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SOME EFFECTS OF TUBOCURARINE ON THE ELECTRICAL ACTIVITY OF THE CAT'S BRAIN

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Recently Feldberg & Sherwood (1954) have shown that intraventricular injections of tubocurarine produce convulsions in the unanaesthetized cat. In the present series of experiments the effect of tubocurarine given by the intraventricular route was studied with respect to changes in electrical activity of the brain in anaesthetized cats. Cortical and subcortical potentials were recorded, and the effect of tubocurarine on the response evoked by peripheral nerve stimulation was examined. Control experiments were also carried out with topical cortical application.

METHODS

The cats were anaesthetized with 40 mg/kg pentobarbitone intraperitoneally and supplementary doses were given when required. The trachea and left femoral vein were cannulated. The bone over the upper aspect of the left cerebral hemisphere was removed and a cannula inserted into the right lateral ventricle, as described by Feldberg & Sherwood (1953). The cat was mounted in a stereotaxic instrument. The edges of the scalp incision were tied to a ring so as to form a liquid paraffin pool under which the dura was opened and reflected. The cortical surface was kept clear of excess cerebrospinal fluid, exudate, and blood by continuous suction through a fine glass capillary placed at the bottom of the paraffin pool. Its temperature was maintained throughout the experiment by an electrical heating ring which consisted of a resistance wire enclosed in a glass tube which lay submerged in the liquid paraffin.

The right peroneal and posterior tibial nerves were exposed and divided and held on platinum electrodes for stimulating in a warm liquid paraffin pool.

Cortical potentials were recorded between a fine platinum wire (gauge 26) lightly touching the surface of the cortex and an indifferent electrode on the scalp. Mid-brain potentials were recorded with a deep or focal electrode as described by Brooks & Eccles (1947), from the lateral posterior thalamic or supramammillary regions, an indifferent electrode being placed on the scalp. The deep or focal electrode consisted of a glass capillary with a diameter of 15–30 μ filled with indium, whose tip had been pulled out and platinum-plated. Such electrodes were first described by Dowben & Rose (1953). It was placed in the desired position with the stereotaxic instrument. For localization we relied on the drawings by Jimenez-Castellanos (1949) and on maps of the brain supplied to us by Dr R. S. Snider (Chicago). The potentials were coupled by cathode followers of a high input impedence to the amplifier, amplified and displayed on a double-beam oscilloscope and recorded on 35 mm film.

The drugs were dissolved in Locke's or Tyrode solution. For the intraventricular injections volumes of between 0·2 and 0·3 ml. were used and slowly injected. Care was taken not to raise the intraventricular pressure unduly by the injection; usually the fluid was allowed to run in by gravity. Tubocurarine was used as the chloride and L-noradrenaline as the bitartrate. All values refer to the salt.

When tubocurarine was given intravenously, artificial ventilation was used. The completeness of neuromuscular block was controlled by stimulation of the lower cut end of the left peroneal nerve.

To ascertain the position of the tip of the electrode in the mid-brain the head was perfused at the end of the experiment with 10% formol-saline from a carotid artery and later embedded in paraffin or in celloidin. In some experiments the brain in the region of the tip of the microelectrode was coagulated by means of a current passed through the electrode before the formol-saline perfusion. Serial sections were cut in the plane of the needle track and stained with either haematoxylin and eosin or by van Gieson's method.

Assay of tubocurarine in fluid collected from the exposed cortex. The exposed cortex was irrigated with 0·3 ml. of Locke's solution delivered from a fine hypodermic needle under the paraffin pool every 10 min; the fluid under the paraffin, consisting now of the Locke's solution and cerebrospinal fluid, was then aspirated and tested on the eserinized frog rectus muscle preparation. For each test the fluid collected (between 0·1 and 0·3 ml.) during two consecutive 10 min periods was pooled and tested together as follows. The muscle was first made to contract every 5 min by a standard dose of ACh kept in the bath for 1½ min, and then by a solution containing, in addition, the fluid collected from the exposed cortex. When this fluid caused a depression of the ACh contraction, the depression was matched against depressions produced by given doses of tubocurarine added to the standard dose of ACh.

Topical application of tubocurarine. Filter-paper disks of a diameter of 3 mm were soaked in warm tubocurarine solution and applied to the surface of the cortex. Records were obtained from the curarized area either after removal of the disk, or through the disk with the recording electrode on top.

RESULTS

In cats under pentobarbitone anaesthesia an intraventricular injection of tubocurarine results in increased motor activity. After an injection of 15–20 μg the effect started within a few minutes with twitching of the ears and tremor of the head and neck, and increased during the first hour. There were occasional movements of the head and occasional waves of coarse tremor and clonus, particularly in the hind legs. During the periods in which these motor effects were absent the following tests were made. Flexion of a hind leg, and maintaining the flexion by pressure, provoked tremor or clonic contraction of the leg or of the whole body, sometimes lasting for up to 20 sec. Tapping of the patellar tendon elicited a clonic response and pinching the hind paws resulted in a strong withdrawal reflex with crossed extension, followed by tonic or clonic extension of both hind legs and sometimes tremor of the whole body. Rotation of the neck to one side produced extension of the foreleg of the other side. Some of the responses to these tests resemble those seen in decerebrate cats. In addition, handling the cat sometimes evoked miaowing and howling. There was salivation and micturition.

With larger doses of tubocurarine the motor effects consisted of intense tremor, general twitching, continuous clonic leg movements, and acceleration of respiration and pulse rate. For instance, in one deeply anaesthetized cat

with no blink reflex and a respiratory rate of 6½ and a pulse rate of 48/min, the injection of 50 μg of tubocurarine produced a gradual increase of respiratory and pulse rate which began after a few minutes and continued over an hour, by which time the respiratory rate had reached 44 and the pulse rate 146/min. The blink reflex also appeared a few minutes after the injection and became gradually brisker. The motor effects began as generalized shivering which was accentuated with the inspiratory movements. There were continuous twitching and later clonic contractions of the whole body; the cat remained in this condition for more than 2 hr.

When an injection of 100 μg of noradrenaline was given intraventricularly at the height of the tubocurarine effect, the cat at once became limp and the previously rather rigid limbs became flaccid. A similar effect of noradrenaline has been observed by Felberg & Sherwood (1954) in the unanaesthetized cat. The knee jerk could still be elicited but was no longer accentuated, and pinching the paws produced only a weak withdrawal response.

Effects of intraventricular injections of tubocurarine on electrical activity of the brain

Stimulation of the muscle afferents of the peroneal nerve evoked a small cortical response in a limited region of the primary sensory area of the posterior sigmoid gyrus. In Fig. 3 this region is shown as a shaded area. The evoked response lasted for about 100 msec or more and varied from experiment to experiment; in typical cases it consisted usually of a surface positive wave followed by a variable surface negative wave and, later, irregular small fluctuations (controls in Figs. 1 and 5). The focal electrode recorded a few groups of spikes (control in Fig. 5); in some experiments they occurred without stimulation, in others they were evoked by the afferent nerve stimulation. Slow potentials such as were evoked in the cortex were not recorded by the deep electrode, although there were usually small slow waves.

The intraventricular injection of 15 μg of tubocurarine, which led to increased motor activity, produced no consistent changes in the cortical record nor in the evoked cortical response: but 200 μg produced pronounced changes after a latency which varied in different experiments from a few minutes to over half an hour. These changes lasted for 1–2 hr and were obtained whether or not muscular activity was apparent, since they also occurred when this activity was suppressed by an intravenous injection of 1–2 mg of tubocurarine.

In the experiment of Fig. 1, the intraventricular injection of 200 μg of tubocurarine affected the evoked cortical response within 1 min, but the maximal effect occurred only after 20 min and consisted of an increase in both the surface positive and negative waves. The surface positive wave increased about twice and the surface negative wave more than ten times; there frequently followed a large and longer lasting positive wave.

When a second stimulus was given at an interval of 200 msec or less after the first stimulus to the peripheral nerve, it still evoked the initial surface positive response; but the subsequent negative response was greatly reduced or absent, as illustrated in the experiment of Fig. 2.

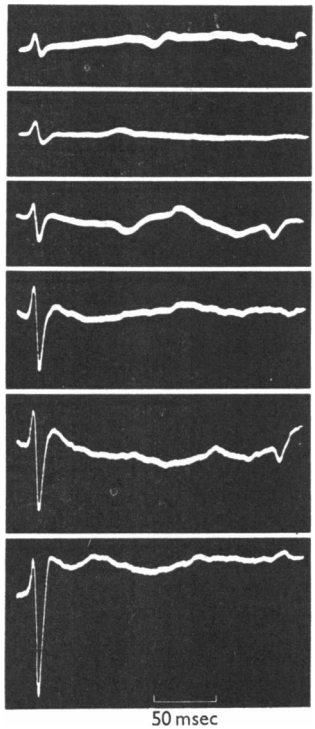

Fig. 1

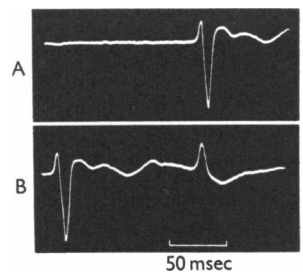

Fig. 2

Fig. 1. Effect of intraventricular injection of 200 μg tubocurarine on the evoked cortical respones recorded from the primary sensory area (shaded area in Fig. 3). The two upper records are before, the following 1, 11, 16 and 20 min after the injection. Downward deflexion recording electrode negative.

Fig. 2. Cortical response to a stimulus applied to the peroneal nerve after a similar conditioning stimulus. (A) Response to test stimulus alone; (B) response to test stimulus 130 msec after conditioning stimulus. Downward deflexion recording electrode negative.

Tubocurarine also greatly increased the area from which the evoked cortical response could be recorded. As shown in Fig. 3, after tubocurarine stimulation of the peroneal nerve not only produced a response in the whole primary sensory area but also in the anterior supra-sylvian, the lateral, and the anterior sigmoid gyri. However, the latency increased with distance from the primary sensory area, as illustrated in the diagram of Fig. 4, in which the latencies are given in msec at the various points examined. The increase in latency is also evident from the record tracings of column D of Fig. 10. Further, whereas

normally there was little overlap of the areas from which evoked responses could be obtained by stimulation of different peripheral nerves, for instance the peroneal and posterior tibial, this was no longer so after tubocurarine.

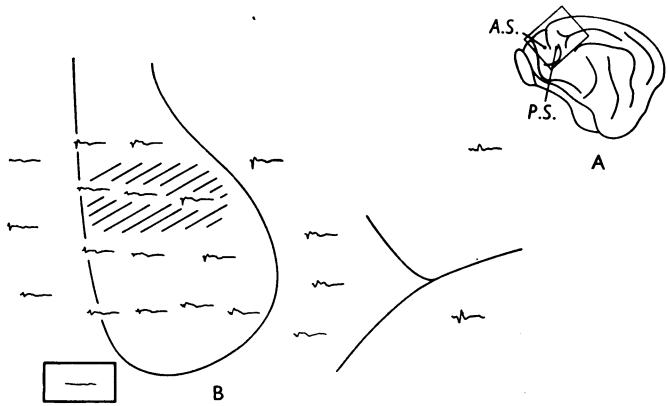

Fig. 3. Diagram of cat's left cerebral cortex (at A). *A.S.* and *P.S.* anterior and posterior sigmoid gyrus. At B enlargement of area in rectangle of A. Spread of the evoked cortical response after intraventricular injection of 200 μg tubocurarine. Shaded area, region from which an evoked cortical response to peroneal nerve stimulation occurred before tubocurarine. The responses shown were from the points at which each record begins. Downward deflexion recording electrode negative. Inset record obtained without stimulation.

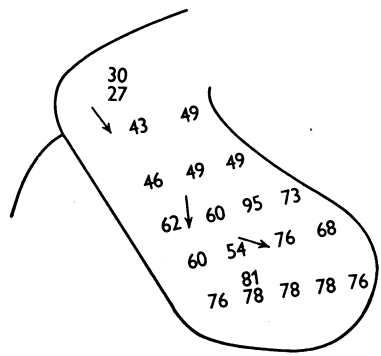

Fig. 4. Diagram of posterior sigmoid gyrus. The numbers give in msec, at their respective positions, the interval between stimulus and start of surface negative wave of the evoked cortical response after an intraventricular injection of 250 μg of tubocurarine. The arrows indicate a possible direction of spread.

The injection of tubocurarine also led to the appearance of 'spontaneous' cortical activity, i.e. unconnected with peripheral stimulation. The waves of this 'spontaneous' activity were often similar in pattern to the evoked responses. A typical effect is illustrated in Fig. 5. Usually the 'spontaneous' activity was not continuous, but there were times of varying length during

which the cortex was relatively quiescent. A period of activity was often set off by peripheral nerve stimulation. The full evoked response could not be obtained if stimulation fell within a period of 'spontaneous' activity.

On the focal record the intraventricular injection of 15 μg of tubocurarine caused an increase in spike activity, but since such an increase occurred also on intraventricular injection of warm Locke's solution we cannot be certain to what extent this increase was due to the tubocurarine. The injection of 200 μg of tubocurarine, however, produced definite changes in the records from the subcortical structures. These effects again were independent of the muscular contractions since they also occurred when these were abolished by intravenous injections of tubocurarine.

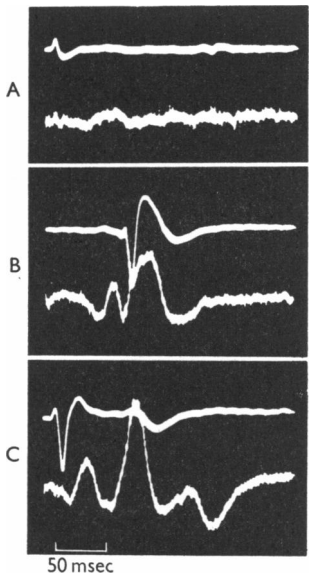

Fig. 5. Effect of intraventricular injection of 200 μg of tubocurarine on cortical response from primary sensory area (upper records) and on focal response from the nucleus ventralis pars postero-lateralis of the thalamus (lower records). (A) control; (B) and (C) about 2 hr after tubocurarine injection. (B) 'spontaneous activity', and (C) response evoked by stimulation of peroneal nerve. Downward deflexion recording electrode negative.

When records were taken from the centrum medianum, 200 μg of tubocurarine did not lead to an evoked response, but there were periods of increased activity, particularly in response to stimulation. When records were taken from the supramammillary or the lateral posterior thalamic region strong evoked responses could be obtained after 200 μg of tubocurarine. The response consisted of several waves and varied in amplitude and duration. In the same experiment the complex of the response was relatively constant, but it varied in different experiments. A typical experiment is illustrated in Fig. 5. The

response consisted regularly of three slow waves and was preceded by a group of small spikes.

In addition to these evoked responses there were periods of 'spontaneous' activity consisting of a group of slow waves often, but not always, showing a pattern similar to the evoked response. Such a group of 'spontaneous' activity was usually associated with the typical 'spontaneous' cortical activity, and is illustrated in Fig. 5 B. In this experiment the activity in the focal record preceded the cortical activity; in other experiments the cortical activity preceded that of the focal record, possibly depending on the precise site of the deep electrode.

None of the changes in electrical activity seen after the intraventricular injections of tubocurarine could be reproduced by intravenous injections of this drug in doses which produced complete neuromuscular block.

Assay for tubocurarine of cerebrospinal fluid collected from the exposed cortex

The finding that an intraventricular injection of tubocurarine was followed by pronounced changes in electrical activity of the cortex raised the possibility that the tubocurarine was carried to the exposed cortex and acted there: particularly since preliminary experiments showed that after an intraventricular injection of a solution of indigocarmine this dye appeared after a few minutes on the exposed cortex, first in the posterolateral sulci and later spreading to cover the greater part of the surface.

Cerebrospinal fluid collected from the exposed cortex was therefore assayed for tubocurarine by its effect in reducing the ACh contractions of the frog's rectus muscle. Such an assay has to take into account the fact, first observed for cerebrospinal fluid of man (Feldberg & Sherwood, unpublished experiments), that this fluid, although ineffective by itself, increases the sensitivity of the muscle to ACh when added to the bath in which the rectus muscle is suspended. This sensitizing effect, however, does not invalidate the assay provided cerebrospinal fluid is added to the control solutions of tubocurarine.

Fig. 6 illustrates the effects of cerebrospinal fluid collected from the exposed cortex before and after an intravenous and an intraventricular injection of tubocurarine into the cat, and shows that after an intraventricular injection of 300 μg small amounts of tubocurarine are carried to the exposed cortex.

The sensitizing effect of cerebrospinal fluid collected before any tubocurarine was injected is shown at (S 1) and illustrates its typical delayed development and disappearance. The first two records are contractions produced by 0·3 μg ACh alone, the third by 0·3 μg ACh together with the fluid collected during 20 min from the exposed cortex, the subsequent six contractions again by 0·3 μg ACh added without cerebrospinal fluid. Some increase is shown in the contraction produced by ACh given with the cerebrospinal fluid, but the main increase occurs with the next two contractions to ACh. The subsequent

four contractions show that the sensitivity of the muscle to ACh returns to its previous level. The sample of cerebrospinal fluid collected after the intravenous injection contained no detectable amounts of tubocurarine, as shown by the fact that when it was added to the ACh solution an increased contraction resulted. A further increase was obtained with the subsequent two contractions caused by ACh alone. The sensitizing effect is smaller than on the previous application of cerebrospinal fluid. This is due not to the fact that

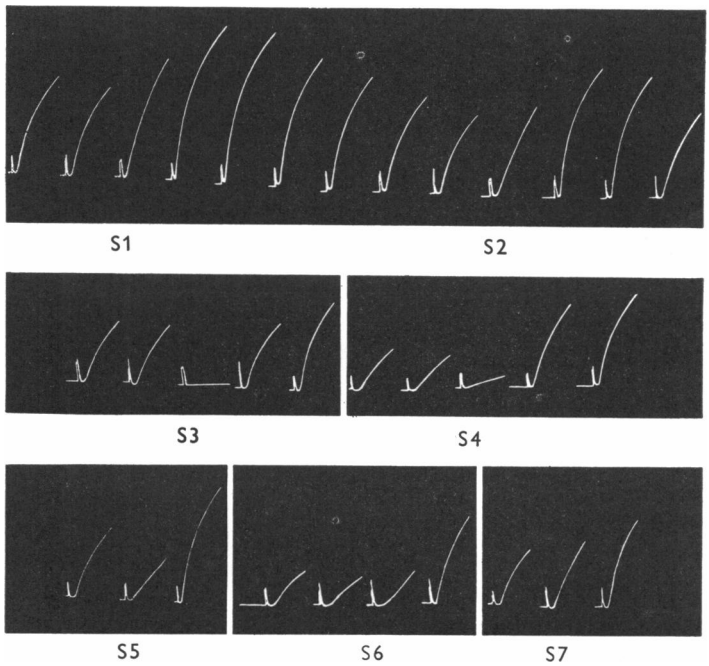

Fig. 6. Contractions of the eserinized frog rectus muscle suspended in 5 ml. bath to 0·3 μg ACh. The contractions S1 to S7 are by 0·3 μg ACh, together with fluid collected during successive 20 min periods from the exposed cortex. S1 before, S2 after intravenous injection of 1·4 mg of tubocurarine, S3 to S7 after intraventricular injection of 300 μg of tubocurarine into a 2·3 kg cat. Between successive blocks several control contractions of 0·3 μg ACh omitted from the figure.

the tubocurarine was injected intravenously but to the diminution of the sensitizing effect which occurs regularly with repeated addition of cerebrospinal fluid. Tubocurarine, however, can be detected in the cerebrospinal fluid collected from the cortex after an intraventricular injection of 300 μg. The greatest amounts are present in the samples collected during the first or second 20 min period. In the experiment of Fig. 6, the greatest amount of tubocurarine was present in the sample collected during the first 20 min, there was less in the subsequent two 20 min samples, and none in the following samples. This muscle was particularly sensitive to tubocurarine when tested at the end

of the experiment and showed a depression of the contraction to ACh in the presence of only 0·3 μg tubocurarine; but the actual amounts of tubocurarine present in the samples of cerebrospinal fluid were not assayed in this experiment. This, however, was done in other similar experiments, when it was found that the maximal amounts of tubocurarine present in the fluid collected from the cortex during a 20 min period after injection of 300 μg tubocurarine varied between 2 and 4 μg. Such an assay is shown in Fig. 7. In this experiment also there is no evidence for the presence of tubocurarine in the cerebrospinal fluid collected after an intravenous injection of tubocurarine (S1).

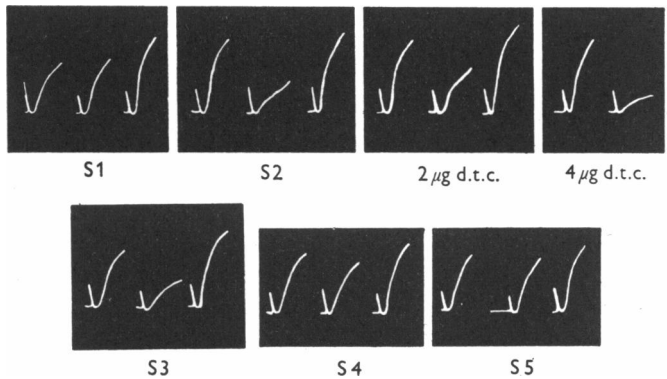

Fig. 7. Contractions of the eserinized frog rectus muscle suspended in 5 ml. bath to 0·5 μg ACh. The contractions S 1 to S 5 are by 0·5 μg ACh, together with the fluid collected during successive 20 min periods from the exposed cortex. S 1 after intravenous injection of 1·5 mg, S 2 to S 5 after intraventricular injection of 300 μg of tubocurarine into a 2·3 kg cat. The second contractions in the third and fourth blocks are by 0·5 μg acetylcholine, together with 2 and 4 μg tubocurarine (d.t.c.) respectively. Between successive blocks several control contractions of 0·5 μg acetylcholine omitted from the figure.

The first and the second 20 min samples collected after the intraventricular injection of tubocurarine, however, caused a depression which was more pronounced than that produced by 2 μg and less pronounced than that produced by 4 μg tubocurarine. The third 20 min sample collected after the intraventricular injection produced only a slight depression, and the fourth sample no longer produced any depression.

Effect of topically applied tubocurarine on the electrical activity of the cortex

The appearance on the surface of the cortex of detectable amounts of tubocurarine after an intraventricular injection of 300 μg raised the question of a local cortical action of tubocurarine following its intraventricular administration; particularly since Chang (1953) found that topical application of tubocurarine to the cortex greatly increased the response evoked by cortical stimulation. Filter-paper disks soaked with tubocurarine solution were therefore placed on the cortex over the area from which the evoked cortical response

was recorded. When they were removed 5–10 min later a pronounced change in the response was noted. The threshold concentrations necessary to produce the effect varied between 10 and 20 μg/ml. and the effect increased with increasing concentration. Fig. 8 illustrates a typical experiment in which a solution of 20 μg/ml. produced a definite increase in the sharpness, and a shortening of the surface negative wave: but a solution of 40 μg/ml. was required to produce an increase in the size of the response, particularly of the surface negative wave. It increased so much in the following 3 min that the gain had to be reduced by 30%. When the cortex was then washed several

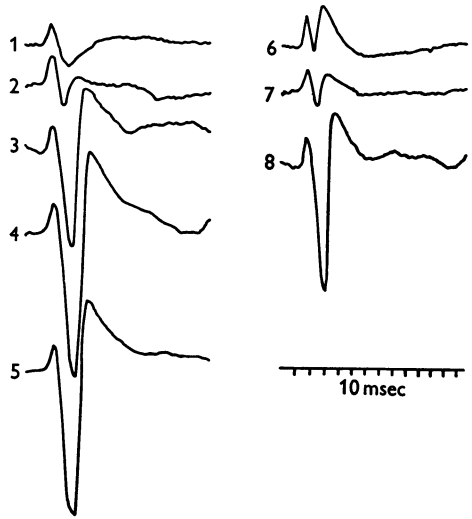

Fig. 8. Effect of topical cortical application of tubocurarine by paper disks on the evoked cortical response in the primary sensory area. (1) Control; (2) after 5 min application of a disk soaked in 20 μg/ml.; (3), (4) and (5) after 7 min application of a disk soaked in 40 μg/ml. tubocurarine; (6) and (7) about 50 min later, after washing the cortex; (7) recorded through a filter-paper disk soaked in Locke's solution; (8) recorded through a disk soaked in 10 μg/ml. tubocurarine. Downward deflexion recording electrode negative.

times with Locke's solution the effect gradually diminished, but 50 min after the removal of the tubocurarine some of the effect was still present. The later positive wave, which had also been greatly increased by the tubocurarine, had not yet returned to normal.

Instead of recording after removal of the filter paper disk, records 7 and 8 were obtained with a disk left in position on the cortex, the electrode being placed on the filter-paper. The disk used in record 7 was soaked in Locke's solution, that used in record 8 in a solution containing 10 μg/ml. tubocurarine. A comparison of records 6 and 7 shows that there was some attenuation, but hardly any distortion when recording through a filter-paper disk soaked in Locke's solution. This was regularly observed. Record 8, which was taken

through the disk soaked in 10 μg/ml. tubocurarine, shows that this solution produced a greater effect than 20 μg/ml. applied to the cortex at the beginning of the experiment. This increase in sensitivity to tubocurarine may have been due to the previous treatment; it was not due to the change in the method of recording.

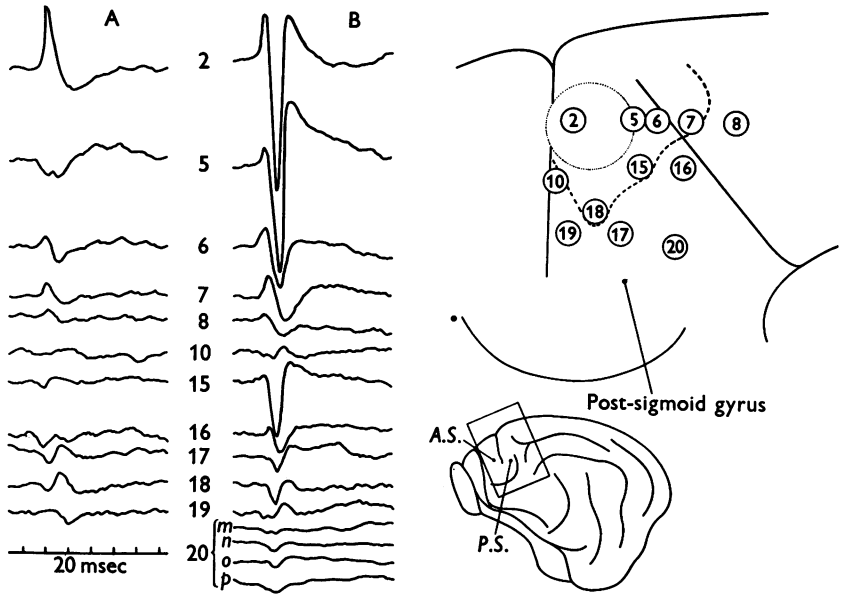

Fig. 9. Evoked cortical responses before (column A) and after (column B) topical application by filter-paper disk of 100 μg/ml. tubocurarine. The numbers beside the columns designate the point in the accompanying diagram from which the records were obtained. The dotted circle in the diagram indicates the area covered by the disk, and the broken line the area from which evoked responses could consistently be obtained. *A.S.* and *P.S.*, anterior and posterior sigmoid gyrus. Downward deflexion recording electrode negative. (For details see text.)

The tubocurarine effect obtained by topical application was limited to that area of the cortex from which the evoked response could originally be recorded. This is illustrated by the experiment of Fig. 9. Column A shows records from various points on the posterior sigmoid gyrus, indicating the limits of the area in the response. In the diagram this area is enclosed by the broken line. The right-hand column shows the effect of a solution of 100 μg/ml. tubocurarine applied by a filter-paper disk covering the area enclosed by the dotted line in the diagram. The tubocurarine effect does not spread to the points 8, 16, 17 and 19, which lie beyond the original limits of the responsive area.

When tubocurarine was applied to an area outside the original limits of responsiveness it had no effect. This is illustrated by the four last records, *m*, *n*, *o* and *p*, of Fig. 9. Records *m* and *n* are taken from point 20 in the

diagram, *m* without and *n* through a filter-paper disk soaked in Locke's solution. Record *o* is from this area through a disk soaked in a solution of 100 μg/ml. tubocurarine and kept on the cortex for 15 min. Record *p* is from the same area immediately after removal of the filter-paper disk.

The strict localization of the tubocurarine effect on the evoked cortical response with topical application to the cortex contrasts strikingly with the spread of the evoked cortical response after an intraventricular injection of tubocurarine, as illustrated in the experiment of Fig. 10, in which the tubocurarine was first applied to the cortex and later given intraventricularly.

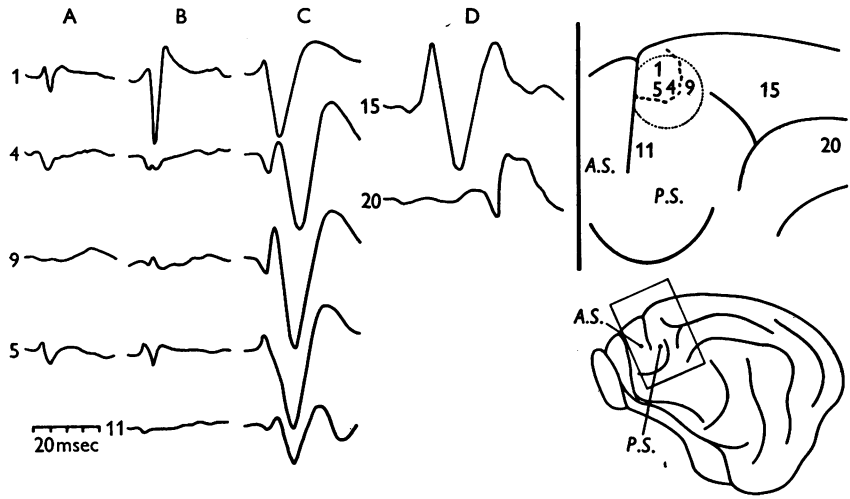

Fig. 10. Evoked cortical responses (A) before, (B) after topical application by filter-paper disk of 100 μg/ml. tubocurarine, (C) and (D) after intraventricular injection of 300 μg tubocurarine. The numbers on the left of the records correspond to the numbers in the accompanying diagram, indicating the points from which they were obtained. *A.S.* and *P.S.* anterior and posterior sigmoid gyrus. Dotted circle and broken line as in Fig. 9. Downward stroke recording electrode negative. (For details see text.)

A comparison of the records in columns A and B shows that in this experiment the effect of cortical application of a filter-paper disk soaked in a solution of 100 μg/ml. tubocurarine remained even more localized than in the experiment of Fig. 9. After the filter-paper disk was removed the whole exposed cortex was covered with a solution of 100 μg/ml. tubocurarine by introducing several ml. of this solution under the paraffin pool for 10 min. Even this flooding of the cortex with tubocurarine solution did not produce further changes nor any spread of the evoked cortical response, as shown by the records taken after the tubocurarine had been removed. On the other hand, when 300 μg tubocurarine was subsequently injected intraventricularly the surface negative wave of the evoked response increased in duration and could be recorded from areas previously not reached by the afferent nerve impulse. This is shown in

column C of Fig. 10. Whereas after topical application of tubocurarine the surface negative wave of the evoked response was increased in size but not in duration, after intraventricular application the duration of the surface negative wave also increased about two to three times. Further, an evoked response was recorded from areas far outside the original limit of the area of responsiveness, as illustrated by the two records of column D. These records also illustrate the later appearance of the evoked response as the distance from the originally responsive area increases.

DISCUSSION

Convulsive muscular activity as a result of intraventricular injections of tubocurarine has been observed in unanaesthetized and anaesthetized cats. In unanaesthetized cats this effect could be obtained with 5–30 μg (Feldberg & Sherwood, 1954). In anaesthetized cats such convulsions had hitherto been shown to occur only on cisternal or intraventricular injection of larger doses. Von Euler & Wahlund (1941) injected 0·1–0·3 ml. of a 0·2 % solution of curare chloride, and Salama & Wright (1950) 0·4 mg of tubocurarine in cats under chloralose anaesthesia. The experiments presented here show that cats under nembutal anaesthesia are highly sensitive to tubocurarine when given by the intraventricular route, since muscular contractions were regularly obtained with 15 μg. As in the unanaesthetized cat, these contractions were abolished by intraventricular administration of noradrenaline.

So far we have not been able to locate a primary site of action of intraventricularly injected tubocurarine in causing the muscular contractions. After doses sufficient to produce these contractions, regular changes in electrical activity were not recorded from either the supramammillary or the posterior thalamic region. The cortical response evoked by stimulation of the peroneal nerve was also not significantly altered by doses of tubocurarine sufficient to produce muscular contraction. Our experiments thus do not demonstrate that these doses of tubocurarine act on the pathway of the muscle afferents to the cortex. Johnson (1955) recently found that the prolonged high-voltage paroxysm induced by small amounts of intravenous strychnine occurred in the reticular substance but not in the nucleus ventralis lateralis of the thalamus. It is possible that tubocurarine acts at the same site as strychnine.

The most striking changes in the electrical activity of the brain produced by the larger doses of tubocurarine consisted of a large increase in the evoked cortical response, particularly in the surface negative wave, and the appearance of large evoked responses in the posterior thalamic and supramammillary regions. Further, the evoked response spread to areas outside the original limit of responsiveness. In addition, there were periods of increased electrical activity at both sites without afferent nerve stimulation. This increased

activity showed a pattern often resembling that of the evoked responses. These changes were not caused by the muscular contractions, as they occurred also when these were abolished or prevented by an intravenous injection of tubocurarine.

It had to be decided whether these electrical changes in the cortex were brought about by tubocurarine reaching the cortex and then acting locally, particularly since it was found that the fluid collected from the exposed cortex after intraventricular injection of larger doses contained tubocurarine. The results obtained with topical application to the cortex of a solution in an apparently comparable concentration produced changes which, although they had some features in common with the effects of intraventricular injection, differed in three important respects from the response following intraventricular injection: (1) The surface negative component of the evoked cortical response increased greatly in size but not in duration; (2) no expansion was found of the area from which an evoked response could be obtained; and (3) topical application caused no periods of 'spontaneous' activity. Therefore, after an intraventricular injection an action of tubocurarine on subcortical structures must be assumed to account for the cortical responses, even though the possibility of a local contributing action on the cortex cannot be excluded, especially since the changes after the intraventricular injection occurred usually only after a delay of a few minutes to over half an hour.

The finding that an intraventricular injection of tubocurarine so greatly increases the evoked cortical response, especially the surface negative phase, suggests that the number of surface elements, cells, or their processes which are activated by an afferent volley is increased. Further, the fact that with two consecutive stimuli, appropriately spaced, only a surface positive response is elicited by the second stimulus indicates that the surface negative response must involve elements, such as dendrites and small neurones, with a relatively long recovery cycle. Finally, the finding that after tubocurarine a large evoked response is elicited in the supramammillary and thalamic regions as well, suggests that the increase in the cortical response depends upon subcortical structures. The large waves evoked on the focal record after tubocurarine again suggest that here also slow conducting elements such as the dendrites and small neurones are activated. The fact that they are recorded from subcortical regions with microelectrodes, i.e. under conditions where relatively few units can contribute to the record, is in accord with this concept.

The finding that the evoked response arrives in the originally responsive area earlier than in the area in which responses appear only after an intraventricular injection of tubocurarine, suggests multisynaptic relay systems, for instance transcortical transmission conditioned by subcortical-cortical activity.

The evoked cortical and focal responses, and the 'spontaneous' activity seen in both records after tubocurarine may well have part of a pathway in

common: or it may even be that the actuating mechanism which synchronizes the 'spontaneous' activity is also brought into action by the evoked responses, not only because of the similar pattern of the electrical waves but also because the 'spontaneous' activity occludes the evoked responses.

We do not know how the changes recorded in these regions are produced by tubocurarine. The finding that large areas are involved in the excitatory changes does not mean that tubocurarine is a general excitant to all central neurones. When given intraventricularly it probably has a selective action on nerve cells near the ventricular lining, and the fact that the changes spread over wide areas of the cortex with varying latencies signifies only that some focus is probably acting as a powerful 'pace maker'. We know that repeated strong stimuli will recruit an increasing number of neurones, gradually activating larger areas of the cortex (Dempsey & Morison, 1942; Starzl & Magoun, 1951; Verzeano, Lindsley & Magoun, 1953). Chang (1953) found that topical application to the cortex of tubocurarine in the absence of afferent stimuli caused a long lasting, repetitive discharge of cortical units. Such an effect of tubocurarine, when given by the intraventricular route, could account for the general excitation of the cortex. The fact that the injections of tubocurarine increase also the activity in subcortical regions suggest that it greatly increases the excitability of many brain stem neurones as well, and that many more neurones respond to afferent volleys.

SUMMARY

The effects of intraventricular and topical cortical application of tubocurarine were examined in cats anaesthetized with pentobarbitone, and records of electrical activity were taken from the cortex and from the lateral posterior thalamic and supramammillary regions. Evoked cortical responses in the primary sensory area were elicited by stimulation of the peroneal nerve.

1. The intraventricular injection of 15 μg of tubocurarine was sufficient to produce increased motor activity which consisted of clonic contractions, waves of tremor and an increase of muscle tone-producing rigidity. These muscular effects were abolished by an intraventricular injection of 100 μg of noradrenaline, whereupon the cat at once became limp.

2. The intraventricular injection of 15 μg of tubocurarine produced inconstant changes in the cortical record, in the evoked cortical response, and in the activity in the subcortical regions from which records were taken. A primary site of the central action of tubocurarine in producing muscular contractions has thus not been located.

3. The main changes in electrical activity recorded after an intraventricular injection of 200 μg of tubocurarine consisted in (*a*) a large increase in the evoked cortical response especially of its surface negative component; (*b*) a spread of the evoked cortical response to areas previously not reached

by the afferent nerve volley; (*c*) the appearance of large, evoked responses in the lateral posterior thalamic and supramammillary regions; (*d*) the appearance in the cortex and in the lateral posterior thalamic and supramammillary regions of 'spontaneous' waves, often of a pattern resembling those of the evoked response.

4. The electrical changes elicited by intraventricular injection of these larger doses of tubocurarine in the cortex and in the subcortical regions still occurred when the muscular contractions produced by the injection were prevented or abolished by intravenous tubocurarine.

5. After an intraventricular injection of 300 μg of tubocurarine some of it reaches the exposed cerebral cortex.

6. Filter-paper disks soaked in tubocurarine solutions (10–100 μg/ml.), applied to the primary sensory area of the cortex, produced an increase in the evoked response, particularly its surface negative component.

7. The effect of topical cortical application of tubocurarine, whether applied locally on a filter-paper disk, or by flooding the cortex with tubocurarine, differed from the effect seen after its intraventricular injection in that the surface negative wave of the evoked cortical response increased in size but not in duration, and the response remained strictly localized in the originally responsive area. Topical application also did not cause periods of 'spontaneous' activity. Therefore an action on subcortical structures is assumed to account for the cortical responses seen after intraventricular injection of tubocurarine, though the possibility of a local contributory action on the cortex is not excluded.

REFERENCES

Brooks, C. McC. & Eccles, J. C. (1947). Electrical investigation of the monosynaptic pathway through the spinal cord. *J. Neurophysiol.* **10**, 251–273.

Chang, H. T. (1953). Similarity in action between curare and strychnine on cortical neurones. *J. Neurophysiol.* **16**, 221–233.

Dempsey, E. W. & Morison, R. S. (1942). The production of rhythmically recurrent cortical potentials after localised thalamic stimulation. *Amer. J. Physiol.* **135**, 293–300.

Dowben, R. M. & Rose, J. E. (1953). A metal filled microelectrode. *Science*, **118**, 22–24.

Euler, U. S. von & Wahlund, H. (1941). Über zentrale Kurarewirkungen. *Acta physiol. scand.* **2**, 327–333.

Feldberg, W. & Sherwood, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol.* **120**, 3–5*P*.

Feldberg, W. & Sherwood, S. L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol.* **123**, 148–167.

Jimenez-Castellanos, J. (1949). Thalamus of cat in Horsley-Clarke coordinates. *J. comp. Neurol.* **91**, 307–330.

Johnson, B. (1955). Strychnine paroxysms in brain stem. I. Anatomical distribution. *J. Neurophysiol.* **18**, 189–199.

Salama, S. & Wright, S. (1950). Action of D-tubocurarine chloride on the central nervous system of the cat. *Brit. J. Pharmacol.* **5**, 49–61.

Starzl, T. E. & Magoun, H. W. (1951). Organization of the diffuse thalamic projection system. *J. Neurophysiol.* **14**, 133–136.

Verzeano, M., Lindsley, D. B. & Magoun, H. W. (1953). Nature of recruiting response. *J. Neurophysiol.* **16**, 183–195.