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Microinjections of tubocurarine, leptazol, strychnine and picrotoxin into the cerebral cortex of anaesthetized cats

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Summary

1. In cats anaesthetized with intravenous chloralose, microinjections of tubocurarine, leptazol, strychnine or picrotoxin, in a volume of 1 μl, were made into the grey matter of the cerebral cortex and the electrical activity was recorded from the site of injection with the microinjection cannula which, insulated except at its tip, served as recording electrode.

2. Routinely the injections were made into the gyrus splenialis or into the underlying gyrus cinguli close to the mid-line, because the injections would then most likely be in grey and not in white matter. Injected in this way all four drugs set up foci of excitation which gave rise to synchronous firing of a large number of neurones with the result that high voltage negative spikes were recorded from the microinjection cannula.

3. On injection into the gyrus splenialis the threshold dose was about 0·04 μg for picrotoxin, about 0·2 μg for tubocurarine, about 5 μg for strychnine and 25 to 50 μg for leptazol. Following the injection of larger doses the spike discharge continued for a few hours after picrotoxin and tubocurarine, for over an hour after strychnine, but for a few minutes only after leptazol. On injection into the gyrus cinguli the threshold doses were slightly greater and with larger doses the spikes occurred at greater frequency but were of lower voltage than in the gyrus splenialis.

4. With large doses of picrotoxin injected into the gyrus splenialis the spikes developed an after-positivity and an after-discharge which sometimes passed into a short period of fast activity.

5. The foci of excitation set up by the drugs were restricted to the site of injection because on raising or lowering the microinjection cannula the spikes recorded from it quickly decreased in voltage and then disappeared. When the injections were made close to a sulcus and the microinjection cannula, on being lowered, traversed the sulcus, the spikes changed their polarity.

6. The spike discharge appears to be a consistent response to the injections of the drugs into grey matter of any part of the cerebral cortex since it was also obtained on their injection into the pyriform cortex, amygdala and area retro-

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limbica anterior, but not on their injection into white matter or caudate nucleus, thalamus or hypothalamus.

Introduction

The present experiments show that microinjections of tubocurarine, leptazol, strychnine and picrotoxin into the grey matter of the cerebral cortex of anaesthetized cats set up a focus of excitation which gives rise to a synchronous firing of a large number of cells, and when the cannula used for the microinjection serves as a recording electrode and acts as a lead from the site of injection, it records a discharge of high voltage negative spikes. The discharge occurs only when the microinjections are made into the grey, not when they are made into the white matter, as shown previously for tubocurarine (Feldberg & Fleischhauer, 1967).

When a microinjection cannula is inserted a few millimetres deep into a gyrus of the cerebral cortex it is not certain that the injections will always be in grey matter. However, the chances of this happening are relatively great when the gyri chosen for injection are those lying closest to the mid-line and the microinjection cannula is inserted as far medially as possible. For routine testing of the drugs, therefore, the gyrus splenialis and the underlying gyrus cinguli (the gyrus fornicatus of Winkler & Potter (1914)) were chosen, and the microinjection cannula was inserted into these gyri close to the mid-line. A spike discharge as recorded from these sites appears to be a universal response to foci of excitation set up by drugs in the grey matter of any part of the cerebral cortex, since it was obtained also when the microinjections were made into the pyriform cortex, amygdala or into the area retrolimbica anterior.

Methods

The experiments were done on cats of both sexes weighing between 2·4 and 3·5 kg. Anaesthesia was with intravenous chloralose (60 mg/kg) induced with ethyl chloride and ether to allow cannulation of the right femoral vein. After cannulation of the trachea the head of the cat, lying on its belly, was fixed to the ear bars and mouthpiece of the Moruzzi-Dell stereotaxic instrument.

Microinjections. The method was that used by Myers (1966) modified for electrical recording by Feldberg & Fleischhauer (1967). The microinjection device consisted of an infusion pump discharging 1 μl of fluid in 60 s from a 10 μl Hamilton syringe filled with absolute alcohol, and of two steel cannulae, one inside the other. The outer tube served as a guide for the inner one, the injection cannula. The two cannulae are shown diagrammatically in Fig. 1. The outer cannula was of 22 gauge, 0·7 mm stainless steel tubing. It was fixed in a Perspex block and attached to a micromanipulator of the stereotaxic instrument. The lower part of the cannula, below the Perspex block, was insulated and its insulation was checked before each experiment. The tip of the guide tube together with the protruding end of the inner tube acted as a lead from the site of injection. The diagram shows the electrode screw; it contacts the non-insulated part of the guide tube inside the Perspex block and is used for leading off. The inner tube was of 28 gauge 0·35 mm stainless steel tubing and connected to the syringe by fine polyvinyl chloride tubing (Braun P.I. 0·95 mm outer and 0·4 mm inner diameter). The inner tube and polyvinyl tubing were filled with a solution of the drug to be injected. The alcohol in

the syringe was prevented from entering the brain substance during the injections because the length of the tubing was at least 50 cm and contained over 100 μl fluid, whereas the amount of fluid injected was 1 μl each time.

Before inserting the cannulae into the brain substance the guide tube was put into an exactly vertical position in the desired saggital and frontal plane without touching the brain surface after the bone and the dura had been removed in this region. The inner tube, filled with the drug solution, was then passed through the entire length of the guide but not beyond, and the two cannulae were lowered together into the brain to a depth 1 mm above the point of injection. Finally, the inner tube was lowered so as to protrude 1 mm beyond the guide, and after a control period, 1 μl of the drug solution was injected. The injections could be repeated several times at the same point. Control injections of 1 μl of 0·9% NaCl solution did not affect the electrical activity recorded from the microinjection cannula.

The site of injection was verified at the end of each experiment. For this purpose the brain was fixed by perfusion of the head from the aorta with saline solution followed by formalin-saline (1/3 v/v) after opening the thorax, clamping the heart and cutting the jugular veins. Sections of the brain were cut on the freezing microtome and stained by the method of Klüver & Barrera (1953). The diagrams of Fig. 2 were drawn from such sections.

Recording electrical activity. Electrical activity was recorded with monopolar electrodes connected to an Offner pen recorder. The right metal ear bar of the stereotaxic instrument was earthed and served as an indifferent electrode. The time constant of the Offner pen recorder was set at 0·3 s and the high frequency limit at 500 Hz. For recording the activity from the surface of the cerebral cortex epidural electrodes consisting of platinum wire held in a nylon screw were inserted

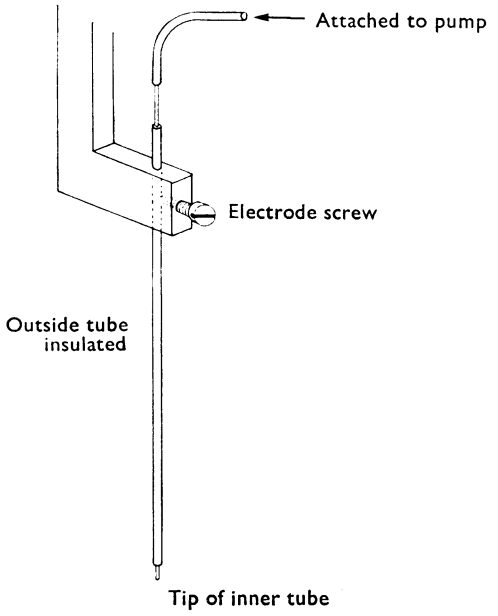

FIG. 1. Diagram of the microinjection cannula. (For details see text.)

through a burr hole on to the dura. For recording from deep structures of the brain either the microinjection cannula or a stereotaxically inserted insulated steel needle electrode was used with a free tip of about 0·5 mm.

Drugs. (+)-Tubocurarine hydrochloride (Burroughs Wellcome & Co., London), strychnine hydrochloride (Hopkin & Williams Ltd., Essex). The amounts given in the text refer to the salt. Picrotoxin (Ralph N. Emanuel Ltd., Alperton, Middlesex); leptazol (Boots, Nottingham). The drugs were freshly dissolved in 0·9% NaCl solution before injection.

Results

Tubocurarine

A few minutes after a microinjection of tubocurarine (1 μg) into the grey matter of the gyrus splenialis, large negative spikes were recorded from the microinjection cannula. The development of this discharge varied somewhat from experiment to experiment, but the most usual occurrence was that the first spikes had a voltage of over 1 mV, that the voltage of the subsequent spikes increased within a few minutes to over 3 mV, and further that the first new spikes occurred at intervals of 10 to 20 s, but that the frequency increased within minutes to between 15 and 25 spikes/min. Once the discharge had fully developed it continued for hours. On simultaneous recording from the surface of the ipsilateral or contralateral frontal or occipital cortex, or from a needle electrode inserted to the same point of the contralateral gyrus splenialis no synchronous spike discharge was recorded in any of these leads. The threshold dose of tubocurarine with which the spike discharge was obtained was 0·2 μg. With near threshold doses the discharge developed differently in that the frequency of the discharge was greater at the onset than with the larger doses, but that the voltage of the spikes was small and only gradually increased.

The focus of excitation which gives rise to a synchronous firing of a large number of neurones is restricted to the site of injection, because on raising or lowering the microinjection cannula, millimetre by millimetre, the spikes recorded in this lead quickly decreased in voltage. This is illustrated in Fig. 2.

In experiment 1, the beginning of tracing (a) shows a spike discharge recorded 12 min after an injection of 1 μg tubocurarine 4 mm deep into the gyrus splenialis. Recorded from the site of injection the spikes had a voltage of over 3 mV, but on raising the cannula by 1 mm, their voltage decreased to less than 1·5 mV, and on further raising the cannula twice by 1 mm to less than 0·5 mV. When the cannula was then returned to the original position the voltage of the spikes became again over 3 mV (tracing b). Lowering the cannula from this position for 1 mm brought little change (not illustrated), but lowering it for another millimetre and then twice for 0·5 mm to a depth of 7 mm, reduced the voltage of the spikes to 2, 1·5 and 1 mV (tracings c and d). When the cannula was lowered still further, the polarity of the spikes reversed and they became positive before they disappeared. The reversal happened at a depth of 8 to 9 mm (tracings e and f). As shown in the diagram on the right, the gyrus splenialis ends at about this depth, and as the gyrus is covered by the pia, which acts as a membrane, the reversal of polarity of the spikes is a sign that the top of the cannula passes out of the splenium into the sulcus and then into the gyrus cinguli.

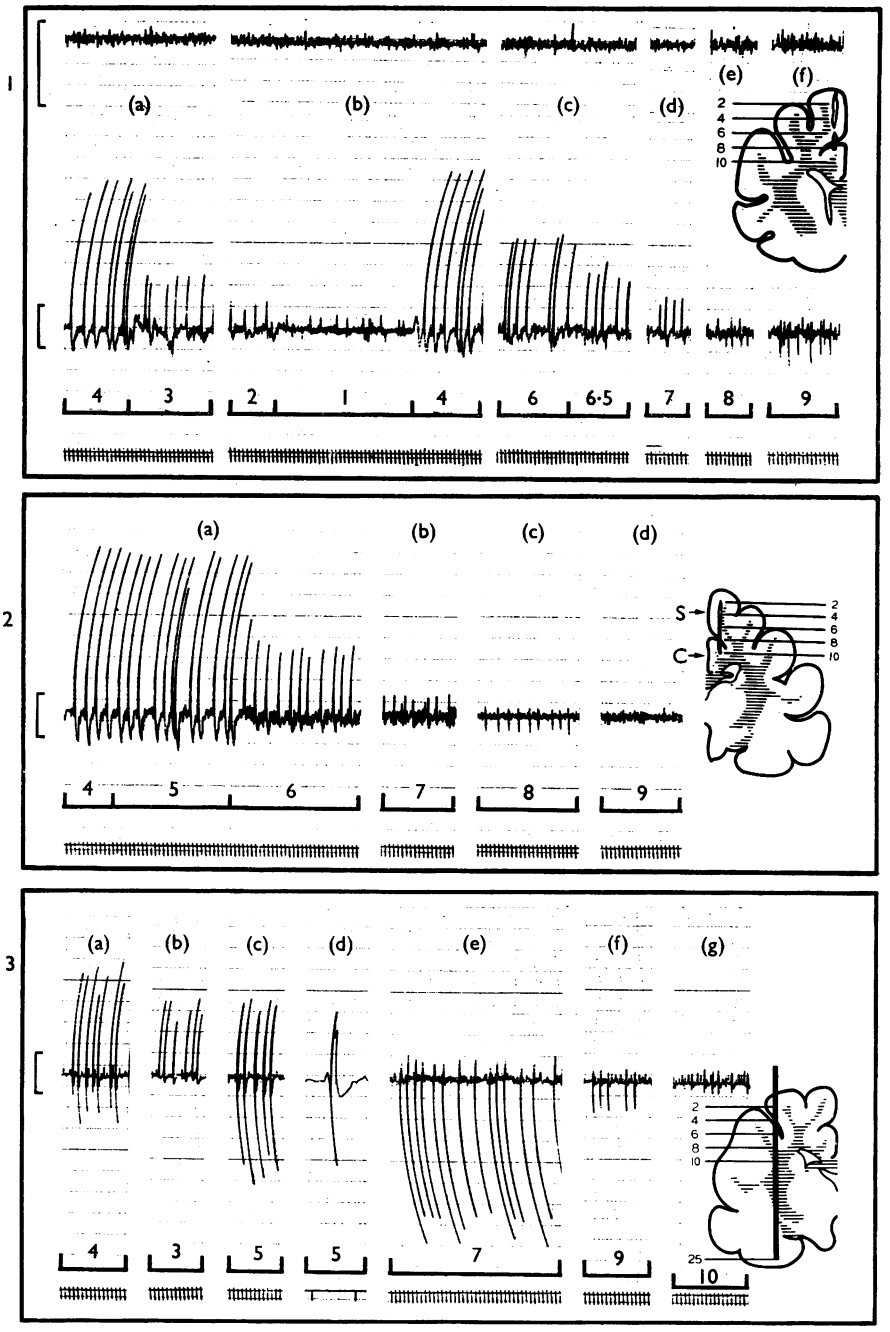

FIG. 2. Electrical activity recorded from the tip of a microinjection cannula after injection of tubocurarine (tbc) 4 mm deep into the cerebral cortex and subsequently raising and/or lowering microinjection cannula in three cats anaesthetized with chloralose. Experiment 1: injection of 1 μg tbc into right gyrus splenialis (11 mm anterior of interaural and 1·5 mm lateral of mid-line) 10 min before beginning of record a. Upper records from surface of right occipital cortex. Experiment 2: injection of 10 μg tbc into left gyrus splenialis (11 mm anterior of interaural and 1·5 mm lateral of mid-line) 35 min before beginning of record a. Experiment 3: injection of 10 μg tbc into gyrus adjacent to left gyrus splenialis (12 mm anterior of interaural and 10 mm lateral of mid-line) 15 min before beginning of record a. The figures below the records refer to the depth of the tip of the cannula in mm from the brain surface, and the diagrams on the right show the cannula tract (experiments 1 and 2) or the position of the cannula (experiment 3). S, Gyrus splenialis; C, gyrus cinguli. Calibration 1 mV, negativity upwards. Time marker in seconds.

The focus of excitation remained localized even when the amount of tubocurarine was increased 10 to 20-fold, as illustrated in the tracings of experiment 2 (Fig. 2) which were taken over half an hour after an injection of 10 μg tubocurarine 4 mm deep into the gyrus. The spikes recorded from the site of injection (first four spikes in tracing a) had attained a voltage of nearly 4 mV. Lowering the cannula for 1 mm had scarcely any effect, but lowering it for another millimetre reduced the voltage to under 2 mV, and at a depth of 7 mm the voltage was 0·5 mV only (tracing b). On further lowering the cannula the polarity of the spikes first reversed (tracing c) and then the spikes disappeared (tracing d).

A reversal of polarity of the spikes was not observed when the focus of excitation was so far away from the sulcus that the spikes disappeared on lowering the cannula before its tip had reached the sulcus. On the other hand, when the focus of excitation was close to the sulcus the positive spikes after the reversal were particularly great. In experiment 3 of Fig. 2, the tubocurarine (10 μg) was injected into the gyrus adjacent to the gyrus splenialis, 4 mm deep at a point so close to the underlying sulcus that some of the injected tubocurarine may have passed into it, and the spikes recorded from the site of injection showed a positive and a negative deflection (tracing a). The positive deflection disappeared and the negative deflection decreased in voltage (tracing b) on raising the cannula, but on lowering it the positive spikes increased in voltage up to 2 mV, and the negative deflection disappeared (tracings c, d and e). On recording at faster speed the positive deflection is seen to precede the negative one (tracing d).

When applied by microinjection into the gyrus cinguli it required about twice the dose of tubocurarine to elicit a spike discharge, which usually developed somewhat differently in that the voltage of the spikes was not high from the beginning, increased gradually and did not attain the same height as in the gyrus splenialis, but the frequency of the spikes was high from the beginning and, when the discharge was fully developed, nearly twice that recorded in the gyrus splenialis. Figure 3 shows the difference between a fully developed discharge following a microinjection of 1 μg tubocurarine into the gyrus splenialis (tracing a) and that following injection into the gyrus cinguli (tracing b) of the same animal. The discharge set up in the gyrus cinguli thus somewhat resembled that often obtained on injection of near threshold doses into the gyrus splenialis.

Once a discharge had appeared external stimuli such as clapping the hands, tapping the table or the spine of the cat with a metal rod, elicited spikes. When the stimuli were applied at regular but somewhat shorter intervals than those between two spikes, the discharge could be " driven " by 10 and sometimes even by 20 stimuli, and spikes occurred not only in the lead from the injection site but also from the same point in the contralateral cortex. Such an experiment is illustrated in Fig. 4. Tracing a shows that tapping the spine every second or two before the tubocurarine injection produced small spikes in all four leads, but not each time. Tracing b was obtained 5 min after a microinjection of 1 μg of tubocurarine into the left gyrus splenialis when a spike discharge had developed in the lead from the site of injection. Now each of the ten taps, given at 2 s intervals, resulted in a spike in this lead, so that the discharge was " driven " by the external stimuli. The driven spikes were somewhat smaller than those obtained before or after the tapping ; the difference was not always as pronounced as in this experiment. Each tap now gave rise also to a spike of varying voltage in the lead from the contra-

lateral gyrus splenialis, but in the leads from the surface of the occipital cortices the response to the taps was unchanged. On tapping the spine at shorter intervals, for instance, every second, the discharge in the gyrus splenialis was often "driven" for the first few taps only, in this experiment for the first six taps (tracing c). The more frequent discharge produced by a microinjection of tubocurarine into the gyrus cinguli was usually driven for a longer period by external stimuli applied every

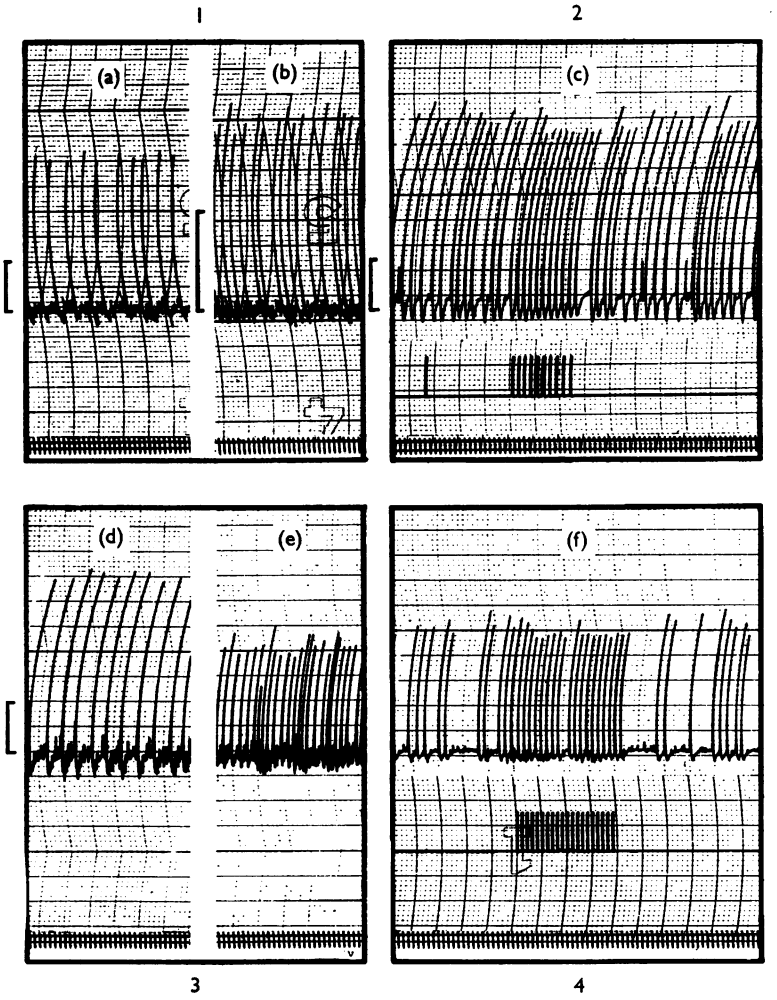

FIG. 3. Electrical activity recorded in four cats anaesthetized with chloralose from the tip of a microinjection cannula after injection of tubocurarine (tbc) into the cerebral cortex. Experiment 1: injection of 1 μg tbc 5 mm deep into left gyrus splenialis (10 mm anterior of interaural and 1 mm lateral of mid-line) 30 min before beginning of tracing a. Between a and b cannula lowered 4 mm into gyrus cinguli and then 1 μg tbc injected 25 min before beginning of tracing b. Experiment 2: same cat as experiment 3 of Fig. 2 which in the diagram gives position of cannula. Injection of 10 μg tbc into left pyriform cortex (12 mm anterior of interaural and 10 mm lateral of mid-line, 25 mm deep) 20 min before beginning of tracing c. Experiment 3: injection of 50 μg leptazol 4 mm deep into right gyrus splenialis (11 mm anterior of interaural and 1 mm lateral of mid-line) 5 min before beginning of tracing d. Between d and e cannula lowered 7 mm into cingulum and then 100 μg leptazol injected 25 min before beginning of tracing e. Experiment 4: injection of 10 μg strychnine 5 mm deep into right gyrus splenialis (10 mm anterior of interaural and 1 mm lateral of mid-line) 25 min before beginning of tracing f. The signals in the middle of tracings c and f indicate taps on the table with a metal rod (c) or claps of the hands (f) at 1 s intervals. Calibration 1 mV, negativity upwards. Time marker in seconds.

second. Tracing d, which was obtained after the microinjection cannula had been lowered and 1 μg tubocurarine had been injected into the gyrus cinguli, shows this effect for twenty taps. Spikes occurred with all but two taps, which may have failed because they may have been less vigorous.

A spike discharge was also obtained with microinjections of tubocurarine into the pyriform cortex, amygdala, or, as illustrated previously (Feldberg & Fleischhauer, 1967), into the area retrolimbica anterior. The discharge on injection into the pyriform cortex as well as the "driving" of the discharge by external stimuli is shown in tracing c of Fig. 3. On stepwise raising the microinjection cannula the voltage of the spikes first diminished and then disappeared, but there was no reversal of polarity as the cannula tip did not traverse a sulcus.

No spike discharge was obtained with microinjections, even of 10 μg tubocurarine, into the hypothalamus, thalamus or caudate nucleus. This had previously been observed for the caudate nucleus (Feldberg & Fleischhauer, 1967).

Leptazol

To obtain a spike discharge with leptazol much larger doses than those of tubocurarine had to be injected and the effect lasted 2 to 12 min only. On injection

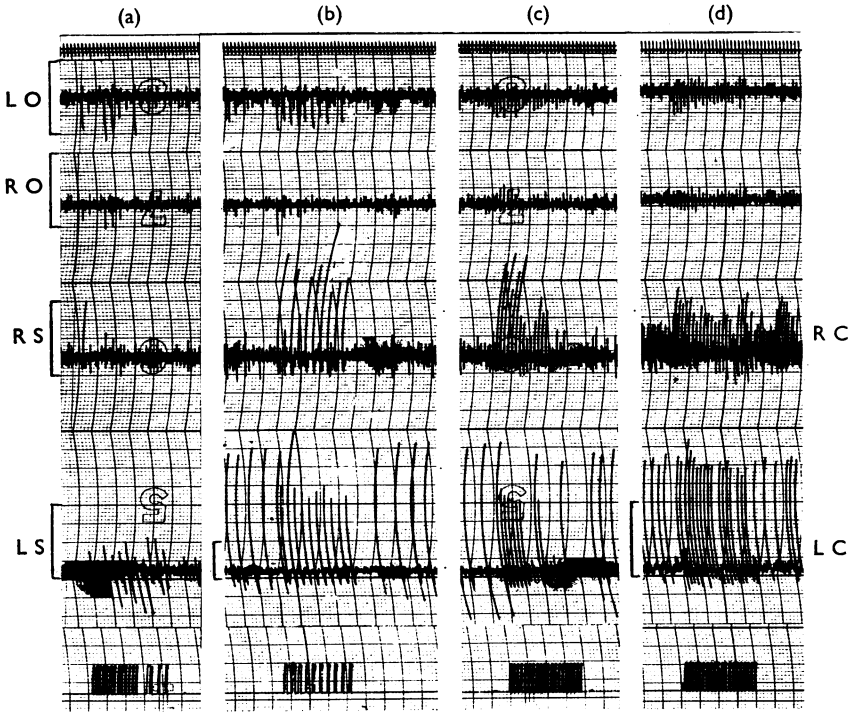

FIG. 4. Electrical activity recorded in a cat anaesthetized with chloralose from surface of left and right occipital cortex (LO and RO) and from the tip of a microinjection cannula inserted first into the left gyrus splenialis (LS) and then into gyrus cinguli (LC) and from a needle electrode inserted first into the right gyrus splenialis (RS) and then into gyrus cinguli (RC). Cannula and needle inserted to same position (10 mm anterior of interaural and 1 mm lateral of mid-line; a to c, 5 mm deep into gyrus splenialis; d, 10 mm deep into cingulum). Tracing a obtained 5 min before, tracings b and c 5 and 10 min after an injection of 1 μg tubocurarine into the left gyrus splenialis. Between c and d, cannula needle lowered 5 mm. Tracing d obtained 20 min after an injection of 1 μg tubocurarine into gyrus cinguli. Signals at the bottom of the tracings indicate tapping of the spine of the cat with a metal rod. Calibration 1 mV, negativity upwards. Time marker in seconds.

into the grey matter of the gyrus splenialis the threshold dose varied between 25 and 50 μg. Sometimes a microinjection of even 50 μg was ineffective when given for the first time but produced a spike discharge when given the second or third time. On injection into the grey matter of the gyrus cinguli, the same difference in sensitivity, frequency and voltage of the spikes was observed as with tubocurarine ; the sensitivity was slightly less, the frequency of the spikes was greater but their voltage was lower. Tracings d and e of Fig. 3 show a fully developed discharge 5 min after an injection of 50 μg leptazol into the gyrus splenialis and of 100 μg into the cingulum.

Strychnine

Its microinjection into the grey matter of the gyrus splenialis, gyrus cinguli or pyriform cortex produced discharges of negative spikes similar to those produced by tubocurarine and leptazol. The threshold dose on injection into the gyrus splenialis was 5 μg, and on injection of 10 or 20 μg the discharge continued for over an hour, which is much longer than after leptazol but shorter than after tubocurarine. As with these drugs the discharge was recorded only from the site of injection and could be driven by external stimuli. Tracing f of Fig. 3 illustrates a discharge obtained by 10 μg strychnine injected into the gyrus splenialis and the "driving" of the discharge by clapping the hands every second.

No spike discharge was recorded from the site of injection when the strychnine (10 or 20 μg) was injected into the caudate nucleus or into the hypothalamus.

Picrotoxin

Microinjections of picrotoxin into the grey matter of the gyrus splenialis or gyrus cinguli produced a long-lasting discharge of negative spikes in the lead from the site of injection. As with tubocurarine, leptazol or strychnine, the spike discharge was localized and disappeared on raising or lowering the microinjection cannula, thus recording from above or below the injection site.

On injection into the gyrus splenialis the threshold dose of picrotoxin was about 0·04 μg ; with 0·02 μg there was some increase in background activity interspersed occasionally by a single negative spike. With near threshold doses (0·05 to 0·1 μg) the discharge continued for over 2 and with larger doses (1 or 2 μg) for over 3 h. As with tubocurarine, the discharge developed differently according to the dose injected. With large doses the first spikes occurred usually at a low frequency, but they had a high voltage, as shown in tracing a of Fig. 5. With near threshold doses, on the other hand, the voltage of the spikes was low at the onset and only gradually increased, but the frequency was high from the beginning.

In about half the experiments with larger doses the spikes developed a strong after-positivity and an after-discharge that sometimes passed into a short period of fast activity. These features which were not observed with tubocurarine, leptazol or strychnine are illustrated in Fig. 5. The nine spikes of tracing a give the onset of the discharge, 1·5 min after the microinjection of 2 μg picrotoxin. Tracings b and c show the development of the after-positivity ; in tracing c the spikes have an after-discharge on the descending limb which stands out more clearly when recording was at faster speed, as in tracing d. Twice the after-discharge developed into

a short period of fast activity, the second period being shown in tracing e. Later on, in tracing f and g, the after-discharge disappeared and the after-positivity diminished. The frequency of the spikes was influenced by the after-discharge. A frequency of about 20/min which was reached before the after-discharge had developed (tracing b), decreased to 12/min during its development (tracing c) and when the after-discharge had disappeared the frequency increased again and reached 25 and 30/min (tracing f).

On simultaneous recording from the surface of the ipsilateral or contralateral frontal or occipital cortices or from a needle electrode inserted to the same point of the contralateral gyrus splenialis, no abnormal spikes were recorded in any of these leads. The upper tracings of Fig. 5 show the absence of abnormal spikes in the lead from the contralateral gyrus splenialis.

On the other hand, when a needle electrode was inserted into the ipsilateral gyrus splenialis anterior to the injection cannula, it recorded positive spikes synchronous with the negative ones recorded from the site of injection. This is shown for experiments 1 and 2 in Fig. 6. In both the microinjection was made at a depth of 5 mm. The positive spikes in experiment 1 were recorded from a needle electrode inserted to the same depth 3 mm anterior to the cannula. On raising the needle 2 mm the spikes disappeared. In experiment 2 the positive spikes were recorded from a needle electrode inserted only 3 mm deep but 5 mm anterior to the cannula ; they disappeared on lowering the needle to a depth of 5 mm.

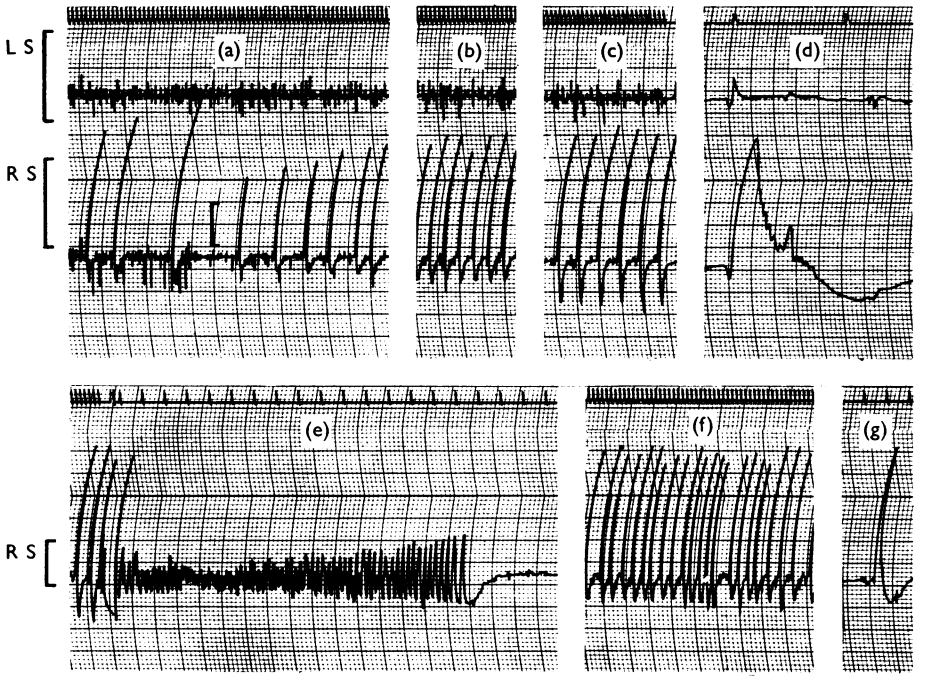

FIG. 5. Electrical activity recorded in a cat anaesthetized with chloralose from the tip of a microinjection cannula (RS) after injection of 2 μg picrotoxin 5 mm deep into the right gyrus splenialis (5 mm anterior of interaural and 1·3 mm lateral of mid-line) and from a needle electrode (LS) inserted at same point into the left gyrus splenialis. Tracings a to g obtained 1·5, 5, 16, 18, 25, 55 and 60 min after the injection. Calibration 1 mV, negativity upwards. Time marker in seconds.

The gyrus cinguli was slightly less sensitive to picrotoxin than the gyrus splenialis and the frequency of the spike discharge was usually greater. The discharge produced by large doses (1 to 2 μg) differed from that recorded from the splenium in that the spikes did not develop strong after-positivity or after-discharges. Further, in contrast to the results obtained on the gyrus splenialis, a synchronous discharge of positive-negative spikes was recorded from a needle electrode inserted into the contralateral gyrus cinguli to the same depth as the injection cannula. This is illustrated in experiment 3 of Fig. 6. In experiment 4, the needle electrode was inserted into the ipsilateral gyrus cinguli to the same depth as the injection cannula but 3 mm anterior to it ; it also recorded positive-negative spikes synchronous with the negative spikes recorded from the site of injection.

Discussion

Methods of introducing drugs by microinjection into the cerebral cortex have been used by many authors. The present method differs from previous ones in that the microinjection cannula serves as a recording electrode as well, and acts as a lead

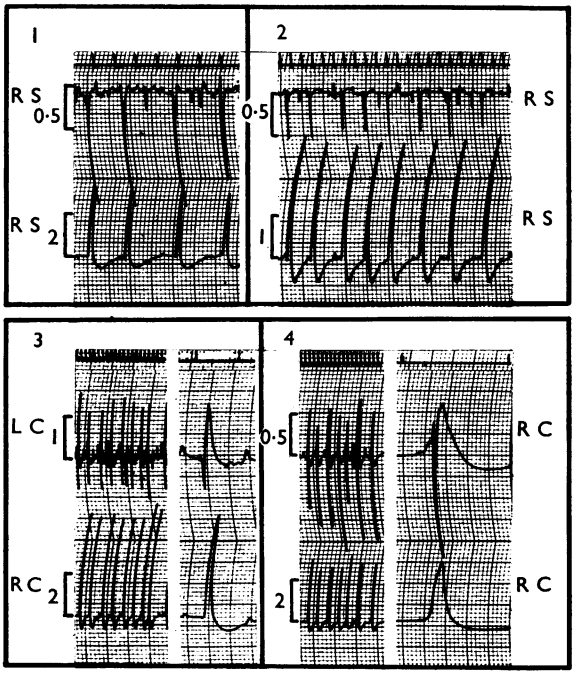

FIG. 6. Electrical activity recorded in three cats anaesthetized with chloralose from the tip of a microinjection cannula (lower records) and from a needle electrode (upper records) after injections of 2 μg picrotoxin into the cerebral cortex. Experiment 1: cannula and needle inserted 5 mm deep into right gyrus splenialis (cannula 3 mm and needle 8 mm anterior of interaural, and both 1·3 mm lateral of mid-line); tracing begins 15 min after the injection. Experiment 2: cannula inserted 5 mm, needle 3 mm deep into right gyrus splenialis (cannula 3 mm and needle 8 mm anterior of interaural, and both 1·3 mm lateral of mid-line); tracing begins 30 min after the injection. Experiment 3: cannula inserted into right and needle into left gyrus cinguli 10 mm deep, 5 mm anterior of interaural and 1·5 mm lateral of mid-line; tracing begins 37 min after the injection, interval between the two tracings about 1 min. Experiment 4: continuation of experiment 2, after lowering cannula and needle into gyrus cinguli, both to a depth of 10 mm ; tracing begins 23 min after a new injection, interval between the two tracings about 1 min. Calibration in mV (0·5–2), negativity upwards. Time marker in seconds.

from the injection site. Further, by making the injections into the gyrus splenialis or into the gyrus cinguli close to their medial border, the drugs are nearly always introduced into grey matter. Thus a simple and efficient method is obtained for finding out whether drugs set up foci of excitation in the grey matter and for assaying the potency of drugs having this action. This is the value of the method for pharmacology in general.

Of the four drugs injected, three, strychnine, leptazol and picrotoxin, are convulsants, and the fourth, tubocurarine, would be a convulsant too, if it were to pass the blood-brain barrier and if it were not to block neuromuscular transmission. But when the barrier is circumvented and peripheral actions are excluded, as in the present experiments, all four drugs produced the same effect, differing only in potency and duration of action. They all set up foci of excitation which gave rise to a synchronous firing of a large number of neurones and led to a discharge of high voltage negative spikes recorded in the lead from the injection site. With large doses of picrotoxin the spikes developed after-positivity and after-discharges which sometimes passed into short periods of fast activity. For this to happen, the picrotoxin had to be injected in about fifty times the threshold dose, and even then it occurred in only about half the experiments and only on injection into the gyrus splenialis, not into the gyrus cinguli. Since picrotoxin was the most potent of the four drugs, and the gyrus splenialis was more sensitive than the gyrus cinguli, these features of the discharge may not be specific for picrotoxin nor for the gyrus splenialis, they may merely signify the effect of an excessively high concentration of an " excitatory drug " in the grey matter of the cerebral cortex, a concentration not attained when picrotoxin was injected into the gyrus cinguli, nor with the other drugs injected into the gyrus splenialis.

The excitatory action of strychnine and tubocurarine on the cerebral cortex is not a new effect, but has been observed with various methods. Chang (1953) demonstrated the similarity of action between tubocurarine and strychnine when applied topically to the surface of the cerebral gyri of anaesthetized rabbits and cats. With either drug the negative wave of an evoked cortical response, due to activity of intercortical neurones, became enhanced and spontaneous discharges appeared. According to Chang, those produced by curare may be called " curare spikes " in analogy with the " strychnine spikes ". Recently, Bhargava (1969) again pointed out the similarity between the effects of topically applied tubocurarine and strychnine, except that curare was effective in weaker concentrations. A similar condition pertains for the hippocampus. Microinjections of tubocurarine, strychnine or picrotoxin into this structure set up spontaneously discharging foci, and, as in the present experiments, picrotoxin was the most and strychnine the least potent of the three (Baker, Kratky & Benedict, 1965 ; Baker & Benedict, 1967).

Other neuromuscular blocking agents do not share with tubocurarine the property of setting up a spike discharge in the cerebral cortex or in the hippocampus. This does not occur with dihydro-β-erythroidine, gallamine triethiodide, decamethonium or succinylcholine (Baker & Benedict, 1967 ; Bhargava, 1969). Substances having mainly a stimulating or depolarizing action on the motor endplate also do not produce " tubocurarine- or strychnine-like " spikes. They were not seen following microinjections into the cerebral cortex of nicotine or acetylcholine, the latter injected either alone or with physostigmine ; nor were they produced by microinjections

of nikethamide, amphetamine or cocaine (Feldberg & Georgiev, unpublished experiments).

A common mechanism of action would therefore appear to be responsible for the characteristic spiking produced by tubocurarine, strychnine, picrotoxin and leptazol. The mechanism could be disinhibition, that is interference or blockade of post-synaptic or presynaptic inhibition.

Strychnine has such an action on the spinal cord, since it depresses the activity of post-synaptic inhibitory synapses which are formed by various pathways converging on motoneurones (Bradley, Easton & Eccles, 1953 ; Eccles, Fatt & Koketsu, 1954 ; Curtis, 1959, 1962), and Bradley *et al.* (1953) suggested that strychnine competes with the inhibitory transmitter " for the same steric configurations on the inhibitory post-synaptic membrane ". A number of observations, recently reviewed by Curtis (1969), point to glycine as the inhibitory transmitter in the spinal cord and suggest that strychnine competes with the glycine. For instance, the distribution of glycine in the spinal cord can be related to the presence of inhibitory neurones (Aprison & Werman, 1965; Graham, Jr, Shank, Werman & Aprison, 1967; Davidoff, Graham, Jr, Shank, Werman & Aprison, 1967), glycine hyperpolarizes spinal motoneurones, and strychnine blocks the hyperpolarizing effect of glycine on spinal motoneurones, interneurones and Renshaw cells (Werman, Davidoff & Aprison, 1967, 1968 ; Curtis, Hösli & Johnston, 1967, 1968 ; Curtis, Hösli, Johnston & Johnston, 1967, 1968). On the other hand, picrotoxin does not block the depressant effect of glycine on the spinal cord (Curtis, 1969) nor does it interfere with spinal post-synaptic inhibition, but it reduces spinal pre-synaptic inhibition (Eccles, 1962).

Depression of post-synaptic inhibitory potentials is thought to occur also in cortical neurones by the action of strychnine as well as of tubocurarine (Purpura & Grundfest, 1956, 1957; Morlock & Ward, 1961). However, the position is not the same as in the spinal cord. Glycine cannot be regarded as the inhibitory transmitter, and there are a number of observations according to which the activity of post-synaptic inhibitory synapses is resistant to strychnine. This result was obtained for the inhibition of the mitral cells and of the cells of the plexiform layer of the olfactory bulb in response to stimulation of the lateral olfactory tract and of the anterior commissure (Green, Mancia & von Baumgarten, 1962 ; von Baumgarten, Green & Mancia, 1962), for the inhibiting action on hippocampal pyramidal cells, cerebellar Purkinje cells and ventral-basal thalamic relay cells (Andersen, Eccles, Løyning & Voorhoeve, 1963), and for the inhibition of cortical neurones, that is, for the hyperpolarizing post-synaptic potentials of Betz cells, produced by cortical or pyramidal stimulation. Picrotoxin also did not affect the inhibition produced by local stimulation in the cortex of the cerebrum and cerebellum (Crawford, Curtis, Voorhoeve & Wilson, 1963 ; Brooks & Assanuma, 1965 ; Krnjević, Randić & Straughan, 1966 ; Phillis & York, 1967 ; Biscoe & Curtis, 1967).

Not all authors agree with the view that post-synaptic inhibition in the cerebral cortex and the higher centres of the brain is strychnine resistant. Susuki & Tukahara (1963) found that sub-convulsive doses of intravenous strychnine abolished the prolonged depression of the Betz cells, and on topical application the hyperpolarizing inhibitory post-synaptic potentials, produced in cortical neurones by cortical or pyramidal stimulation, were found not only to be depressed, but often replaced by depolarizing potentials (Stefanis & Jasper, 1965 ; Pollen & Ajmone

Marsan, 1965 ; Pollen & Lux, 1966). Pollen & Ajmone Marsan explain this double action of strychnine as follows. The generation of the inhibitory potential is achieved by selective transport of the small hydrated K^+ and Cl^- ions through " pores " which exclude the larger hydrated Na^+ ions. Strychnine damages the membrane so that the restriction of the Na^+ movements is removed with the result that the inhibitory potential is converted into an excitatory one. This hypothesis provided an alternative to the one proposed by Bradley *et al.* (1953), according to which strychnine combines at receptor sites and sterically blocks the action of the inhibitory transmitter. Sawa, Maruyama, Kaji & Nakamura (1966) also describe a double action of strychnine. Applied to the cerebral cortex, strychnine first facilitated excitatory and inhibitory cortical processes produced by stimulation of pyramidal and non-pyramidal neurones, but later the inhibitory processes were blocked whilst the excitatory synaptic transmission remained active. An inhibition which has been regarded as post-synaptic inhibition is that of the evoked acoustic response following stimulation of the olivo-cochlear bundle. This inhibition is abolished by strychnine (Desmedt & Monaco, 1960 ; Katsuki, Tanaka & Miyoshi, 1965 ; McKinstry & Koelle, 1967 ; Brown, Daigneault & Pruett, 1969), but not by picrotoxin (Desmedt & Monaco, 1960, 1961), nor by tubocurarine (Katsuki *et al.*, 1965).

Another possibility which has been suggested is that " disinhibition " by strychnine and similarly acting substances is restricted to cholinergic and monoaminergic synapses. As far back as 1954, Chatfield and Purpura put forward the view that the cerebral cortex contains an intrinsic inhibitory system which exerts a tonic influence on a specific afferent system which is probably cholinergic and blocked by strychnine, tubocurarine, atropine and related substances. More recently, Phillis & York (1967, 1968) showed that strychnine and tubocurarine reduced or abolished the depressant action of acetylcholine, 5-HT and noradrenaline on the firing of cortical neurones, as well as the long-lasting inhibitions produced in cortical neurones by repetitive stimulation of the pyramidal tract or of the mesencephalic reticular formation and by stimulation of the lateral hypothalamus. The cortical neurones inhibited by stimulation of the pyramidal tract and of the reticular formation were thought to be cholinergic because their activity was depressed by acetylcholine ; on the other hand, the inhibition produced by stimulation of the lateral hypothalamus was thought to occur at monoaminergic synapses since 5-HT- and noradrenaline-containing pathways are known to project from the lateral hypothalamus to the cerebral cortex (Andén, Dahlström, Fuxe, Larsson, Olson & Ungerstedt, 1966).

Although strychnine, tubocurarine and picrotoxin have the ability to depress inhibitory potentials, this does not prove that the localized rhythmic spike discharge, following their introduction into the cerebral cortex by microinjection, is solely the result of " disinhibition ". Otherwise one would have to assume that firing at a rate of about once every two seconds is the uninhibited activity of the cortical cells, or firing at an even higher rate, if the periods of fast activity obtained with large doses of picrotoxin were the result of more complete disinhibition. The same conclusion would apply to the hippocampal cells, since strychnine, tubocurarine, picrotoxin and leptazol, when acting on the hippocampus, produce a similar spike discharge interrupted by periods of fast activity (Feldberg & Fleischhauer, 1962, 1963 ; Baker *et al.*, 1965 ; Feldberg, Hall & Reit, 1966 ; Feldberg & Georgiev, 1970). It would even be necessary to consider the possibility that other excitatory effects of

these substances result from disinhibition, for instance, the scratching movements produced by an action on superficial structures in the upper cervical cord and the shivering produced by an action on structures in the walls of the third ventricle. These effects were obtained with tubocurarine (Feldberg & Fleischhauer, 1960; Carmichael, Feldberg & Fleischhauer, 1962) and with picrotoxin (Feldberg & Georgiev, 1970). Feldberg, Hall & Reit (1966) also obtained shivering with leptazol but not with strychnine; they did not examine the action of the two compounds on the upper cervical cord.

Phillis and York found that a number of substances which have little or no central excitatory actions produced the same disinhibitions as strychnine and tubocurarine, for instance, atropine and hyoscine as well as gallamine triethiodide and dihydro-β-erythroidine, two neuromuscular blocking agents, which are always contrasted with tubocurarine as lacking its central excitatory action. To produce a spike discharge in the cerebral cortex it may therefore not be sufficient that inhibition is abolished; it may be necessary that, in addition, the inhibitory potentials are converted into excitatory ones, or that excitatory processes are facilitated as well. Thus in spite of the extensive work on disinhibition the problem of how the spike discharges are brought about in the cerebral cortex by strychnine, tubocurarine, picrotoxin and leptazol still defies solution.

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