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EXPERIMENTS ON THE SITE OF ACTION OF TUBOCURARINE WHEN APPLIED VIA THE CEREBRAL VENTRICLES

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In the present experiments an attempt was made to locate the site where tubocurarine acts to produce the convulsive effects and the accompanying changes in electrical activity of the brain on injection into the lateral cerebral ventricle. Two different approaches were made. By recording the electrical activity from two points on either side of the mid line, it could be demonstrated that the activity produced by the unilateral injection occurred with similar latencies on both sides. This excludes structures lining the lateral ventricles as the sites at which the activity originated. In the course of these experiments it was found that records obtained from the cellular layer of Ammon's horn during the action of tubocurarine showed a characteristic pattern different from the patterns found in any other region so far examined.

In the second approach the tubocurarine was perfused through a cannula ending in the lateral ventricle and the effluent was collected from various points between the third ventricle and the cisterna. In these experiments the muscular effects were recorded on a myograph and changes in excitability tested by the response of the quadriceps muscle to tapping its tendon.

METHODS

The experiments were performed on cats under chloralose anaesthesia (60 mg/kg intravenously). In experiments in which records were taken of the electrical activity of the brain and in which evoked responses to peripheral nerve stimulation (peroneal nerve) were recorded before and after an intraventricular injection of tubocurarine, the methods used were those previously described (Feldberg, Malcolm & Smith, 1957), and tubocurarine was injected intravenously. In those experiments in which tubocurarine was perfused through a cannula in the lateral ventricle and the effluent was collected from the cisterna or the aqueduct, the methods were essentially those described by Feldberg & Bhattacharya (1958), but the perfusion fluid used was not always Locke's solution but often an 'artificial cerebrospinal fluid' introduced by Merlis (1940) and later used by Leusen (1949). Its composition was as follows (g/l.): NaCl 8·10, KCl 0·25, $CaCl_2$ 0·14, $MgCl_2$ 0·11, $NaHCO_3$ 1·76, NaH_2PO_4 0·07, $(NH_2)_2CO$ 0·13, glucose 0·61. No difference was observed in the effects of tubocurarine when Locke's solution or artificial c.s.f. was used for perfusion. The rate of

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perfusion was 0·1 ml./min. At the end of the perfusion experiments the tubocurarine solution was replaced by a solution containing 0·1 % Evans Blue and perfusion was continued for 20 min before the head was perfused from a carotid artery with alcohol-formalin-saline fixative. From the staining it was then possible to say which regions had been reached by the perfusing tubocurarine.

In some experiments the fourth ventricle was perfused with a tubocurarine solution which was prevented from entering the aqueduct. Two procedures were adopted. In the one, illustrated in Fig. 1, a double-bore cannula was used, the inner tube being used for the infusion of the tubocurarine solution, the outer tube for collecting the outflow. After the tip of the collecting tube had been passed through the opened atlanto-occipital membrane into the fourth ventricle, the inner tube, of fine polythene, was passed through the collecting tube so that its tip reached 2–3 mm beyond the tip of the collecting tube. The tubocurarine was perfused through the fine inner tube at a rate of 0·1 ml./min whilst a perfusion with artificial c.s.f. alone was maintained from the lateral ventricle at a rate of 0·05 ml./min, so as to prevent the tubocurarine from entering the

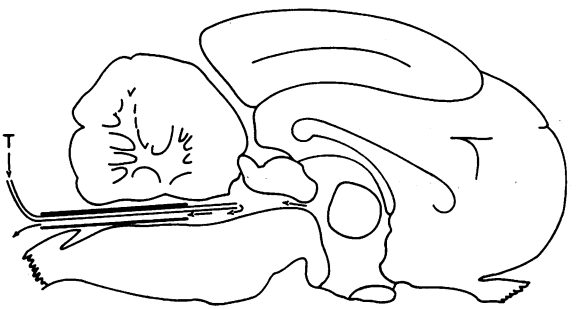

Fig. 1. Diagram of the double-bore cannula used for perfusion of the fourth ventricle with tubocurarine (T). Both cannulae made from polythene tubing.

aqueduct. With this method a limited region of the fourth ventricle was perfused with the tubocurarine solution; in addition, there was some leakage through the lateral recesses, as was shown by staining of parts of the outside of the brain stem when, at the end of the experiment, the tubocurarine solution was replaced by a solution containing 0·1 % Evans Blue and perfusion was continued for 20 min.

With the other method no double-bore tube was used; the wider tube alone was placed with its tip in the fourth ventricle. Perfusion was carried out through this tube with the tubocurarine solution at a rate of 0·1 ml./min and the outflow was allowed to drain from the widely opened cisterna, the tubocurarine solution again being prevented from entering the aqueduct by a slower counter-flow from the ventricular cannula. With this method large areas of the fourth ventricle and of the outer surface of the medulla oblongata and spinal cord were reached by the tubocurarine solution, as was shown by the staining when later the tubocurarine solution was replaced with a solution containing Evans Blue.

In order to record muscular effects both quadriceps muscles were fixed on a Brown–Schuster myograph and their contractions were recorded on a smoked drum. Every few seconds a myotatic reflex was elicited by tapping the patellar tendon with an electrically driven hammer.

In some experiments two needles were inserted subcutaneously into the sole of the left foot and an electrical stimulus was applied through them in the intervals between the tapping of the patellar tendon. The stimulus strength was just sufficient to elicit a small reflex response.

When respiration was recorded a stethograph was placed across the chest, and the volume changes were recorded on the smoked drum with a small metal bellows recorder.

RESULTS

Electrical recordings from the brain after intraventricular tubocurarine

Records from Ammon's horn. The outstanding feature in the electrical activity of certain parts of Ammon's horn after an intraventricular injection of 200 μg of tubocurarine was the appearance of groups of high-voltage spikes. They came simultaneously with the bursts of increased electrical activity recorded from the cerebral cortex and from sub-cortical structures, and referred to previously as 'episodes' (Feldberg *et al.* 1957).

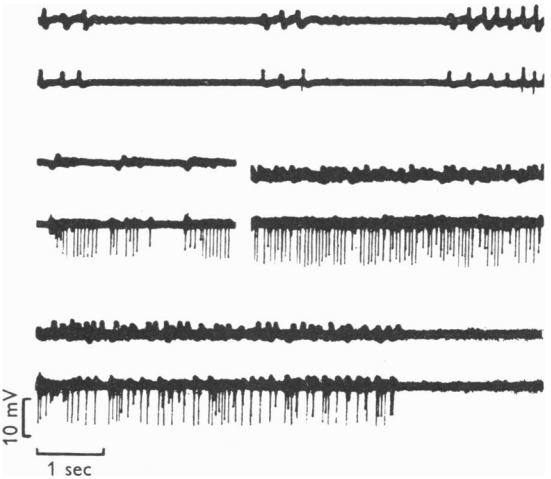

Fig. 2

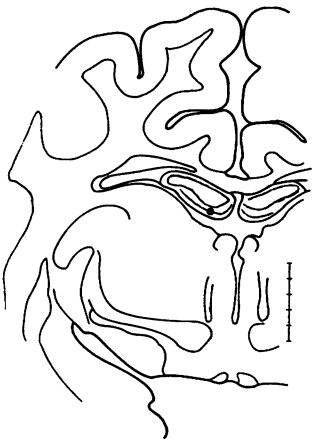

Fig. 3

Fig. 2. Part of an episode of activity after an intraventricular injection of 200 μg tubocurarine in a cat under chloralose anaesthesia. In each record the upper tracing is from the primary sensory area of the cerebral cortex and the lower tracing from a cellular layer of Ammon's horn. The position of the tip of the electrode is shown in the diagram Fig. 3. Voltage calibration refers to the focal electrode. For details see text.

Fig. 3. Diagram of coronal section of cat's brain 4 mm in front of the Horsley–Clarke zero plane. The black point in the cellular layer of Ammon's horn gives the position of the tip of the micro-electrode in experiments of Figs. 2 and 4.

Figure 2 illustrates an episode recorded simultaneously from the primary sensory cortex and from a cellular layer of Ammon's horn. The position of the tip of the electrode in Ammon's horn, as ascertained by subsequent histological examination of the perfused brain, is shown in the diagram of Fig. 3 by the black spot. The episode begins with bursts of a few slow waves in both records. From the slow waves recorded from Ammon's horn, spikes develop which increase in size and, during the height of the episode, dominate the focal record. The episode stops abruptly, and immediately afterwards no responses can be evoked by stimulation of the contralateral peroneal nerve. The development

of the spike activity is shown on a faster sweep in Fig. 4. The top record shows two small positive slow waves the first of which gives rise to a small single spike. In the middle record, taken several seconds later, a few spikes develop after the beginning of the slow waves and increase in size. The bottom record shows the spike activity at the height of the episode.

When the tip of the electrode was in a cellular layer of Ammon's horn, the evoked responses were also characterized, after the tubocurarine injection, by groups of large-voltage spikes arising from the slow evoked wave. On traversing

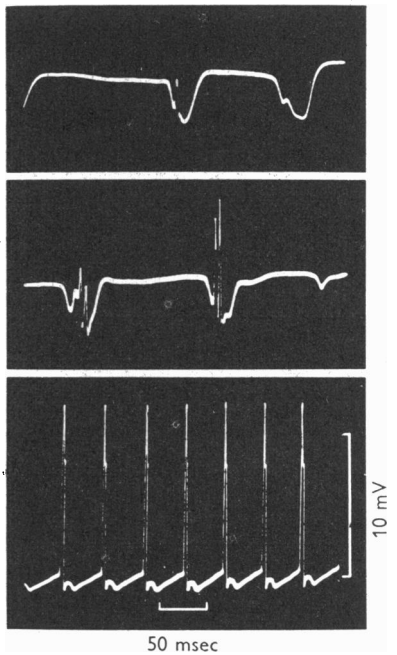

Fig. 4. Same experiment as Fig. 2 but on a faster sweep. Development of large spike-like activity in electrode from the cellular layer of Ammon's horn during an episode. (For details see text.)

Ammon's horn a point was always reached at which the spikes were maximal and diphasic. Both above and below this point, the spikes decreased in size and usually became first negative and then positive while the slow wave remained. This is illustrated in Fig. 5. The evoked response at *C* is obtained with the tip of the electrode within, or very close to a cellular layer, as ascertained by subsequent histological examination of the perfused brain. With the records *A*, *B*, *D* and *E*, the exact distance of the tip of the electrode from this cellular layer of Ammon's horn was not known.

Records from two points on either side of the mid line. Table 1 indicates the location of the tip of the electrodes in six experiments in which records were taken simultaneously at points on either side of the mid line. In each of these

experiments the first signs of altered electrical activity, spontaneous or evoked, after an injection of 200 μg of tubocurarine, occurred at the same time on both sides although the tubocurarine was always injected into the left lateral ventricle. It is therefore unlikely that the tubocurarine produced the changes in electrical activity by acting on structures lining the left lateral ventricle.

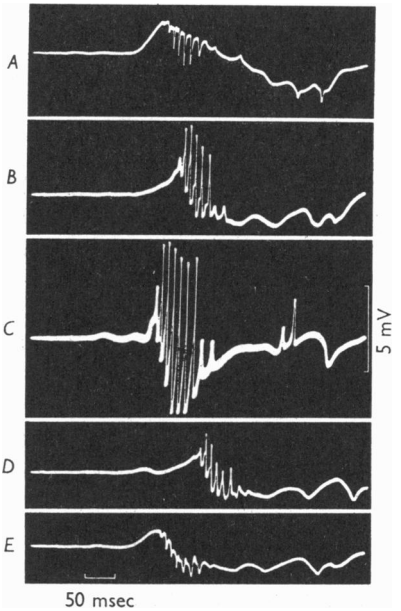

Fig. 5. Record of responses from left Ammon's horn evoked by stimulation of the contra-lateral peroneal nerve after an intraventricular injection of 200 μg tubocurarine. The record at *C* was obtained when the tip of the electrode was in, or very close to a cellular layer. The other records were obtained after lowering or raising the electrode 50 or 100 μ, the traverse being at an angle of 30° from the vertical. The electrode was first lowered 50 μ between *C* and *D* and between *D* and *E*, and then withdrawn beyond the point recorded from at *C*, first 100 μ (Record *B*) and then 50 μ (Record *A*). (For details see text.)

TABLE 1. Regions on the left and right side of the mid line from which simultaneous records were taken in six experiments after injection of 50 μg tubocurarine into the left lateral ventricle

Expt.	Left	Right
1	Ammon's horn	Sup. colliculus
2	Sup. colliculus	Sup. colliculus
3	Pulvinar	Ammon's horn
4	Nucleus centralis medialis	Pulvinar
5	Ammon's horn	Nucleus reuniens
6	Ammon's horn	Nucleus centralis medialis

Observations on muscular activity during perfusion of the cerebral ventricular system with tubocurarine

Outflow from cisterna. When a solution of tubocurarine 1:10,000 was perfused through the cannula in the lateral ventricle and the outflow was collected

from a cannula in the cisterna, the effects were similar to those described with single intraventricular injections of 20 μg tubocurarine in 0·2 ml. into cats anaesthetized with chloralose (Feldberg *et al.* 1957). Within a few minutes of the start of perfusion with tubocurarine, tapping the quadriceps or soleus tendon produced increased reflex responses with repetitive contractions,

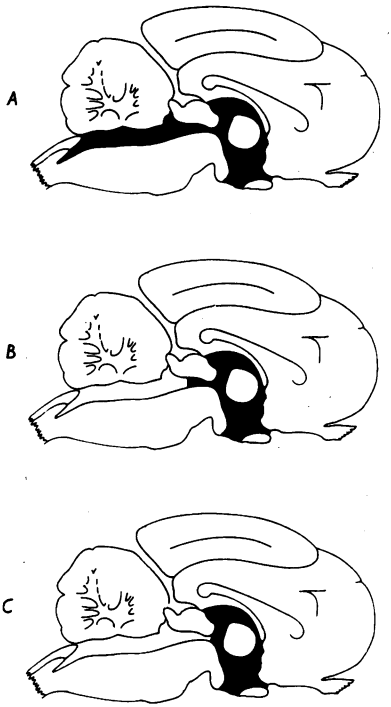

Fig. 6. Parts of the cerebral ventricular spaces (indicated in black) reached by the tubocurarine during perfusion from the lateral ventricle with the tip of the outflow cannula in the cisterna (at *A*), at the mid-collicular level of the aqueduct (at *B*) and at the entrance to the third ventricle (at *C*).

followed within a few minutes by continuous fasciculation of the quadriceps muscle. Later the continuous fasciculation involved the respiratory muscles as well and jerky movements occurred.

Tip of outflow cannula at mid-collicular level. With the outflow cannula in the cisterna the tubocurarine passes not only over the fourth ventricle, as shown in Fig. 6*A* by the black area, but also over the outer surface of the brain stem. The general picture of the effects of tubocurarine, however, remained unchanged in experiments in which the tip of the outflow cannula was pushed into the aqueduct as far as the mid-collicular level. Under this condition, which is illustrated in Fig. 6*B*, the tubocurarine no longer reaches the fourth ventricle or the outer surface of the brain stem. Its first effect was again an

increase in the height of the contractions of the quadriceps in response to tapping its tendon, as illustrated in Fig. 7 at *a*. In addition the reflex responses showed great irregularities. The changes began within 2 min of perfusion with tubocurarine and continued to increase during the following 15 min. When perfusion was then continued with the artificial c.s.f. alone it took over half an hour before the reflex responses returned to their original size. When the perfusion was restarted with tubocurarine the reflex responses again increased in size and showed irregularities, as in Fig. 7 at *b*.

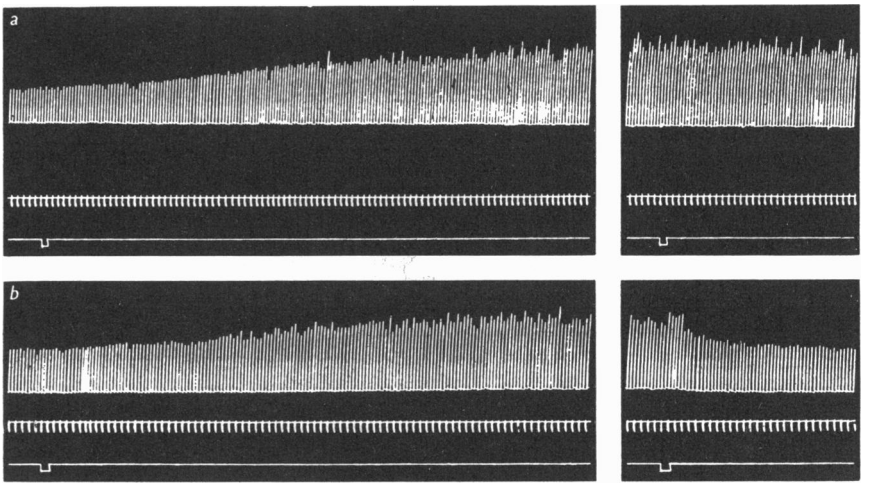

Fig. 7. Responses of the left quadriceps to tapping of the patellar tendon during perfusion of the cerebral ventricular spaces with the tip of the outflow cannula at the mid-collicular level of the aqueduct. From the first to the second signal, perfusion with tubocurarine 1:10,000; from the second signal onwards, perfusion in *a* with artificial c.s.f. alone and in *b* with tubocurarine 1:10,000 plus noradrenaline 1:2000. Between the two sections in *a* an interval of 5 min; and in *b* of 1 min. Time marker, 10 sec.

In several experiments the augmentation of the knee jerk was less pronounced, as for example in the experiment of Fig. 8, and the main effect was that, after a variable interval of time, each response was followed by a series of small tremor-like contractions. At first these involved only the hind-limb muscles, but later they spread to the muscles of the forelimb and trunk. This activity increased in duration and gradually became a continuous background of activity which persisted when the tendon tap was stopped.

The development of this activity is illustrated in the records of Fig. 8 by the irregularity and broadening of the base line. Record *b* was obtained after perfusion for about 5 min, record *c* after about 15 and record *d* after about 50 min with tubocurarine 1:10,000. At the signals in record *c* the tendon tap was stopped, but already the after-discharge had become a continuous background

of activity which persisted while the tendon taps had ceased, although there was as yet little activity in the contralateral quadriceps; but later, in record *d*, the activity had spread to the other limb as well. The tremor-like activity, particularly when it became continuous, occluded to a varying degree many responses of the quadriceps muscle to the tendon tap, as is shown in record *d* of Fig. 8. Sometimes, at later stages of perfusion, the finer tremor-like movements became more synchronous and led to clonic jerks which involved also the muscles of the upper extremities and the trunk.

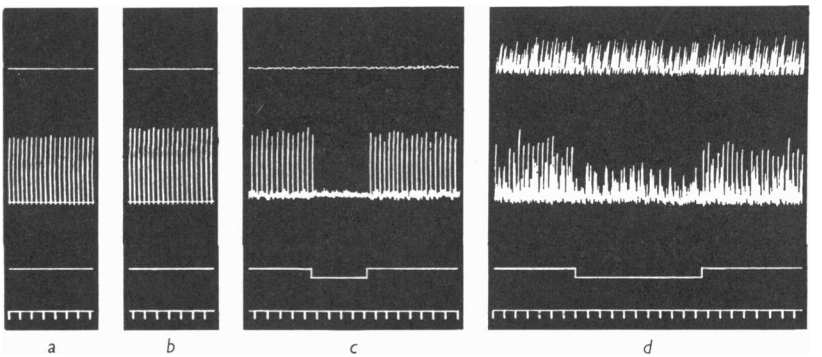

Fig. 8. Upper record from right and lower record from left quadriceps before and during perfusion of the cerebral ventricular spaces with the tip of the outflow cannula at the mid-collicular level of the aqueduct. Tapping of left patellar tendon every few seconds except at the signals when tapping was stopped. Records *a* before, and *b*, *c* and *d* after, perfusion for about 5, 15 and 50 min with tubocurarine 1:10,000. Time marker, 10 sec. (For details see text.)

At the height of the tubocurarine action many types of stimuli, such as tapping the table or the back of the animal, or sudden loud noises, produced generalized vigorous jerks. Similarly, cutaneous sensory stimuli produced by an electrical pulse to electrodes inserted into the skin of the foot, which before the tubocurarine produced only a slight muscular response in the foot, caused a larger response which involved the contralateral limb as well.

One of the first effects which often preceded the activity in the limb muscles was a change in the pattern of the respiratory movements. The inspirations were preceded by small inspiratory muscular efforts, or there were occasional sharp inspiratory efforts, during an inspiration. Later each inspiration was followed by tremor-like contractions of the muscles of the thorax and pectoral girdle.

In the course of the tubocurarine perfusion the pupils became gradually wider and with each general muscle jerk there was a sudden short-lasting dilatation of the pupils. When there was a continuous background of tremor-like activity there were also continuous oscillations of the pupillary diameter. This phase was frequently accompanied by nystagmus-like movements of the eyeballs. The blink reflex often became brisker.

Tip of outflow cannula at entrance of third ventricle. In these experiments the tip of the outflow cannula had been passed throughout the whole length of the aqueduct to reach the posterior commissure. The diagram of Fig. 6*C* illustrates that under this condition the tubocurarine does not reach the aqueduct. Perfusion with tubocurarine 1:10,000 no longer produced the muscular effects; but when the concentration was increased to 1:2000 contractions of the thoracic muscles occurred, yet only after about 20 min perfusion.

Perfusion of the fourth ventricle. In two experiments the perfusion with tubocurarine was carried out with the double-bore cannula. The areas stained

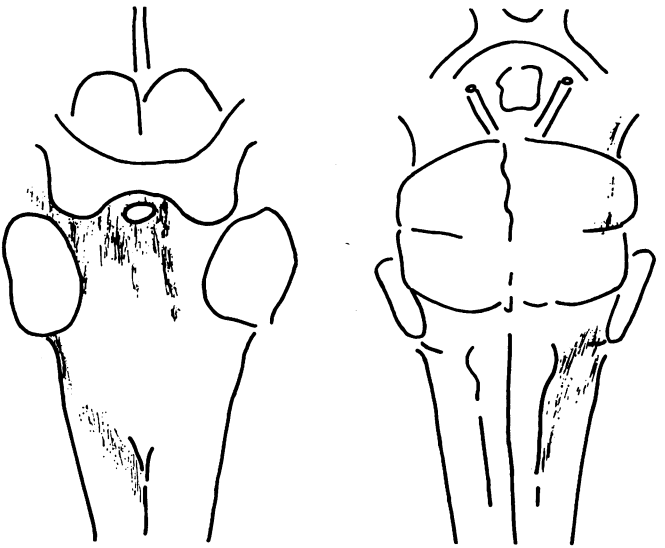

Fig. 9. Shaded areas indicate regions of the floor of the fourth ventricle and of the outside of the brain stem which were stained blue after perfusion of the fourth ventricle for 20 min with Evans Blue, using the double-bore cannula shown in Fig. 1.

after 20 min perfusion with Evans Blue at the end of the experiment were about the same for both experiments. They are shown for one experiment in Fig. 9. The perfusion with tubocurarine 1:8000 for 40 min produced no muscular or respiratory effects, but when the tubocurarine was later added to the fluid perfusing the lateral and third ventricles and the aqueduct the typical muscular effects appeared. Even when the tubocurarine reached larger areas of the surface of the brain stem, as in those experiments in which a single-bore cannula was used and the whole outflow was allowed to drain from a widely opened cisterna, it produced no muscular contractions but greatly affected the depth and rate of respiration. This is illustrated in the upper part of Fig. 10. The lower part shows diagrammatically the areas which became stained when at the end of the experiment Evans Blue was perfused through the cannula. Large areas of the fourth ventricle, the whole ventral surface of the medulla

oblongata and parts of the pons were found to be stained, as is indicated diagrammatically in Fig. 10. The staining, however, did not extend to the spinal cord. In one experiment, in which the staining involved the whole

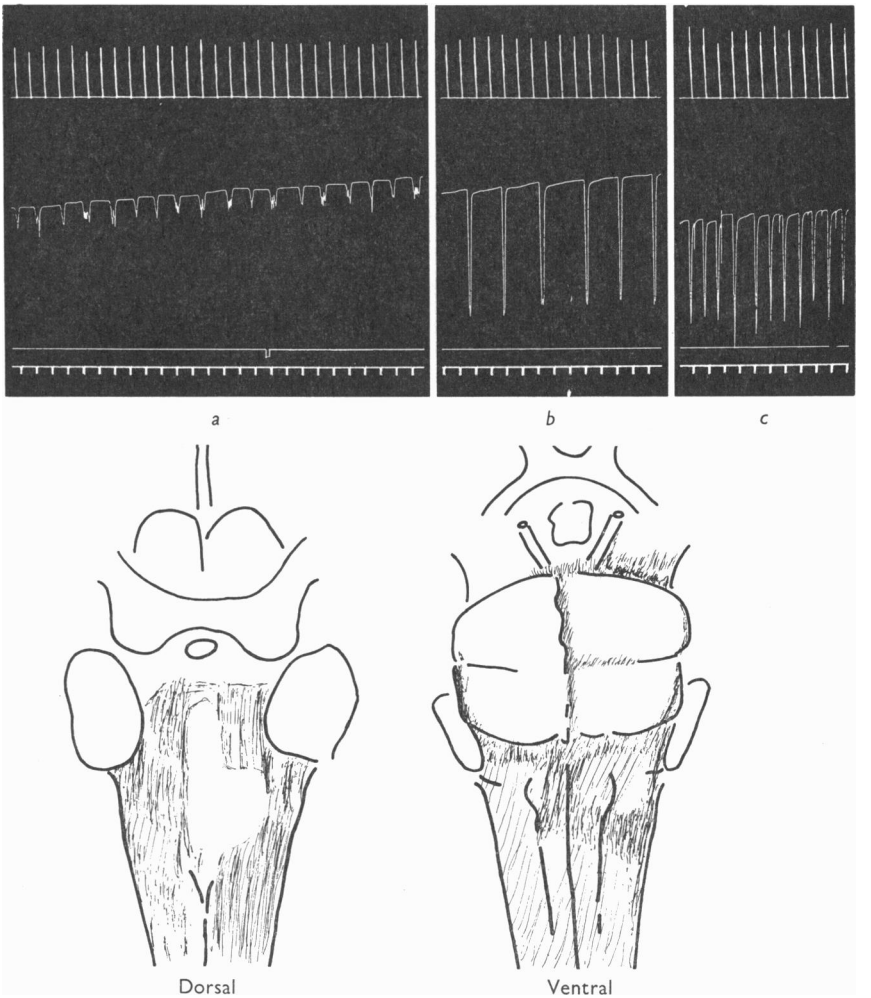

Fig. 10. Effect on knee-jerk (upper record) and respiration (lower record) on perfusion of fourth ventricle with tubocurarine 1:8000 started at the signal: *b* and *c* about 20 and 40 min later. Time marker, 10 sec. The shaded areas in the diagram below indicate the region of the floor of the fourth ventricle and of the outside of the brain stem which were stained blue after perfusion for 20 min with Evans Blue at the end of the experiment.

spinal cord down to the thoracic region, the perfusion with tubocurarine had produced an increase in the quadriceps response to the tendon tap; subsequently respiration became more rapid and deeper, and at the height of action

typical tremor-like clonus developed and the whole animal jerked at each tendon tap. A cutaneous sensory stimulus, however, produced a transient inhibition of the tremor. This is illustrated in Fig. 11. This effect was obtained in one experiment only and it is therefore difficult to interpret this inhibitory action of the cutaneous stimulus on the tremor, and to associate it definitely with the fact that the perfusion with tubocurarine had extended to the spinal cord.

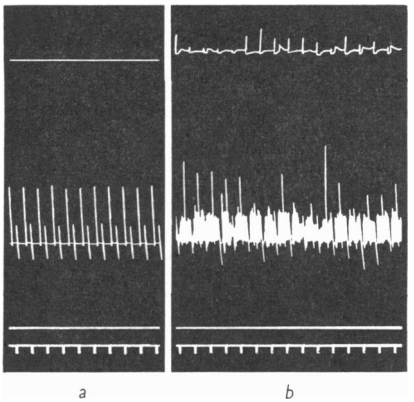

Fig. 11. Inhibitory effect of a cutaneous sensory stimulus on tremor-like activity produced by tubocurarine; perfusion of cerebral ventricular spaces with tip of outflow cannula at the mid-collicular level of aqueduct. Upper record from right, lower from left quadriceps (*a*) before and (*b*) during perfusion with tubocurarine 1:8000. Tapping the left patellar tendon approximately every 10 sec resulted in the large upward strokes. Between taps a cutaneous sensory stimulus was applied to paw of left hind limb, resulting in the smaller upward and downward strokes in *a*, and in inhibition of tremor-like activity in *b*. Time marker, 10 sec.

Effects of noradrenaline and adrenaline

In experiments with perfusion from the lateral ventricle and with the tip of the outflow cannula at the mid-collicular level of the aqueduct, noradrenaline added to the perfusion fluid in concentrations of from 1:10,000 to 1:2,000 did not affect the normal quadriceps response to the tendon tap but it influenced the response to tubocurarine. If tubocurarine had enhanced the response, noradrenaline reduced it to normal (Fig. 7*b*); if it had produced tremor-like activity with occlusion of the quadriceps responses, noradrenaline abolished the tremor and the occlusion, but it did not reduce the generalized jerks. In fact, they often became stronger, probably because they were no longer occluded by the tremor (Fig. 12). The effects of noradrenaline were obtained whether the tubocurarine was maintained in the perfusion fluid or not. When a period of perfusion with noradrenaline 1:10,000 had preceded the perfusion with tubocurarine 1:10,000 the appearance of the full tubocurarine effects was

delayed, sometimes for over an hour, and during this time the only muscular effects of the tubocurarine were generalized jerks.

In one experiment in which adrenaline 1:10,000 was used it affected the tubocurarine response in the same way as noradrenaline.

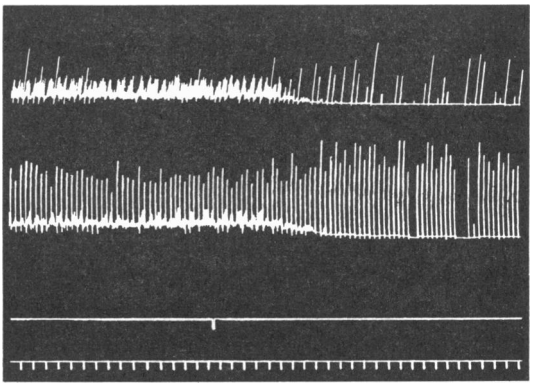

Fig. 12. Effect of perfusion of cerebral ventricular spaces with noradrenaline 1:10,000, started at signal, on muscular activity produced by prolonged perfusion with tubocurarine 1:10,000. Tip of outflow cannula at mid-collicular level of aqueduct. Upper record from right, lower record from left quadriceps; tapping of left patellar tendon every few seconds. Time marker, 10 sec.

DISCUSSION

When, whilst examining the changes in electrical activity of the brain produced by tubocurarine, records were taken from Ammon's horn, a pattern of activity was recorded, on traversing this structure, which was characterized by high-voltage spikes. The position of the tip of the electrode from where this type of activity was obtained was in or near the cellular layers, which are such a characteristic feature of this part of the cerebrum. The high-voltage spikes thus are a sign of activity in a compact layer of cells discharging synchronously. Studies of the activity of Ammon's horn may be helped by this knowledge that its activation, at least from subcortical structures, can easily be recognized when records are taken from its cellular layers.

The main aim of the present experiments was to obtain information about the site or sites of action of tubocurarine in the brain, when on injection into the lateral ventricle it produces strong muscular activity which leads to convulsions, and the profound changes in electrical activity of the brain which bear such a close resemblance to the abnormal discharge of the epileptic seizure. From the results of our experiments it appears that we can eliminate, as the main sites of action for these effects, structures lining the lateral ventricles, the fourth ventricle and the outer surface of the brain stem. The main sites of

action appear to be structures which are reached when the tubocurarine passes through the aqueduct.

In order to know which structures are affected we ought to know how deeply the tubocurarine, when passing through the aqueduct, penetrates into the surrounding tissue of the mesencephalon. No such penetration experiments have been carried out with tubocurarine nor, as far as penetration from the surface of the aqueduct into the mesencephalic tissue is concerned, with any other substance. However, M. Draskoci, W. Feldberg, K. Fleischhauer and P. S. R. K. Haranath (unpublished) have recently measured the penetration of histamine into the tissue of the diencephalon from the third ventricle when a solution of histamine 1:1000 was perfused for 1 hr from the lateral ventricle to the aqueduct. They found that the histamine penetrated about 3 mm into the diencephalon. If we were to assume a similar penetration of tubocurarine into the mesencephalic region, the structures reached would lie beyond the grey matter around the aqueduct and the tubocurarine would reach that region in the central core of the brain stem which consists of a diffuse aggregation of cells of different types and sizes separated by a wealth of fibres travelling in all directions and termed the reticular formation of the tegmentum. It is known that muscular effects are obtained from stimulation of this region, so that—on the assumption that tubocurarine, in contrast to its paralysing action at motor end-plates, has an excitatory action on central synapses—the concept of muscular activity resulting from penetration of a central excitatory substance into the tegmentum is in accord with physiological observations.

Experiments on cats have shown that the rostral portion of the mesencephalic tegmentum and its continuation into the hypothalamus are involved in the act of walking (Hinsey, Ranson & McNattin, 1930), and that unilateral stimulation of the tegmentum results in ipsilateral flexion with contralateral extension of the forelimbs, in varying muscular responses of the hind limbs and in bending of the body with the concavity of the curvature to the side of stimulation. The older literature on tegmental stimulation has been reviewed by Hinsey, Ranson & Dixon (1930). They themselves stimulated in decerebrate cats the cut surface of the mesencephalon and found that the tegmental response was elicited when the electrode was in the region of the red nucleus. They concluded that the descending pathway is ipsilateral down into the reticular formation, from which it may be continued via the reticulo-spinal tracts. On the other hand, Ingram, Ranson, Hannett, Zeiss & Terwilliger (1932), working on anaesthetized cats with the brain intact, found that the response was not specifically related to the red nucleus. In fact, they had considerable difficulty in obtaining the response from this part of the tegmentum, whereas it was easily obtained from the reticular formation of the tegmentum dorsal and lateral to the red nucleus, that is, from almost everywhere in the reticular formation back to the caudal part of the pons.

When describing the effects obtained with tubocurarine, it was repeatedly pointed out that some of the muscular activity appeared in the form of tremor, first as a repetitive response to stimuli, but later as a continuous activity. It is therefore of particular interest that tremor is a typical effect of stimulation of the tegmental reticular formation (Folkerts & Spiegel, 1953; Jenkner & Ward, 1953; Wycis, Szeleky & Spiegel, 1957; Ward, 1957), and that the tremor is assumed to be the result of activation of reticular neurones rather than of other fibres coursing through this region. In order to explain the finding (Ward, McCulloch & Magoun, 1948; Peterson, Magoun, McCulloch & Lindsley, 1949) that not only stimulation of, but also lesions in, the tegmentum may result in tremor, Jenkner & Ward (1953) and Ward (1957) assume that these lesions interrupt projections to those neurones in the reticular formation, stimulation of which evoked rhythmic tremor, and that these de-afferentated cells subsequently become supersensitive to acetylcholine. This latter conclusion they based on the effectiveness of antagonists of acetylcholine in reducing tremor in patients with Parkinsonism. The finding of Feldberg & Sherwood (1954*b*) that the anticholinesterases eserine and DFP produce tremor on intraventricular injection would be in favour of this conclusion, provided it could be shown that the anticholinesterases also act after penetrating into the mesencephalon from the aqueduct. This finding would then strongly support the view that the nerve cells in the reticular formation of the tegmentum, the discharge of which results in tremor, are impinged upon by cholinergic neurones.

There are other effects observed with intraventricular tubocurarine which may also result from an action of the drug penetrating from the aqueduct into the mesencephalon. In unanaesthetized cats an intraventricular injection of tubocurarine may produce bouts of loud calling both before and between periods of convulsive seizures (Feldberg & Sherwood, 1954*a*) and it is known that vocalization can be elicited by mesencephalic stimulation. For instance, Ingram, Ranson, Hannett, Zeiss & Terwilliger (1932) showed that in cats stimulation of areas of the tegmental reticular formation, near the mid-line, led to cries, and Magoun, Atlas, Ingersol & Ranson (1937) found that stimulation of the tegmentum below the aqueduct yielded facio-vocal responses. More recently, Jenkner & Ward (1953) evoked in monkeys forceful rhythmic vocalization by electrical stimulation of the tip of an electrode in the tectum.

The finding that in the course of perfusion with tubocurarine the pupils become gradually wider can also be explained by a gradual penetration of the drug into the tegmen. reticular formation, since Ingram *et al.* (1932) state that 'dilatation of the pupils occurred on stimulation practically everywhere in the reticular formation' of the tegmentum. This interpretation is strengthened by the observed association of pupillary movements with the muscular activity, sudden jerks resulting in short-lasting dilatations of the pupils and the

background tremor-like activity resulting in oscillations of the pupillary diameter. On the other hand the possibility cannot be excluded that the effect is brought about by an action of the tubocurarine on diencephalic structures as well, through penetration from the third ventricle, since Ingram *et al.* (1932) obtained pupillary dilatation also on stimulation of the grey matter of the wall of the third ventricle in the hypothalamus, in various hypothalamic nuclei and in other regions of the diencephalon.

The changes in the pattern of the respiratory movements which were among the first effects observed during perfusion with tubocurarine may also result from an action of the drug on structures of the tegmental reticular formation. This applies to the small respiratory effects preceding the inspiration and to the occasional sharp inspiratory effects during an inspiration as well as to the tremor-like contractions which later on followed each inspiration. This conclusion is based not only on the obvious association of the respiratory movement with the other muscular activity and the tremor, but also on the fact that Ingram *et al.* described respiratory effects, cessation as well as acceleration of respiration, as a result of tegmental stimulation.

Can all the effects on the body musculature observed in our perfusion experiments with tubocurarine, and, in unanaesthetized cats, with intraventricular injection, be attributed to an action on structures in the mesencephalon, or may some of the contractions perhaps have resulted from an action on more rostral structures by penetration from the lateral walls of the third ventricle into the diencephalon? When in our experiments on perfusion from the lateral ventricle the tip of the outflow cannula had entered the third ventricle, thus excluding the aqueduct from the perfusion, no muscular activity occurred with the usual concentration of 1:10,000 tubocurarine in the perfusing fluid. However, when the concentration was 1:2000 contractions occurred although only after a delay of about 20 min. This delayed effect with the higher concentration might indicate an action of tubocurarine on deeper structures in the diencephalon reached by penetration through the lateral walls of the third ventricle. But it might as well result from an action on the most rostral zone of the mesencephalic tegmentum, the drug penetrating into this region from the posterior wall of the third ventricle at the zone of transition between diencephalon and mesencephalon. In agreement with this interpretation is the finding by Ingram *et al.* (1932) that the weak currents used for obtaining muscular effects on stimulation of the tegmentum had no such effects when applied to various regions of the diencephalon, to the hypothalamus, the subthalamus and to the most ventral part of the thalamus as far as the pre-optic area. On the other hand, our results do not exclude the possibility that a facilitating effect is brought about by the action of tubocurarine on diencephalic structures, thereby increasing the excitability of the mesencephalic structures to tubocurarine. Such a facilitating effect may also result

from an action of tubocurarine on structures caudal to the mesencephalon; this apart from the fact that tubocurarine can apparently produce contractions by acting on the spinal cord itself when reaching the subarachnoidal space surrounding it.

The results of the present experiments do not enable us to decide the pertinent question of where the tubocurarine acts when, on intraventricular injection, it produces the profound changes in electrical activity of the brain which we presume to be associated with the convulsive activity. We know that these changes must be produced by an action on subcortical structures, and it may well be that these structures are mainly or wholly situated in the mesencephalon.

The finding that no respiratory effects were produced on perfusion of the fourth ventricle with tubocurarine when the perfusion fluid reached limited areas of the floor of the ventricle, but that strong respiratory changes occurred when the fluid passed over large areas of the floor of the fourth ventricle and entered the subarachnoidal space, can be explained by the fact that the tubocurarine in order to be active must reach an extensive region of the floor of the fourth ventricle. There is, however, another explanation. Loeschcke and his co-workers (Loeschcke, Koepchen & Gertz, 1958; Loeschcke & Koepchen, 1958*a*, *b*) have recently made the interesting observation that solutions of different pH, or solutions containing procaine, veratridine or lobeline, produced no respiratory changes when applied to the floor of the fourth ventricle but did so when the fourth ventricle was perfused, and these solutions passed through the lateral recesses into the adjacent subarachnoidal space of the base of the brain. Injections of these solutions into the lateral recesses were also effective. They conclude that the hydrogen ions and the drugs did not act on the respiratory centre proper in the floor of the fourth ventricle but 'on a very superficial sensitive substrate in the region of the lateral recesses of the fourth ventricle and their neighbourhood', i.e. on structures beyond the fourth ventricle at the side or base of the brain stem.

The sympathomimetic amines, noradrenaline and adrenaline, seemed to exert a selective antagonistic effect on the muscular activity produced by tubocurarine. They abolished the augmentation of the knee-jerk and the tremor-like activity but did not reduce the generalized muscle jerks. Since these occurred only late in the perfusion, in contrast to the other two muscular activities, they may require a deeper penetration of the tubocurarine into the mesencephalon, to structures not reached by the noradrenaline or adrenaline on subsequent perfusion. These amines, however, have some action on muscle jerks as well, since Feldberg & Sherwood (1954*a*) found in unanaesthetized cats that intraventricular adrenaline reduced, although it did not abolish, the convulsive activity of a small dose of intraventricular tubocurarine.

Whatever the explanation, the dramatic abolition of the tremor-like activity

without reduction of the muscle jerks suggests a selective action of noradrenaline and adrenaline on the tegmentum, probably on its reticular formation. The finding that intraventricular adrenaline abolished the bouts of loud calling produced by intraventricular tubocurarine (Feldberg & Sherwood, 1954*a*) is another central adrenaline-tubocurarine antagonism which also points to an action of adrenaline on structures in the tegmentum.

Does the effect of noradrenaline, particularly its ability to abolish the tremor-like activity, point to a corresponding physiological function of this amine? That it was used in large concentrations need not prevent us from considering this possibility, since the tubocurarine, too, was used in large concentrations. The pharmacological effect of noradrenaline in abolishing the tremor-like activity produced by tubocurarine may indeed imitate a physiological function exerted by the noradrenaline present in the brain stem; moreover it may give us an understanding, in physiological terms, of the disturbance underlying the tremor of Parkinsonism.

The anatomical region from which tremor-like movements can be elicited on electrical stimulation has been mapped out in the brain of the monkey by Jenkner & Ward (1953). It comprises the more medial portions of the reticular formation between the red nucleus and the nucleus of the abducens nerve, regions in the brain stem in which noradrenaline was found by Vogt (1954) in dogs. Could it then be that its physiological function here would be to suppress a latent tremor? Jung (1941) has postulated that tremor represents a phylogenetically early form of movement (cf. movements of fins of fish) which is suppressed by the elaborate development of the nervous system in higher forms. If one of the functions of the noradrenaline in the brain stem were to suppress this phylogenetically early form of movement, tremor, we should expect it to become manifest when this brake is removed, that is, when the brain stem is depleted of its noradrenaline; and this seems to be the case. Reserpine is known to deplete the noradrenaline in the brain stem and other nervous tissues (Holzbauer & Vogt, 1956; Shore, Olin & Brodie, 1957; Muscholl & Vogt, 1957) and one of the effects it produces is tremor. This is a well-known toxic side effect of reserpine treatment in psychotic patients (Kline & Stanley, 1954; Hollister, Krieger, Kringel & Roberts, 1954; Flach, 1954; Kinross-Wright, 1954; Weber, 1954; Barsa & Kline, 1955) and has been observed in most species (Chusid, Kopeloff & Kopeloff, 1955; Woodson, Youngken, Schlittler & Schneider, 1957). But since in animals reserpine causes a fall in body temperature (von Bein, Gross, Tripod & Meier, 1953; Schneider & Earl, 1954) it is not certain whether a distinction between tremor and shivering has always been made.

It is tempting to speculate whether the tremor of Parkinsonism is also a sign of insufficient depression by noradrenaline of a latent tremor. Either noradrenaline may not be present in sufficient amounts in the brain stem, or the dis-

turbance may be in the enzyme system synthesizing it, or the normal rate of release of noradrenaline may be impeded, or the responsive neurones may have become insensitive or less sensitive to it. Should the idea prove to be correct that the physiological function of noradrenaline in the mesencephalon and in the more caudal parts of the brain stem is one of suppression of a phylogenetically early form of movement, then we should have to consider that the function of noradrenaline in the more rostral parts of the brain stem is also one of suppression of motor or sensory functions.

SUMMARY

1. Experiments were carried out in anaesthetized cats in order to locate the site of action of tubocurarine when it produces profound changes in electrical activity of the brain and increased muscular activity.

2. By recording the electrical activity from two points on either side of the mid line it could be demonstrated that the changes in electrical activity produced by unilateral injection of tubocurarine occurred with similar latencies on both sides. This excluded structures lining the lateral ventricles as the sites at which the activity originated.

3. When the tip of the recording electrode was in or near the cellular layers of Ammon's horn during episodes of increased electrical activity of the brain following an intraventricular injection of tubocurarine the records showed groups of high-voltage spikes occurring at regular intervals, a pattern of activity different from that found in any other region of the brain so far examined.

4. When the tip of the recording electrode was in or near the cellular layers of Ammon's horn the records of the responses evoked after the tubocurarine injection showed groups of large-voltage spikes arising from the slow evoked wave. On traversing Ammon's horn with the electrode, a point was always reached where the spikes were maximal and diphasic; both above and below this point the spikes decreased in size and usually became first negative and then positive.

5. The pattern of high-voltage spikes obtained in records from Ammon's horn is explained by the fact that we are recording a synchronous discharge from a compact layer of cells which is a characteristic feature of the histological structure of the Ammon's horn.

6. When perfusing tubocurarine through a cannula in the lateral ventricle and collecting the effluent from various points between the third ventricle and the cisterna it could be shown that the aqueduct was the most sensitive region from which to elicit muscular effects such as increased excitability of the knee-jerk, tremor-like activity and, at later stages of perfusion, generalized jerks.

7. It is suggested that the main site of action of tubocurarine in producing

increased muscular activity is the reticular formation of the tegmentum, stimulation of which is known to produce muscular effects and particularly tremor-like activity resembling that observed after tubocurarine.

8. Noradrenaline as well as adrenaline added to the perfusion fluid prevented or abolished the augmentation of the knee-jerk and the tremor-like activity produced by tubocurarine, without reducing the generalized muscle jerks. It is assumed that noradrenaline and adrenaline when exerting this antagonistic effect act also on the reticular formation of the tegmentum.

9. The possibility is discussed that a physiological function of the noradrenaline present in the brain stem is to depress a latent tremor considered to be a phylogenetically early form of movement, and that the tremor of Parkinsonism is associated with a disturbance in the metabolism of noradrenaline.

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