consists of synchronous fast activity, and is followed by a 25 sec period of electrical silence. When the

rhythmic discharge reappears, the first eleven spikes are single, thereafter becoming multiple again. Record *D* illustrates the phase in which episodes consisting mainly of multiple spikes follow each other at short intervals, and record *E* illustrates the last phase, in which the frequency of the rhythmic discharge of multiple spikes has increased to such an extent that episodes are scarcely discernible.

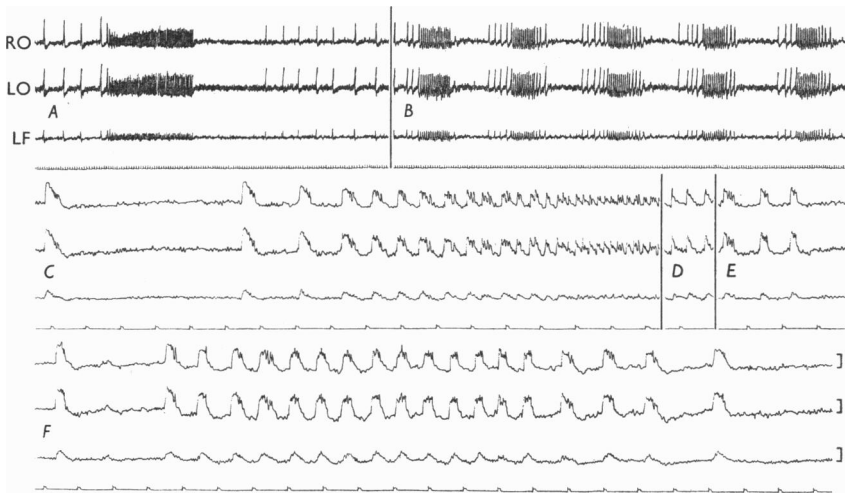


Fig. 5. E.e.g. of a cat prepared as for Fig. 1. Perfusion with tubocurarine 1/1000 from both lateral ventricles to aqueduct. *A* and *B* taken 11 and 27 min, *C-E* 15 min, and *F* 31 min after onset of perfusion. Lettering and calibration as in Fig. 1.

Figure 5 is from one of the few experiments in which perfusion from both lateral ventricles to aqueduct with tubocurarine 1/1000 also led to a phase in which many episodes followed each other in quick succession. *A* and *B*, recorded on slow paper, illustrate both types of episodes and their quick succession. Two episodes are recorded on a fast paper. The activity of the one (*C-E*) consists in its middle part of the fast synchronous activity, that of the other, *F*, of multiple spikes throughout.

The quickening of the rhythmic discharge often develops as follows: interspersed between the large spikes smaller ones appear which later grow to full size. This is illustrated in Fig. 6. Record *A* is taken before the appearance of the small spikes. Records *B* and *C* illustrate small spikes interspersed between the large. The typical sequence at *B* is one, and at *C* two, small spikes between each large one. But this rhythm is not rigid. At *B* two large spikes following each other can be seen, without a small one in between, and at *C* one small spike is interspersed between two large ones. The small spikes are also recorded from the frontal leads. In record *D*

the size of the small spikes has increased, and in *E* they have grown to full size. Record *F*, which shows a further quickening of the rhythmic discharge, shows that the appearance of interspersed small spikes which grow in size is not the sole mechanism by which an increase in the frequency of the rhythmic discharge is effected. *G* and *H* show the activity at *D* and *F* recorded on a fast paper.

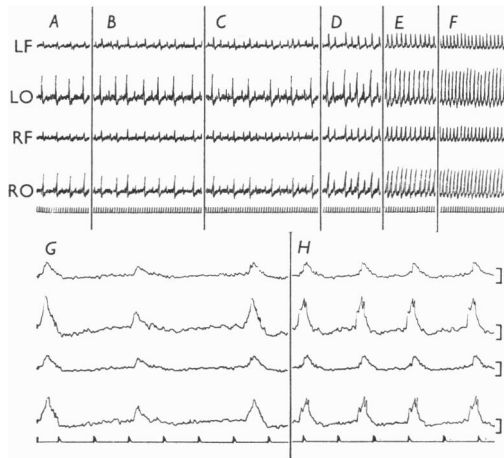


Fig. 6. E.e.g. of a cat prepared as for Fig. 1. Perfusion with tubocurarine 1/1000 from both lateral ventricles to aqueduct. *A-F* taken 13, 21, 35, 37 and 54 min after onset of perfusion; *G* taken immediately after *D*, and *H* shortly before *F*. Lettering and calibration as in Fig. 1.

*Perfusion with tubocurarine from one lateral ventricle.* When both lateral ventricles are perfused simultaneously, one with tubocurarine and the other with artificial c.s.f., the tubocurarine is prevented from entering the lateral ventricle perfused with artificial c.s.f. Visual evidence for this conclusion is obtained when bromophenol blue is perfused in this way instead of tubocurarine. Figure 7 illustrates the result of such an experiment after perfusion for 1½ hr with 1% bromophenol blue from the left, and with artificial c.s.f. from the right lateral ventricle to the aqueduct at a rate of 0.1 ml./min. The figure shows a coronal section of the brain obtained after the perfusion. It shows that dye had not entered the right lateral ventricle, since only the walls of the left lateral and of the third ventricles are deeply stained.

Figure 8*A-E* illustrates the effects of one-sided perfusion with tubocurarine. The abnormal discharge appears on one side only. Perfusion was with tubocurarine 1/1000 from the left lateral ventricle to aqueduct. *A* and *B* show the initial single spikes of the abnormal activity. There is little reflexion of this activity in the right occipital and in both frontal leads.

In record *C*, taken 30 min after *B*, the activity is still confined to the left occipital lead. *D* and *E*, taken 50 and 82 min after the onset of one-sided tubocurarine perfusion, show that when the multiple spikes appear they also are reflected slightly only on the right occipital and on both frontal leads. Between *E* and *F* perfusion from the right ventricle with artificial c.s.f. was changed to perfusion with tubocurarine 1/1000. Within a few minutes large spikes of about similar voltage are recorded from both occipital and smaller spikes from both frontal leads. The spikes occur synchronously in all leads (*G-J*).

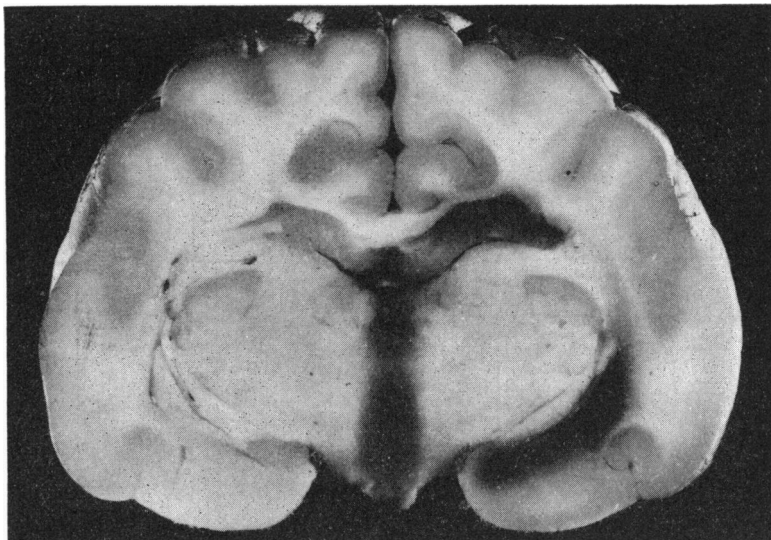


Fig. 7. Coronal section through the brain of a cat after 1½ hr perfusion with artificial c.s.f. from the right, and with 1% bromophenol blue from the left lateral ventricle to aqueduct.

In some experiments in which the one-sided perfusion of tubocurarine was continued for several hours the slight reflexion of the abnormal discharge in the contralateral leads increased gradually in amplitude and at the later stages there was little difference between the abnormal discharge from the two hemispheres in spite of the fact that perfusion with tubocurarine was still confined to one lateral ventricle.

*Perfusion from the third ventricle with tubocurarine.* Further proof that the tubocurarine when producing the abnormal discharge acts on structures reached from the lateral and not from the third ventricle was obtained in a few experiments in which one of the two cannulae, that on the left side, was incorrectly positioned so that its opening was in the third ventricle.

When tubocurarine 1/1000 was perfused from this, and artificial c.s.f. from the other cannula inserted into the right lateral ventricle, no abnormal discharge was recorded on the e.e.g. However, when later tubocurarine was perfused through the right lateral ventricle, the typical abnormal discharge was obtained on the right occipital lead. After perfusion of bromophenol blue through the cannula inserted into the third ventricle, it was

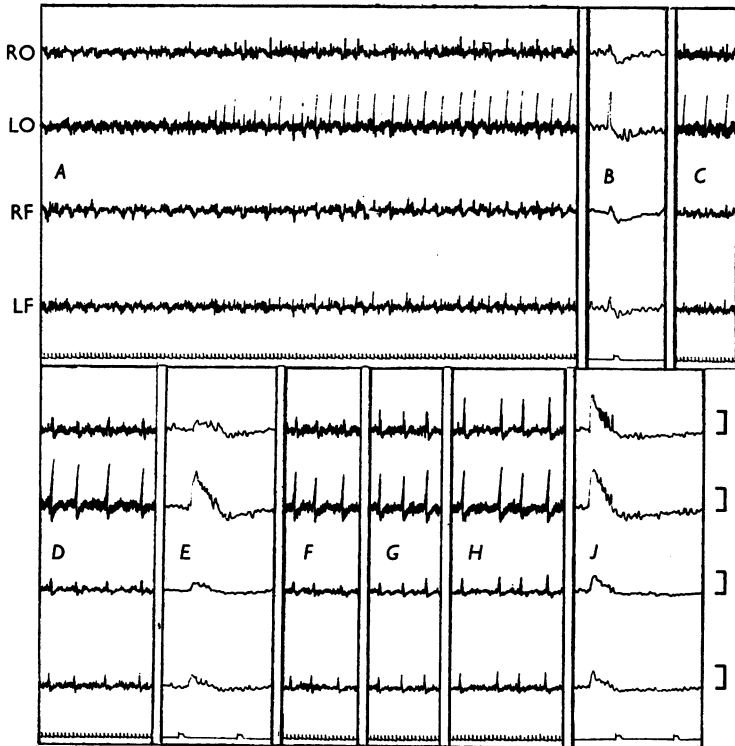


Fig. 8. E.e.g. of a cat prepared as for Fig. 1. Perfusion with artificial c.s.f. from the right, and with tubocurarine 1/1000 from the left lateral ventricle to aqueduct. *A-E* taken  $4\frac{1}{2}$ , 10, 40, 50 and 82 min after onset of perfusion. Between *E* and *F* tubocurarine 1/1000 also perfused from right lateral ventricle; *F-J* taken 2, 4, 6 and 7 min later. Lettering and calibration as in Fig. 1.

found that the dye had deeply penetrated the walls of the third ventricle and the tissue surrounding the anterior part of the aqueduct, but not the walls of the lateral ventricles. The parts of the ventricular system which had been perfused through this cannula are shown diagrammatically in Fig. 9B.

*Exclusion of the posterior half of the lateral ventricle from perfusion with tubocurarine.* Another experiment enabled us to locate the site within the

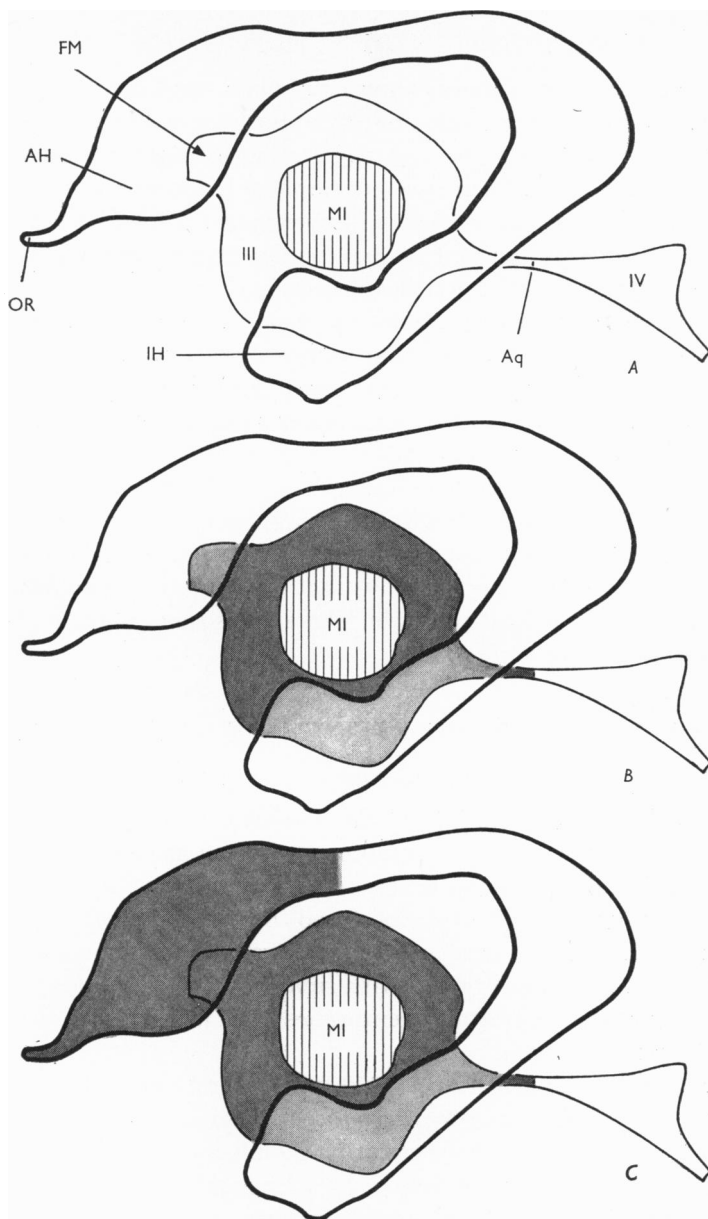


Fig. 9. Diagrams of the ventricular system of the cat's brain. Only one lateral ventricle shown.

AH, anterior horn of lateral ventricle; FM, foramen of Monro; IH, inferior horn of lateral ventricle; MI, massa intermedia; OR, olfactory recess of lateral ventricle; III, third ventricle; IV, fourth ventricle. Aq indicates tip of outflowing cannula in aqueduct.

The shaded area in *B* shows the regions of the ventricular system perfused when the inflowing cannula is inserted into the third ventricle. The shaded area in *C* shows the regions of the ventricular system perfused in the one experiment in which the entrance into the posterior half of the lateral ventricle was blocked.

lateral ventricle where tubocurarine acts when producing the abnormal discharge. Perfusion was carried out from both lateral ventricles to aqueduct with tubocurarine 1/500, yet the abnormal discharge was confined to the left occipital lead. Therefore, bromophenol blue was perfused through the right cannula at the end of the experiment. The walls of the third ventricle and the anterior part of the aqueduct were deeply stained. The walls of the right lateral ventricle were stained in the anterior half only, but no dye had reached the posterior half behind the cannula. The cannula had been inserted in such a way that its opening faced anteriorly, and the perfusion fluid thus passed into the anterior half of the lateral and into the third ventricle. The parts of the ventricular system perfused through this cannula are shown diagrammatically in Fig. 9C.

As the staining in the anterior half of the lateral ventricle included the septum pellucidum and the head of the caudate nucleus, these cannot be the structures on which tubocurarine acts when producing the abnormal discharge. Thus the tubocurarine must act on structures reached from the posterior part of the lateral ventricle. This suggests an action on the hippocampus and/or the amygdala.

## PART II. AUTONOMIC EFFECTS OF TUBOCURARINE

### *Pupils and nictitating membranes*

Before and during perfusion of the cerebral ventricles with artificial c.s.f. the pupils were slit-like and the nictitating membranes were protruded. No change occurred when tubocurarine was injected intracisternally, but when injected into or perfused through the cerebral ventricles, it caused mydriasis and retraction of the nictitating membranes. The pupillary dilatation was obtained with smaller doses of tubocurarine than the retraction of the nictitating membranes; and in perfusions with larger concentrations it occurred earlier.

On perfusion of tubocurarine 1/5000 from lateral ventricle to aqueduct or cisterna, the pupils began to dilate after 15–30 min, before the changes in the e.e.g. occurred, but the nictitating membranes remained protruded even when perfusion was continued for 2 hr and the pupils had become maximally dilated. On perfusion of tubocurarine 1/1000 or 1/500 dilatation of the pupils began within a few minutes and withdrawal of the nictitating membranes followed 5–15 min later. The effect on the pupils began with small oscillatory movements (hippus). At first the pupils became slits in between; later, the oscillations increased and with each oscillation the pupils became and remained wider; and finally, when nearly maximal dilatation was attained, the oscillations again became small.

When tubocurarine was perfused from both lateral ventricles, pupillary

dilatation occurred simultaneously and equally in both eyes, as did withdrawal of the nictitating membranes. This was not always so when tubocurarine was injected into or perfused through one lateral ventricle only; in these experiments the effects often occurred earlier, and were more pronounced on the same side as the tubocurarine injection or perfusion. This finding suggests that the effects were, at least in part, brought about by an action on structures reached from the lateral ventricles. In support of this conclusion was the observation made in the few experiments in which, because of incorrect positioning of the cannula, the tubocurarine passed straight into the third ventricle without entering the lateral. In these experiments tubocurarine produced neither hippus nor retraction of the nictitating membranes.

#### *Arterial blood pressure*

An intracisternal as well as an intraventricular injection of 400  $\mu\text{g}$  tubocurarine resulted, after a latency of at most a few minutes, in a strong rise in arterial blood pressure. Such rises occurred also when tubocurarine 1/500 or 1/1000 was perfused from lateral ventricle to either cisterna or aqueduct. In some experiments the blood pressure rose steeply, in others the rise was more gradual, but in all experiments values of 200 mm Hg or more were reached within 30 min. The high level was maintained for 10–30 min, until the pressure fell again, sometimes below the original level. Intraventricular as well as intracisternal injection of 200  $\mu\text{g}$  tubocurarine, or perfusion with tubocurarine 1/5000 caused little or no rise in arterial blood pressure.

There are thus two sites from which the tubocurarine produces pressor effects. The rise produced by the intracisternal injection must be the result of an action on structures reached from the subarachnoid space, because in this condition little or no tubocurarine reaches the cerebral ventricles. On the other hand, the pressor effect produced by perfusion of tubocurarine from lateral ventricle to aqueduct must be on structures reached from the cerebral ventricle, because no tubocurarine enters the subarachnoid space under these conditions.

Apart from the long-lasting pressor effects there were transient rises in blood pressure during prolonged perfusion with tubocurarine, particularly when the high blood pressure had begun to fall again. Some of these transient rises lasted for about 1 min, others for a few seconds only (Figs. 10 and 11). Intraperitoneal pentobarbitone sodium abolished the short fluctuations, but did not suppress the longer-lasting rises in arterial blood pressure.



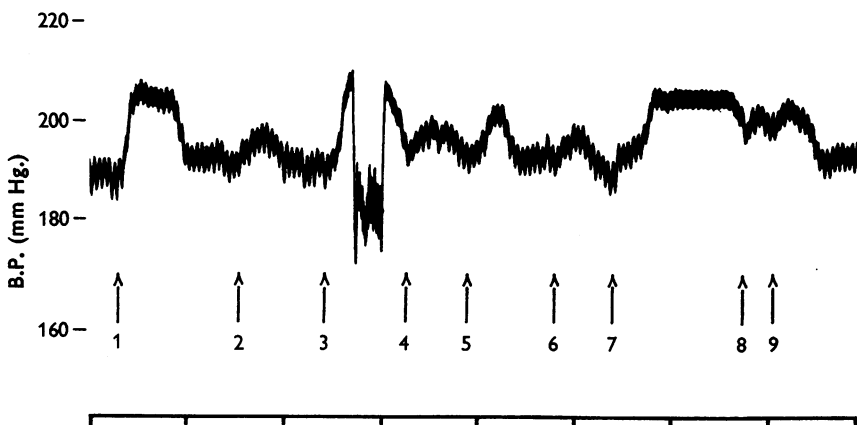


Fig. 10. Arterial blood pressure of a cat prepared as for Fig. 1. The record was obtained 50 min after the onset of perfusion with tubocurarine 1/500 from left lateral ventricle to aqueduct. The arrows indicate a wave or waves of pilo-erection. Time marker, in minutes.

#### *Pilo-erection*

Pilo-erection occurred in waves first on the tail and later on the back as well. It was a late effect of perfusion with tubocurarine to either aqueduct or cisterna. On perfusion with tubocurarine 1/1000 or 1/500 pilo-erection occurred usually after 40–60 min, when the blood pressure had just begun to fall again; on perfusion with 1/5000 it was sometimes 3 hr before pilo-erection was seen.

#### *Correlation between blood-pressure changes and waves of pilo-erection*

Figure 10 gives a blood-pressure record obtained 50 min after the onset of perfusion with tubocurarine 1/500 from the left lateral ventricle to aqueduct. The numbers 1–9 indicate the beginnings of 9 transient rises in arterial blood pressure; some are small, some large, and the duration varies. The sudden fall after the rise at 3 is caused by a dropped beat which is followed by a 25 sec period of bradycardia, after which the heart beat quickens again. With each rise in blood pressure there was one wave or more of pilo-erection in the tail; with each fall, the hairs lay down again. Even the individual variations in the fluctuations of the blood pressure were reflected. The steep large rises at 1 and 3 were associated with several strong waves of pilo-erection following in quick succession and resulting in maximal pilo-erection, maintained until the blood pressure began to fall; then pilo-erection subsided, not smoothly but in steps. Each of the small rises in blood pressure at 2, 4, 5 and 6, was associated with a small wave of pilo-erection. The parallelism went so far that the slightly steeper

and longer rise of blood pressure at 5—as compared to 2, 4 and 6—was associated with a stronger and faster wave of pilo-erection. At 7 pilo-erection began slowly, but was superseded by renewed faster and stronger waves as the rise in blood pressure became steeper. As soon as the blood pressure fell again, pilo-erection decreased, but at 8 and 9, when small rises occurred, the laying down of the hairs was interrupted by small waves of renewed pilo-erection.

*Correlation between the abnormal discharge on the e.e.g. and autonomic effects*

In all experiments there were periods in which the abnormal discharge in the e.e.g. showed a close association with the autonomic effects.

The large multiple spikes in the e.e.g. were associated with brief pupil dilatations and contractions of the nictitating membranes, whilst the onset of an episode was immediately followed by maximal or nearly maximal dilatation of the pupils and retraction of the nictitating membranes; during the subsequent electrical silence the pupils narrowed again and the nictitating membranes relaxed. However, there were always long periods in which the electrical changes occurred without visible effects on pupils and nictitating membranes, and sometimes there were oscillatory movements of the pupils which did not coincide with the rhythmic discharge.

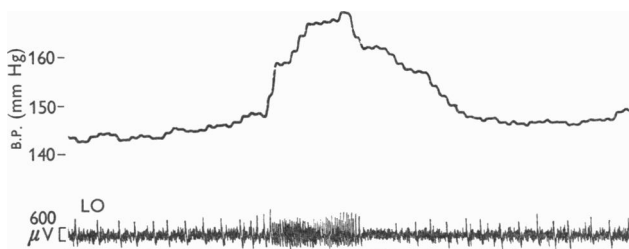


Fig. 11. Mean arterial blood pressure and e.e.g. of a cat prepared as for Fig. 1. The record was obtained 3 hr 10 min after the onset of perfusion with tubocurarine 1/5000 from left lateral ventricle to aqueduct. LO refers to left occipital lead. Time marker, 30 sec.

The episodes, apart from the initial one, were also often reflected on the arterial blood pressure. Their onset coincided with a more or less steep rise in arterial blood pressure which continued or was maintained throughout the episode. As soon as the episode was over, the blood pressure fell again. Sometimes, during the period of electrical silence, it fell below its original level; then, with the reappearance of the rhythmic discharge, it gradually rose again. Figure 11 illustrates an episode with its reflexion on the arterial blood pressure. In experiments in which there were long

periods when episodes occurred at short intervals, it was an impressive experience to follow the reflexion of each episode, its onset and cessation, on the arterial blood pressure.

A reflexion of the rhythmic spike discharge was occasionally seen when anaesthesia lightened and the arterial blood pressure showed short-lasting fluctuations. In this condition each multiple spike in the e.e.g. was followed by a small transient rise in pressure.

Pilo-erection was also closely associated with the abnormal discharge. Often each multiple spike was immediately followed by pilo-erection, a small spike by a small wave on the tip of the tail, a large spike by a strong wave, involving the whole of the tail and spreading to the back. When two spikes quickly followed each other there were two superimposed waves. An episode led to maximal pilo-erection, reached not smoothly but in several steps and then maintained. The hairs stood up, separated from each other, so that the skin became visible. As soon as the episode was over pilo-erection subsided and the hairs lay down during the period of electrical silence, but the reappearance of the first small spikes was again reflected by small waves of pilo-erection in the tip of the tail.

Thus an episode in the e.e.g. was associated with maximal or nearly maximal dilatation of the pupils, full retraction of the nictitating membranes, a rise in arterial blood pressure and intense pilo-erection. When after prolonged perfusion with tubocurarine the rhythmic discharge had become so frequent that the episodes were scarcely discernible, it was not possible to correlate the autonomic effects with the different features of the abnormal discharge. Yet in this condition a close association was sometimes still observed between blood pressure changes and waves of pilo-erection.

### PART III. EFFECTS OF BROMOPHENOL BLUE

When 0.2–1% bromophenol blue was perfused from lateral ventricle to aqueduct or cisterna it produced an abnormal discharge similar to that of tubocurarine. However, the effect of bromophenol blue differed from that of tubocurarine in that the barbiturate spindles did not disappear and in that none of the autonomic effects occurred.

Figure 12 illustrates the abnormal discharge on perfusion with 0.2% bromophenol blue from the left lateral ventricle to cisterna. *A* begins 10 min after the beginning of perfusion and shows the onset of the abnormal discharge. It consists at first of rhythmic single spikes recorded from the left occipital lead. Later the spikes become multiple and are reflected in the other leads. One of these spikes is recorded on fast paper at *B*. Five minutes after the beginning of the abnormal discharge the first episode is recorded at *C*. Its activity consists of fast synchronous spikes. In later

episodes the pattern of activity changes to that of a train of multiple spikes, and the episodes occur at short intervals (*D*).

When a 1% solution of bromophenol blue is used, episodes occur, if at all, during the initial stage only, and on prolonged perfusion the spikes of the rhythmic discharge become gradually smaller. When perfusion is then switched over to tubocurarine, they again increase in size, and all the autonomic effects described appear.

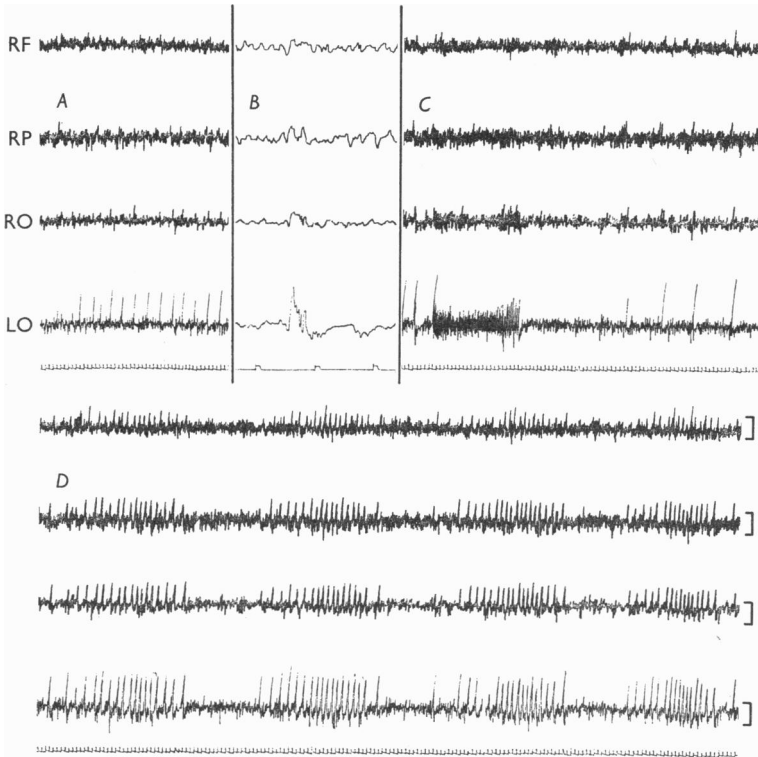


Fig. 12. E.e.g. of a cat prepared as for Fig. 1. Perfusion with 0.2% bromophenol blue from left lateral ventricle to cisterna. *A-D*, taken 10, 14, 15 and 50 min after the onset of perfusion. RP refers to right parietal lead. Other lettering and calibration as in Fig. 1.

In one experiment the cannula used for perfusion with 1% bromophenol blue was incorrectly positioned with its opening facing the foramen of Monro so that the dye did not enter the lateral ventricle, but passed through the third ventricle and upper part of the aqueduct. The regions perfused are illustrated in Fig. 11*B*. In this experiment the abnormal discharge did not appear. Therefore, the discharge produced by bromophenol blue, like that produced by tubocurarine, must be due to an action on structures reached from the lateral ventricles.

## DISCUSSION

The finding of Samson Wright (1955) has been confirmed that an injection of tubocurarine into the lateral ventricle of an anaesthetized cat produces an abnormal discharge in the e.e.g. resembling the seizure discharge of epilepsy. In the present experiments a definite sequence in the development of this discharge was observed, and it could be shown that the sites where tubocurarine acts when producing the discharge must be structures of grey matter easily reached by the tubocurarine from the posterior half of the lateral ventricle. There are three such structures: the hippocampus, the amygdala and the pyriform cortex.

Evidence obtained with other methods by various authors favours the hippocampus and/or the amygdala. For instance, the seizure discharge due to hypoglycaemia originates either in the hippocampus or in the amygdala and only later spreads to the rest of the brain (Tokizane & Sawyer, 1957) and so does the seizure discharge following intracarotid injections of hypertonic solutions (Sawyer & Gernandt, 1956).

In favour of an action of tubocurarine on the hippocampus are the following facts. Its electrical stimulation results in a seizure discharge which, when projected to the cerebral cortex, like the seizure discharge produced by tubocurarine, appears first on the occipital lobe (Creutzfeldt, 1956). The threshold for electrical-seizure discharge is lower in the hippocampus than in any other region of the brain (for references see Green, 1960). Injections of small amounts of acetylcholine together with physostigmine or of carbachol directly into the hippocampus produce a seizure discharge (MacLean, 1957).

In favour of an action of tubocurarine on the amygdala are the following facts. Its electrical stimulation results in seizure discharge, and the threshold also is low, but not as low as that of the hippocampus (for references see Gloor, 1960). The long-lasting one-sidedness of the abnormal discharge produced by tubocurarine during one-sided perfusion would agree with the fact that the discharge from the amygdala is projected mainly to ipsilateral structures (Gloor, 1957). The early occurrence of pupillary dilatation, often more pronounced on the side of tubocurarine perfusion, and the frequently observed association between the abnormal discharge and autonomic effects, also favour an activation of the amygdala, since its electrical stimulation causes mydriasis, withdrawal of the nictitating membranes, rise in arterial blood pressure and pilo-erection (Koikegami & Yoshida, 1953; Gastaut, 1954; Gloor, 1960). Activation of the amygdala by tubocurarine would also explain the observed suppression of the barbiturate spindles, since they are suppressed by electrical stimulation of these structures (Feindel & Gloor, 1954).

Perfusion of the lateral ventricles with bromophenol blue resulted in a seizure discharge similar to that produced by tubocurarine, also appearing first in the occipital leads, also being one-sided with one-sided perfusion, and also failing to appear on perfusion through the third ventricle. But with bromophenol blue the barbiturate spindles did not disappear and none of the autonomic effects were obtained. These findings show that the tubocurarine has a wider action than bromophenol blue and that the seizure discharge is not necessarily linked with the other effects. They may be produced by activation of an additional site.

It would be tempting to assume that the seizure discharge, whether produced by tubocurarine or bromophenol blue, originates from an action on the hippocampus, that the autonomic effects as well as the suppression of the barbiturate spindles produced by the tubocurarine result from an activation of the amygdala, and that bromophenol blue lacks this action. The following findings support this assumption, as they indicate that at least some of the autonomic effects are produced by an action on structures reached from the lateral ventricles. On perfusion with tubocurarine from one lateral ventricle the effects on pupils and nictitating membranes were often more pronounced on the side of tubocurarine perfusion and they failed to appear in the two experiments in which tubocurarine did not enter the lateral ventricle but passed straight into the third ventricle. In this connexion it is worth mentioning that a one-sided effect on the nictitating membrane, indicating an action on structures reached from the lateral ventricle, was also observed by Bein (1957) on injection of reserpine into a cannulated lateral ventricle. The fact that on perfusion with tubocurarine from one lateral ventricle both pupils became sometimes equally dilated could be explained by the fact that the sympathetic discharge produced by the tubocurarine affected the suprarenal glands and that the dilatation was brought about by circulating adrenaline. On the other hand, our experiments so far do not exclude the possibility that the autonomic effects observed when tubocurarine is applied by the intraventricular route are partly at least produced by an action on structures reached from the third ventricle.

Although with one-sided perfusion of either tubocurarine or bromophenol blue the abnormal discharge always appears first on the side of perfusion, there is in time an increasing reflexion on the contralateral side. Since the tubocurarine or bromophenol blue perfusion remains, as far as the lateral ventricle is concerned, one-sided, this reflexion must result from a spread of the discharge to the contralateral side. Volume conduction is unlikely to be the cause of this spread, because the discharge remains small in the frontal lead of the perfused side. The spread must therefore result from excitation of neuronal pathways to the contralateral side,

either from the hippocampus or amygdala, to the corresponding structures of the contralateral hemisphere via the psalterium and anterior commissure or from cortex to cortex via the corpus callosum, or by both routes. With the relatively slow method of e.e.g. recording, the discharge was synchronous in all leads. This, however, does not exclude differences in milliseconds which could only be revealed if more refined methods of recording were used.

In previous experiments (Feldberg *et al.* 1956; Feldberg *et al.* 1957; Feldberg & Malcolm, 1959) in which the effects of intraventricular injections of tubocurarine on the electrical activity of the brain were recorded with micro-electrodes from the sensory areas of the cerebral cortex and from various subcortical structures including the anterior part of the hippocampus, two main effects of tubocurarine were observed: episodes, consisting of bursts of high-voltage activity, and an augmentation of the evoked response to stimulation of sensory nerves from the skin. The episodes most likely correspond to the episodes described in the present experiments. If so, they, too, originate from an action of tubocurarine on structures reached from the posterior half of the lateral ventricle, i.e. from hippocampus and/or amygdala, and are propagated via neuronal pathways to those structures from which the recordings were made. In a recent paper of Cairnie & Malcolm (1960) which deals with the augmentation of the evoked response produced by tubocurarine, the conclusion is reached that the tubocurarine must pass into the subarachnoid space and reach the sensory area of the cortex in order to produce this effect. This conclusion would entail a different site of action of tubocurarine when producing augmentation of the evoked response, and when producing the abnormal e.e.g., i.e. the discharge of rhythmic surface-negative spikes and of episodes.

Both tubocurarine and bromophenol blue are substances which do not occur normally in the c.s.f. and do not pass the blood-brain barrier. Yet the analysis of the site of origin of the seizure discharge produced by these substances when penetrating the brain tissue from the cerebral ventricles provides the basis for an understanding of the mechanism by which a seizure discharge may be produced by many other substances applied by the intraventricular route.

The finding that substances can easily reach the hippocampus and the amygdala from the c.s.f. and in so doing activate these structures raises the question whether, in epilepsy, changes in the composition of the c.s.f. influence the seizure threshold of these most excitable structures of the brain. Since these structures are bathed by the c.s.f., slight but prolonged changes in its composition could well account for some of the periodical changes which occur in the frequency of seizures of epileptic patients, for

instance, during day and night, or during the menstrual cycle. The results obtained with tubocurarine and bromophenol blue, when viewed in conjunction with the other experimental findings concerning seizure discharge originating in the hippocampus and amygdala, point to the possibility that activation of these structures is an essential factor in the seizure discharge of many forms of epilepsy, although the primary focus of irritation may be elsewhere.

#### SUMMARY

1. The effect of tubocurarine applied to the ventricular system of the brain was examined on the e.e.g. in cats anaesthetized with pentobarbitone sodium, immobilized with Flaxedil and artificially ventilated. The e.e.g. was recorded with monopolar leads from epidural electrodes inserted through burr holes over the right and left frontal and occipital lobes.

2. The injection of 200–400  $\mu\text{g}$  tubocurarine into the cannulated lateral ventricle produces an abnormal discharge in the e.e.g. resembling the seizure discharge in epilepsy. The discharge develops according to a definite pattern: first, the barbiturate spindles are suppressed in all leads, then a rhythmic discharge of large surface-negative spikes or sharp waves begins on the occipital leads. These spikes later become multiple and spread to the frontal leads where, however, they are smaller and remain so throughout. The rhythmic discharge is interrupted from time to time by episodes, i.e. bursts of fast high-voltage activity which are often followed by periods of electrical silence. The initial episodes consist mainly of fast synchronous spike activity, the late ones mainly of a train of multiple spikes. Finally, the rhythmic discharge becomes so frequent that the episodes are scarcely discernible. The abnormal discharge is synchronous in all leads and is more pronounced on the side of injection.

3. The abnormal discharge results from an action of tubocurarine on structures reached from the posterior half of the lateral ventricle, for the following reasons:

(a) Structures reached from the subarachnoid space are excluded, because injections of tubocurarine into the cisterna magna did not produce such discharge.

(b) The abnormal discharge must be due to an action on structures reached from the inner surface of the brain, because it occurred on perfusion of tubocurarine from the lateral ventricle to aqueduct.

(c) The tubocurarine must act on structures reached from the lateral ventricle, because the discharge was of equal intensity in both occipital leads when tubocurarine was perfused through both lateral ventricles, but one-sided when tubocurarine was perfused through one lateral ventricle whilst the other was perfused with artificial c.s.f. Further, the



discharge did not occur when the perfusion fluid containing the tubocurarine passed straight into the third ventricle.

(d) The site of action must be on structures situated in the walls of the posterior half of the lateral ventricle, because the discharge did not occur when the perfusion fluid containing the tubocurarine was prevented from entering the posterior half of the lateral ventricle, but passed through its anterior half and through the third ventricle.

4. It is concluded that the abnormal discharge is produced by an action of tubocurarine on the hippocampus and/or amygdala.

5. The following autonomic effects were observed when tubocurarine was injected into the lateral ventricle or perfused through the ventricular system: withdrawal of the nictitating membranes, mydriasis, a rise in arterial blood pressure and pilo-erection. As these autonomic effects were obtained on perfusion from lateral ventricle to aqueduct, they are due to an action on structures reached from the lateral and/or third ventricles. However, a rise in arterial pressure occurs also when tubocurarine is injected into the cisterna magna and acts on structures reached from the subarachnoid space. There are, therefore, at least two central sites from which tubocurarine can elicit this effect.

6. There was often an association of the abnormal discharge on the e.e.g. with the autonomic effects. Spike discharges coincided with sudden small retraction of the nictitating membranes and dilatation of the pupils; episodes were associated with maximal retraction of the nictitating membrane and maximal widening of the pupils. On the blood pressure the episodes were reflected by transient rises. Rhythmic waves of pilo-erection on the tail coincided with large spikes, and episodes with periods of maximal sustained pilo-erection.

7. Perfusion with the dye bromophenol blue from lateral ventricle to aqueduct or cisterna resulted in a seizure discharge similar to that produced by tubocurarine, also appearing first on the occipital leads. It was also one-sided with one-sided perfusion, and absent when the dye was perfused from the third ventricle to the aqueduct. This discharge is therefore also attributed to an action on the hippocampus and/or amygdala; but with bromophenol blue none of the autonomic effects occurred and the barbiturate spindles did not disappear.

#### REFERENCES

- BEIN, H. J. (1957). Effects of reserpine on the functional strata of the nervous system. In *Psychotropic Drugs*, pp. 327-331, ed. GARRATTINI, S. & GHETTI, V. London: Elsevier Publishing Co.
- BHATTACHARYA, B. K. & FELDBERG, W. (1956). Perfusion of the ventricular system of the brain in the anaesthetized cat. *J. Physiol.* **135**, 4-5 P.
- CAIRNIE, A. B. & MALCOLM, J. L. (1960). Site of action of D-tubocurarine that enhances evoked cortical potentials. *J. Physiol.* **154**, 38-39 P.

- CREUTZFELDT, O. (1956). Die Krampfausbreitung im Temporallappen der Katze. *Schweiz. Arch. Neurol. Psychiat.* **77**, 164-194.
- FEINDEL, W. & GLOOR, P. (1954). Comparison of electrographic effects of stimulation of the amygdala and brain stem reticular formation in cats. *Electroenceph. clin. Neurophysiol.* **6**, 389-402.
- FELDBERG, W. & FLEISCHHAUER, K. (1960). Penetration of bromophenol blue from the perfused cerebral ventricles into the brain tissue. *J. Physiol.* **150**, 451-462.
- FELDBERG, W. & MALCOLM, J. L. (1959). Experiments on the site of action of tubocurarine when applied via the cerebral ventricles. *J. Physiol.* **149**, 58-77.
- FELDBERG, W., MALCOLM, J. L. & SHERWOOD, S. L. (1956). Some effects of tubocurarine on the electrical activity of the cat's brain. *J. Physiol.* **132**, 130-145.
- FELDBERG, W., MALCOLM, J. L. & SMITH, I. D. (1957). Effect of tubocurarine on the electrical activity of the cat brain under chloralose. *J. Physiol.* **138**, 178-201.
- FELDBERG, W. & SHERWOOD, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol.* **120**, 3-5 P.
- GASTAUT, H. (1954). Correlations entre le système nerveux végétatif et le système de la vie de relation dans le rhinencéphale. *J. Physiol., Paris*, **44**, 431-470.
- GLOOR, P. (1957). The pattern of conduction of amygdaloid seizure discharge. *Arch. Neurol., Chicago*, **77**, 247-258.
- GLOOR, P. (1960). Amygdala. In *Handbook of Physiology*. Section I, vol. 2, pp. 1395-1420, ed. FIELD, J., MAGOUN, H. W. & HALL, V. E. Baltimore: Waverly Press.
- GREEN, J. D. (1960). The hippocampus. In *Handbook of Physiology*. Section I, vol. 2, pp. 1373-1389, ed. FIELD, J., MAGOUN, H. W. & HALL, V. E. Baltimore: Waverly Press.
- KOIKEGAMI, H. & YOSHIDA, K. (1953). Pupillary dilatation induced by stimulation of amygdaloid nuclei. *Folia psychiat. neurol. Jap.* **7**, 109-126.
- MACLEAN, P. D. (1957). Chemical and electrical stimulation of hippocampus in unrestrained animals. *Arch. Neurol., Chicago*, **78**, 113-127.
- MERLIS, J. K. (1940). The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. *Amer. J. Physiol.* **131**, 67-72.
- SAWYER, C. H. & GERNANDT, B. E. (1956). Effects of intracarotid and intraventricular injections of hypertonic solutions on electrical activity of the rabbit brain. *Amer. J. Physiol.* **185**, 209-216.
- TOKIZANE, T. & SAWYER, C. H. (1957). Site of origin of hypoglycaemic seizures in the rabbit. *Arch. Neurol., Chicago*, **77**, 259-266.
- WRIGHT, S. (1955). Electroencephalographic patterns following intraventricular injections of tubocurarine in the cat. *J. Physiol.* **130**, 35-36 P.