J. Physiol. (1966), **183**, *pp.* 341–359
With 1 *plate and* 11 *text-figures*
Printed in Great Britain

MICRO-ELECTROPHORETIC STUDIES OF NEURONES IN THE CAT HIPPOCAMPUS

By T. J. BISCOE* and D. W. STRAUGHAN†
From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge

(*Received* 10 *June* 1965)

SUMMARY

1. Drugs have been applied micro-electrophoretically to units in the hippocampal cortex of the anaesthetized cat, and their effects on cell firing were recorded simultaneously.

2. L-Glutamate rapidly and powerfully excited hippocampal units, an effect which was quickly reversed on stopping the expelling current. The local application of L-glutamate also excited a fast seizure discharge at 15–50/sec. Both these effects of L-glutamate were strongly depressed by fimbrial stimulation.

3. γ-Aminobutyric acid had a strong depressant action on all the units on which it was tested; the time course of this effect was rapid.

4. ACh excited half the units to which it was applied. Characteristically this excitation developed slowly over many seconds and persisted after stopping the expelling current. Most cholinoceptive units were found to be concentrated in the superficial layer of the cortex corresponding to the hippocampal pyramidal cells and their main dendritic processes.

5. Atropine selectively blocked the excitation of cholinoceptive units by ACh, but not the excitation by L-glutamate. No cholinoceptive units were blocked by dihydro-β-erythroidine, though several were selectively blocked by dimethyl (+)-tubocurarine.

6. The most usual effect seen with 5-HT was depression, though several units were found to be excited. Some of the units tested with 3-hydroxytyramine (dopamine) or noradrenaline were found to be depressed.

INTRODUCTION

The hippocampus has been the subject of many physiological studies in recent years, such as those of Kandel & Spencer (1961), Kandel, Spencer & Brinley (1961) and of Andersen, Eccles & Løyning (1964*a*, *b*); these have

* Present address: Department of Physiology, John Curtin School of Medical Research Australian National University, Canberra, Australia.

† Rothschild Fellow. Present address: Neuropharmacology Laboratory, University Department of Psychiatry, Edinburgh University, Morningside Terrace, Edinburgh, 10.

been recently reviewed by Green (1964). The pharmacological studies of the hippocampus have so far been indirect (see review by Stumpf, 1965); for example, Feldberg & Malcolm (1959) evoked synchronous activity in the hippocampus with (+)-tubocurarine. They deduced that this was an indirect excitation whose origin was in the peri-aqueductal tissue, but more recently Feldberg & Fleischhauer (1963) have concluded that (+)-tubocurarine does act directly on the hippocampal cortex. In addition, MacLean (1957) has found that acetylcholine, carbamoylcholine and acetyl-β-methylcholine can produce seizure discharges when applied within the hippocampus in a concentrated form. We have attempted to define with greater precision some of the pharmacological properties of cells present in this area by using the technique of micro-electrophoresis. A brief report of some of our results has already appeared (Biscoe & Straughan, 1965).

METHODS

Experiments were performed on eleven cats. In the initial experiments the cats were anaesthetized with sodium pentobarbitone (Abbot Labs. Ltd.) 30 mg/kg intraperitoneally or Dial compound (Ciba Ltd), which contained diallyl barbituric acid (0·1 g/ml.) and urethane (0·4 g/ml.), in a dose of 0·7 ml./kg intraperitoneally. In later experiments anaesthesia was induced with ethyl chloride and maintained with ether until the animals were given a mixture of 40 mg/kg chloralose (Hopkin & Williams) and 500 mg/kg urethane (Hopkin & Williams) through a cannula in the radial vein. A sagittal incision was made through the scalp and the skull was opened widely. On each side the overlying cerebral tissue was carefully aspirated until the lateral ventricle was entered. The lateral ventricle was then injected with 3% Bacto Agar (Difco Laboratories, Detroit) in 0·9% NaCl at 38° C. This filled and expanded the ventricle and allowed the dorsal hippocampus to be completely exposed without injuring the alvear fibres. Later, the agar filling the ventricle was removed, and the whole area was re-covered with 3% agar. This procedure helped fixation of the cortex and considerably improved the recording conditions. Bleeding was minimized by tying the sagittal sinus rostrally and caudally, and using a gelatine sponge (Sterispon, Allen & Hanburys Ltd) to promote blood clotting.

The multibarrelled pipettes were made of five hard glass tubes fused over part of their lengths and drawn to a fine tip in an electrode puller similar to that designed by Winsbury (1956). The tips were broken back to give a tip diameter of 4–8 μ and the electrodes were filled by boiling in glass-distilled water. The four outer barrels were filled with concentrated aqueous solutions of the drugs to be tested, acid or alkali being added to produce optimal ionization of the solute compatible with stability. In order to excite quiescent cells one micropipette barrel was always filled with a 1 M solution of Na L-glutamate at pH 8·5, while another barrel was usually filled with 2·7 M-NaCl for applying current controls. The drugs were ejected micro-electrophoretically by connecting the solutions through a silver wire and flexible copper lead to a polarizing source with a 50 MΩ, or in some experiments a 1000 MΩ series resistor. The circuit was completed through an earthing electrode (Ag-AgCl) embedded in the muscles of the neck. The currents could be reversed, and measured with a series Pye galvanometer to an accuracy of 2 nA. Provision was also made for an appropriate retaining current to be applied to prevent outward diffusion of the drugs. Doses of drugs are expressed in nanoamps, for example, 40 nA L-glutamate is interpreted as a current of 40 nA L-glutamate passed for the indicated time.

The central barrel of the micropipette was filled with 2·7 M-NaCl and connected by a short chlorided silver wire to the grid of a cathode follower valve. The electrode assembly and the cathode follower were supported on a manipulator which could be advanced in 1 μ steps.

The output of the cathode follower was led to a pre-amplifier with a short time constant (usually 2 msec), since our main interest was in recording spike potentials. The pre-amplifier output was amplified and recorded on the upper beam of an oscilloscope provided with *Z*-axis modulation to intensify fast transients (Pokrovsky, 1960). The signal from one plate of the vertical amplifier was connected to a rate-meter with a discriminator at the input, to permit the selection of large spikes from small spikes and noise. The rate of firing of units in impulses per sec could then be continuously monitored on a pen recorder. With fast firing rates the spike amplitudes decline, and may fall below the discriminator threshold, and thus fail to be counted by the rate-meter. To avoid this the output of the discriminator was monitored on the lower beam of the oscillosope. Provision was also made for the audiomonitoring of the signals on both beams. The oscilloscope face could be photographed on 35 mm moving film.

Field responses were frequently recorded from the surface of the hippocampus with a silver ball electrode of 0·5 mm diameter. This was connected directly to the high input impedance first stage amplifier and so to the oscilloscope. Arrangements were also made to stimulate the ipsilateral fimbria and contralateral hippocampus through bipolar silver electrodes with square waves of 0·1 msec duration. The temperature of the animals was maintained at 37–38° C with a thermosensitive transistor amplifier controlling the current flowing through a heating blanket (Krnjević & Mitchell, 1961).

The following drugs were used: acetylcholine chloride, acetyl-β-methylcholine chloride, atropine sulphate, carbamoylcholine chloride, dihydro-β-erythroidine hydrobromide, dimethyl(+)-tubocurarine, ergometrine acidmaleate, γ-aminobutyric acid, 3-hydroxytyramine (dopamine) hydrochloride, 5-hydroxytryptamine bimaleinate and creatinine sulphate, noradrenaline bitartrate, nicotine hydrogen tartrate, sodium L-glutamate.

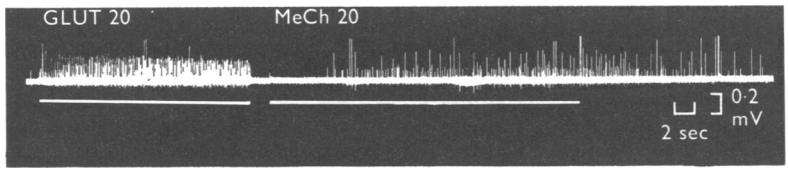
RESULTS

The regions most fully explored were those of fields CA 1, CA 2 and CA 3 (Lorente de Nó, 1934) in the dorsal hippocampus. These fields were recognized by their anatomical position (see Pl. 1*A*) and also by the characteristics of the responses evoked by stimulation of homotopic points in the contralateral hippocampus as described by Andersen (1959, 1960). In CA 1 the normal response is a surface positive–negative wave with one or more spikes, usually negative, superimposed on the positive wave, while in CA 3 there is an early diphasic spike followed by a negative wave with a following late positive wave.

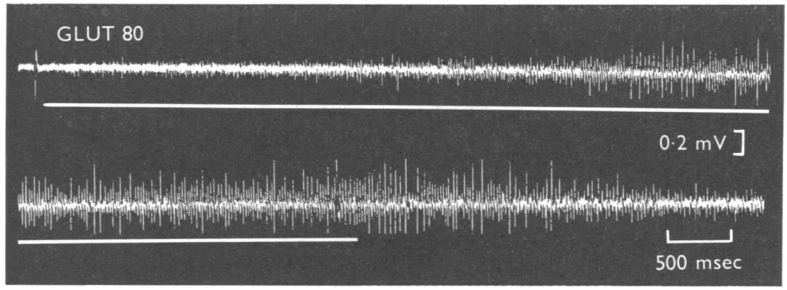
Very few spontaneously firing units were encountered. In addition we were not successful in evoking unit firing by stimulation of the ipsilateral fimbria and hippocampus or the contralateral hippocampus, despite the presence of well marked field responses. However, by releasing L-glutamate continuously from one barrel of the micropipette it was possible to excite one or more units at three or four depths in any one track down to 2·5 mm. In this way 161 single or grouped units were excited. In quiescent units, excitatory or depressant effects could be demonstrated against a background of firing induced by L-glutamate. Single unit responses were seen most readily in those cats which had been lightly anaesthetized with chloralose and urethane rather than with barbiturates.

L-Glutamate responses

The unit firing induced by the application of small (10–30 nA) currents of L-glutamate was quite characteristic, the excitation having a short latency, and ceasing equally quickly within several hundred msec when the expelling current was turned off. With larger currents of L-glutamate the firing rate increased more rapidly, and then the spike amplitude tended to decline.



Text-fig. 1. Filmed record of extracellular spike responses from a group of units at a depth of 100 μ in hippocampal field CA 2–3. Left, response to L-glutamate 20 nA (GLUT 20) for 20 sec; right, response to acetyl-β-methylcholine 20 nA (MeCh 20) for 30 sec. Duration of each drug application indicated by horizontal bar below record.



Text-fig. 2. Filmed oscilloscope record showing small spikes and then synchronized activity induced in CA 3 region of hippocampus, at a depth of 950 μ, by the micro-electrophoretic application of L-glutamate 80 nA.

A typical unit response to L-glutamate 20 nA and acetyl–β–methyl-choline 20 nA is shown in Text-fig. 1. It can be seen that this group of units at 0·1 mm fired almost immediately with the L-glutamate, but there was a delay of 5 sec before the acetyl-β-methylcholine excited. Whereas firing induced by L-glutamate ceased almost immediately on stopping the expelling current, that due to the acetyl-β-methylcholine continued for many seconds.

An interesting observation was that larger currents (60–100 nA) of L-glutamate applied micro-electrophoretically at any depth in the hippo-campal cortex induced a fast synchronized rhythm or seizure discharge (15–50/sec). This is illustrated in Text-fig. 2; here L-glutamate 80 nA was

applied at a depth of 950 μ. After 3·5 sec a seizure discharge commenced; with the continued application of L-glutamate the amplitude of the waves increased, and a peak frequency of 30/sec was reached. On stopping the expelling current of L-glutamate the seizure discharge gradually declined into quiescence over several seconds. This slow time course is in marked contrast to the rapid course of the excitation of unit firing by L-glutamate. Similar fast rhythms in the hippocampal e.e.g. of cats under barbiturate or urethane anaesthesia have been described by Green & Arduini (1954), Stumpf, Petsche & Gogolák (1962), Brooks (1962) and Bradley & Nicholson (1962).

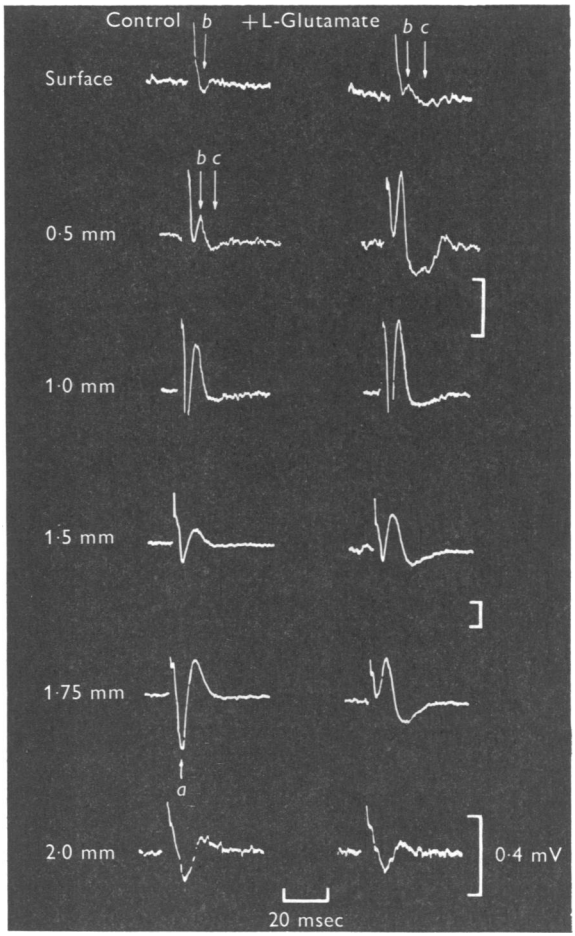
The application of large currents of L-glutamate also had an effect on the responses evoked by fimbrial stimulation. This is illustrated in Text-fig. 3. The control field responses recorded at the surface and at various depths down to 2·0 mm are shown on the left, while the responses recorded during the application of L-glutamate 80 nA are shown on the right. There was an early positive wave (*a*) with a peak latency of about 4 msec, best seen in the records at 1·0, 1·5, and 1·75 mm. This wave was most marked at 1·75 mm and was markedly reduced at this depth only by the application of L-glutamate. There followed a later wave (*b*, *c*) recorded as a positive–negative sequence at the surface. When L-glutamate was applied micro-electrophoretically to the surface this sequence was reversed, possibly because L-glutamate diffused into the superficial layer of the cortex and increased basal dendritic invasion. This surface positive–negative sequence was also reversed when the micro-electrode penetrated the cortex. It can be seen that when L-glutamate is applied into the depth of the hippocampal cortex, the most marked increase in the amplitude of the negative wave occurred at 0·5 and 1·5 mm. The control responses were unaffected either by the current control (80 nA tip negative) or by a similar current of 5-HT (not shown).

γ-Aminobutyric acid (GABA) was tested on ten units. Four of these units were firing spontaneously and in these GABA strongly depressed this spontaneous firing and also firing induced by L-glutamate. Six of the units did not fire spontaneously, and in five of these GABA depressed L-glutamate firing. In several cases even the spontaneous diffusion of GABA from the tip of the micropipette, when the retaining voltage was removed, was sufficient to cause depression. The time course of depression was rapid and comparable to that of L-glutamate excitation.

Inhibition

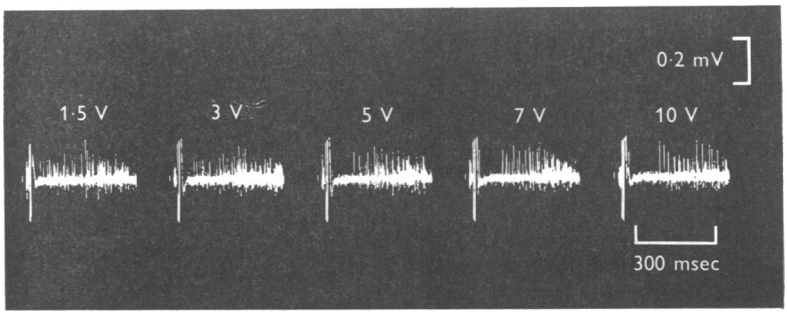
It is well known that stimulation of the fimbria induces a powerful inhibition of hippocampal pyramidal cells with large and prolonged inhibitory post-synaptic potentials (IPSPs) probably mediated via

pyramidal cell axon collaterals (Kandel & Spencer, 1961; Kandel *et al.* 1961; Andersen *et al.* 1964*a*). Crawford, Curtis, Voorhoeve & Wilson (1963) and Krnjević, Randić & Straughan (1964) have shown that post-synaptic inhibition can be conveniently demonstrated by extracellular recording on a background of glutamate induced activity. This technique was employed in these present experiments, and it confirmed that fimbrial stimulation produced a powerful inhibition of pyramidal cell firing, as



Text-fig. 3. Field responses to fimbrial stimulation in hippocampal field CA 3, at depth indicated in mm to left of each pair of responses. The left hand response of each pair is the control; the right-hand response of each pair was recorded after a current of L-glutamate 80 nA had been passed for 12–18 sec through an adjacent barrel of the micropipette. Note, the early positive wave at 1·75 mm (a↑) was abolished by L-glutamate, while the positive (*b*↓)-negative sequence at the surface was reversed to a negative–positive sequence (*b* and *c*↓) by L-glutamate.

shown in Text-fig. 4. This unit at a depth of 1500 μ was excited by the continuous application of L-glutamate. Fimbrial stimulation at 10 V produced an inhibition which lasted for 100 msec, a value which is within the range for the duration of the IPSPs (80–300 msec) given by Andersen *et al.* (1964*a*).



Text-fig. 4. Extracellular spike responses from a unit at a depth of 1500 μ in CA 3. This unit was quiescent but could be excited to fire regularly by the micro-electrophoretic application of L-glutamate from an adjacent barrel of the micropipette. As the intensity of fimbrial stimulation was increased, from left to right, an inhibition of unit firing developed which lasted up to 100 msec. Each record consists of superimposed frames from five successive stimuli. Note the generally larger size of spike immediately after the inhibitory period; also, that the spike amplitudes decline as unit firing rate rises.

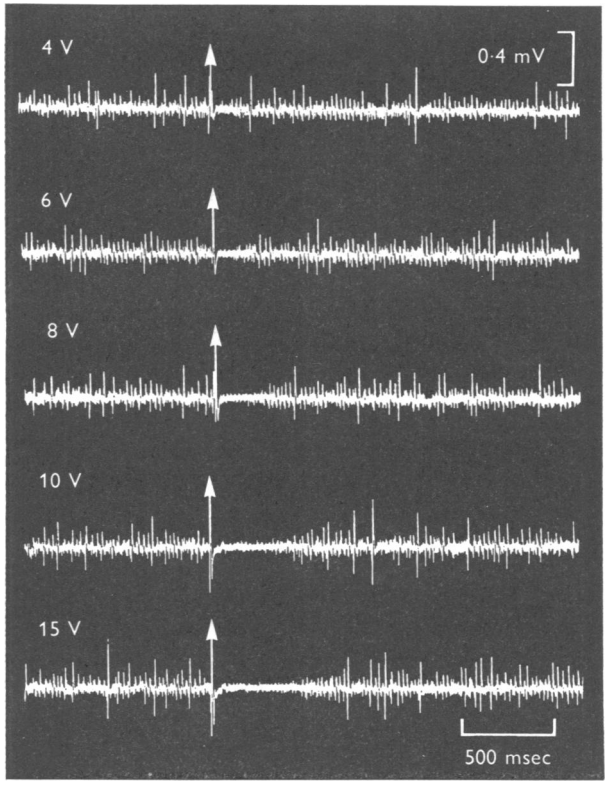
Fimbrial stimulation also inhibits the fast 'seizure discharge' produced by the sustained application of L-glutamate with large micro-electrophoretic currents. This is illustrated in Text-fig. 5. Here the discharge was induced by the application of L-glutamate 80 nA at a depth of 950 μ in CA 1. When the intensity of fimbrial stimulation was 15 V, it can be seen that the discharge was inhibited for 500 msec.

Acetylcholine (ACh)

Acetylcholine excited 49 of the 90 units on which it was tested. In 37 units ACh alone caused excitation, while potentiation of L-glutamate excitation was seen in 12 units.

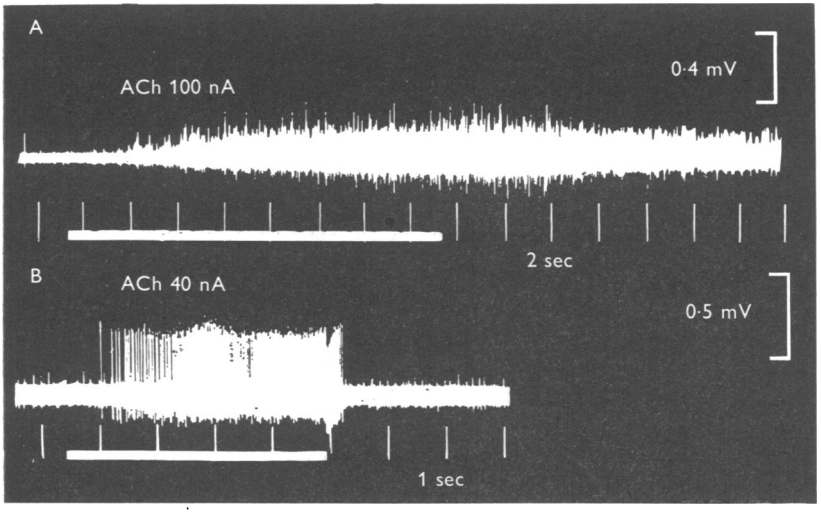
When units were directly excited there was typically a latent period of several seconds before a gradual slow increase in the frequency of firing occurred; in addition, the ACh excitation persisted for 10–15 sec after the current was turned off. This contrasted with the rapid onset and termination of excitation seen with L-glutamate, e.g. Text-fig. 8. Acetylcholine often induced spontaneous firing in previously quiescent units. The delayed time course of ACh excitation is similar to that seen in the thalamus (Andersen & Curtis, 1964*a*), cerebellum (McCance & Phillis, 1964), neocortex (Krnjević & Phillis, 1963*b*) and pyriform cortex (Randić & Straughan,

1965), and contrasts sharply with the rapid initial ACh response seen in the spinal Renshaw cell (Curtis & Eccles, 1958). This point is illustrated in Text-fig. 6, showing at A the response of a hippocampal unit at a depth of 1000 μ to ACh 100 nA and at B the response of a Renshaw cell in the 7th lumbar segment of the spinal cord to ACh 40 nA.

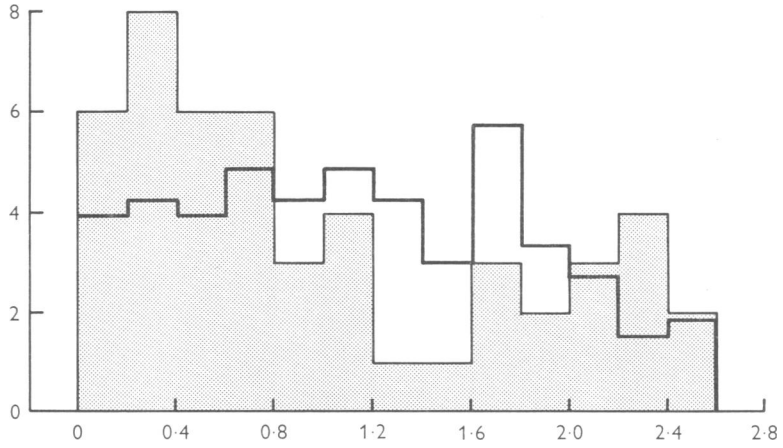


Text-fig. 5. Fast rhythmic discharge induced by the continuous micro-electrophoretic application of L-glutamate 80 nA at a depth of 950 μ in region CA 1. A single stimulus to the fimbria was applied at the arrows, and the intensity of stimulation is indicated above each record. Note that the fast rhythmic discharge is progressively inhibited by stimulation from 6 V upwards.

In Text-fig. 7 the depth distribution of the 49 cholinoceptive units is compared with the depth distribution histogram of all the units excited by L-glutamate. To make the histograms dimensionally comparable, the number of the L-glutamate-excited units at each depth was calculated as a proportion of the actual total of 161, but with the sample size reduced to 49 (the total of the cholinoceptive units). The totals were used in a χ^2 test, assuming that the distribution of units excited by L-glutamate was the



Text-fig. 6. A, the response of a unit at a depth of 1000 μ in field CA 3 of hippocampus to ACh 100 nA with B, the response of a Renshaw cell in the spinal cord to ACh 40 nA (Biscoe & Krnjević, unpublished).



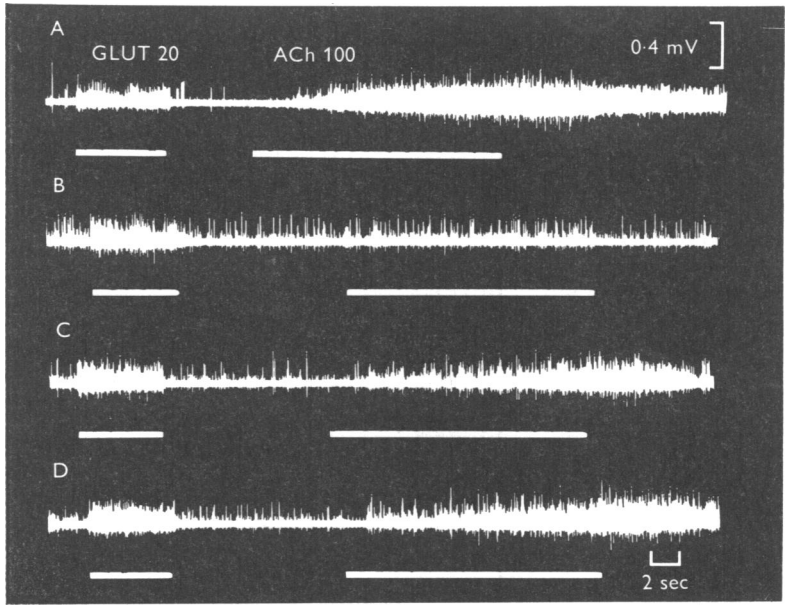
Text-fig. 7. Depth distribution histogram of all the 161 cells excited by L-glutamate (unshaded area, thick line) and of the 49 cells amongst them which were excited by ACh (shaded area, thin line). Abscissa: depth of cells from alvear surface in mm; ordinate: number of cells per 0·2 mm. To make the histograms dimensionally comparable, the proportion of cells excited by L-glutamate at each depth was replotted on a sample size of 49. Hippocampus: depth distribution histogram (excited) L-glutamate (161), ACh (49).

expected distribution. There was a significant difference between the two histograms: $\chi^2 = 12{\cdot}831$, $\varnothing = 5$, $0{\cdot}05 > P > 0{\cdot}025$.

Acetyl-β-methylcholine (*MeCh*) excited six out of fifteen units tested. The time course of this response was similar to that of ACh (see Text-fig. 1). Several units excited by carbamoylcholine or nicotine were also noted.

Cholinolytic drugs

Atropine was tested on eight cholinoceptive units and with all of these the ACh response was selectively blocked while the units still responded to L-glutamate. Text-figure 8A illustrates the response of a group of spontaneously active units to L-glutamate and to ACh. After this first application of ACh the spontaneous firing was enhanced (see B). Between A and B atropine was applied for 3·0 min without blocking ACh excitation; when L-glutamate was now superimposed (B), it still produced its characteristic excitation, thus excluding any non-specific depressant effects of



Text-fig. 8. Extracellular spike responses from a group of units at a depth of 1000 μ in region CA2–3. In each trace A to D: left, excitatory response to L-glutamate 20 nA applied for 5 sec (short bar below record); right, excitatory response to ACh 100 nA applied for 15 sec (long bar). Between A and B, atropine 40 nA applied for 3 min did not block ACh excitation; at B, after 3·5 min atropine, L-glutamate-induced firing still unaffected but excitation by ACh now completely blocked. The spontaneous firing, which had been triggered by the first application of ACh, was unaffected by atropine. C, 3 min after stopping the atropine current; recovery of excitation by ACh nearly complete. D, 4 min after stopping the atropine.

atropine; but the excitatory response to ACh was now almost completely depressed, so that only the spontaneous activity remained. Sequences C and D show progressive recovery of the ACh excitation; in D, when the atropine had been off for 4 min the ACh excitation had recovered to control levels, though it was rather slower in development than in the first control (A).

This selective depression of ACh excitation by atropine is shown in another unit in Text-fig. 9. Here after atropine had been applied for 2·5 min the excitatory response to ACh was almost completely abolished, though glutamate-excitation was unaffected. After the atropine current was turned off nearly full recovery occurred in about 7 min, though the onset of firing was slow and did not outlast the ACh application as markedly as in the control period.

Dihydro-β-erythroidine (*DHβE*) did not depress the cholinoceptive response in any of eight units tested, and in five of these DHβE caused some direct excitation as in the neocortex (Krnjević & Phillis, 1963*c*) and the thalamus (Andersen & Curtis, 1964*b*), and in the pyriform cortex (Legge, Randić & Straughan, 1965).

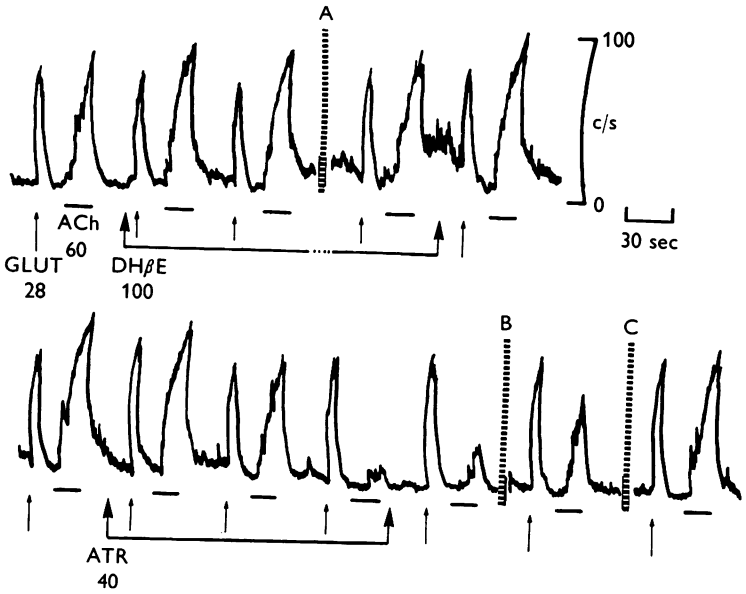
This effect of DHβE is shown in Text-fig. 9. Here, DHβE increased the rate of spontaneous firing, reflected by the rising base line in the rate-meter record. This effect was marked after 4 min, though both ACh- and L-glutamate-excitation were virtually unaltered in terms of peak frequency. This failure of DHβE to block the excitant effect of ACh is in marked contrast to the blocking action of atropine in the same unit mentioned previously.

Dimethyl (+)-tubocurarine (DMTC) was tested on six cholinoceptive units, and in two of these the excitant action of both ACh and MeCh was selectively blocked. In addition the micro-electrophoretic application of DMTC into their environment caused 'direct' excitation in four units and was similar to that noted in the neocortex for (+)-tubocurarine and DMTC by Krnjević & Phillis (1963*c*), and in the thalamus by Andersen & Curtis (1964*b*).

The effect of some biogenic monoamines

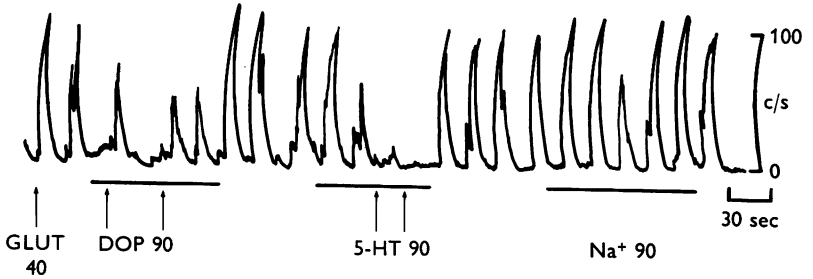
5-*Hydroxytryptamine* (5-HT) was tested on 66 groups of units in eight cats and was found to depress the firing rate in 29 units. Five of these latter units were firing spontaneously; 5-HT depressed this firing in a similar manner to the glutamate-induced firing in these units. The onset of depression was usually delayed for 10–15 sec as in Text-fig. 10, though in a few units depression occurred within a few sec.

In nine other units recorded from three cats, 5-HT caused excitation only, and in some cases this progressed to a seizure discharge similar to



Text-fig. 9. Rate-meter record showing the effects of dihydro-β-erythroidine (DHβE) and atropine on the activity of a group of units at a depth of 144 μ in region CA 2–3. L-Glutamate 28 nA applied for 5 sec periods throughout (indicated by ↑). ACh 60 nA applied for 10 sec periods throughout (short bars below record). In the upper trace DHβE 100 nA applied for 5 min (indicated by ▲ and long bar below record); note that there is a 2 min gap in this record at A. ACh-excitation was not affected by the DHβE, but there was some direct excitation and increased spontaneous activity as shown by the rising base line of the record.

In the lower trace atropine 40 nA was applied for 3 min only (▲ and long bar below record); this did not affect excitation by L-glutamate, though the excitation by ACh was almost completely blocked. Recovery of ACh-excitation began within 1 min and reached 60 % of its control level within 3 min (after gap B in the record). Complete recovery of ACh-excitation occurred after 7·5 min (after gap C). Scale at right of upper trace shows the number of spikes counted/sec by the rate-meter.

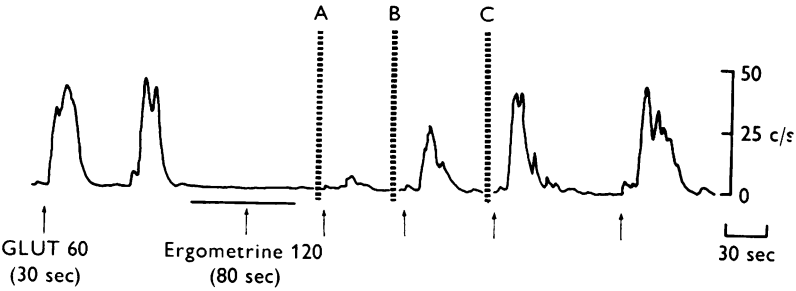


Text-fig. 10. Rate-meter record showing depression of glutamate-induced responses by dopamine and by 5-HT in a unit at a depth of 1750 μ in CA 3. L-Glutamate 40 nA applied for 5 sec in every 15 sec (at ↑). Background current of dopamine 90 nA applied (at left and indicated by bar beneath record). 5-HT 90 nA (centre) caused complete depression of cell firing, while control Na^+ 90 nA (right) was without definite effect. Scale shows number of spikes/sec counted by the rate-meter.

that seen with L-glutamate. In several of these units, only very small currents (10–15 nA) were required to give excitation, though in the remainder the currents required to produce excitation (40–60 nA) were similar to those needed to produce depression.

3-*Hydroxytyramine* (*dopamine*) depressed two out of five units tested. Text-fig. 10 shows the effect of dopamine, 5-HT and a current control on the responses evoked by L-glutamate. Occasional bursts of spontaneous activity were seen with this unit. Dopamine depressed the L-glutamate response strongly, and 5-HT produced an even more profound depression of L-glutamate firing, though the recovery was rapid. The current control was without marked effect.

Thus dopamine depression had a similar time course to that produced by 5-HT, but less pronounced.



Text-fig. 11. Rate-meter record showing the long lasting depression of L-glutamate-induced firing by ergometrine in a group of units at a depth of 1000 μ in region CA 1. L-Glutamate 60 nA applied for 30 sec in every 60 sec (indicated by ↑) and ergometrine 120 nA applied for 80 sec from an adjacent barrel of the micropipette (horizontal bar). The first gap, A, in the record was of 3·5 min duration, while the second and third gaps, B and C, were each of 3 min duration. Full recovery of the control excitation by L-glutamate took 9·5 min. The scale shows the number of spikes counted/sec by the rate-meter.

Noradrenaline was tested on eleven cells; only one showed depression of L-glutamate excitation, and in ten there was no effect. This finding is in keeping with the low order of potency of noradrenaline-induced depression of hippocampal cells briefly reported by Stefanis (1964) and Herz & Nacimiento (1965).

Ergometrine was tested on five units excited by L-glutamate and a strong depression was seen in all; one of these is shown in Text-fig. 11. Here ergometrine completely depressed L-glutamate-induced firing in a group of units within 45 sec; 3·5 min after the ergometrine current was stopped (after A) there was some evidence of recovery, which progressed so that 3 min later (after B) the response had recovered to approximately 50 % of the control peak frequency. After 9·5 min (after C) recovery was virtually

complete. This long-lasting depression contrasts with the much shorter depression produced by 5-HT and is comparable with the long-lasting depression produced by various lysergic acid derivatives in the neocortex (Krnjević & Phillis, 1963*d*) and pyriform cortex (Legge *et al.* 1965).

DISCUSSION

Most of the experiments described in the literature on the effects of various potential neurotransmitters on the hippocampus have involved the administration of drugs either systemically or intrathecally. It is thus difficult to determine whether the observed effects on hippocampal rhythms or single unit activity are due to a direct effect of the drug on the hippocampus. In particular many of the reported effects of adrenergic and cholinergic drugs on hippocampal θ-rhythms appear to be indirect (Stumpf, 1965).

The method of micro-application of cholinergic drugs directly into the hippocampus described by MacLean (1957) has the disadvantage of not allowing the controlled release of graded amounts of drugs for predetermined periods of time. Further, the cause of the observed changes in hippocampal electrical activity could be either cell excitation or depression, but this is not immediately apparent when recording with non-micro-electrodes. For these reasons the micro-electrophoretic method was used to study the effect of various potential neurotransmitters on spontaneous or L-glutamate-induced firing in the hippocampus. Brief reports of other micro-electrophoretic studies in this region have appeared recently (Stefanis, 1964; Herz & Nacimiento, 1965).

At all depths in the hippocampal cortex, cells were excited by L-glutamate and depressed by GABA, and these effects had a rapid time course. This agrees with other reports on the activity of these drugs in the hippocampus by Andersen, Crawford & Curtis (cited by Crawford & Curtis, 1964), Stefanis (1964) and Herz & Nacimiento (1965), and elsewhere in the brain: cerebral cortex (Krnjević & Phillis, 1963*a*) pyriform cortex (Legge *et al.* 1965) and ventrobasal thalamus (Andersen & Curtis, 1964*a*). The use of L-glutamate-induced excitation to identify cells, and as a basis for testing the effects of drugs, seems justified for two main reasons: (1) as far as can be judged L-glutamate excites all nerve cell bodies in the cortex and not axons, and repeated applications of L-glutamate cause excitation without desensitization or apparent damage. It is also possible that L-glutamate could be an excitatory transmitter (Krnjević & Phillis, 1963*a*). (2) Cells activated synaptically or with L-glutamate respond similarly to procedures affecting the post-synaptic membrane. Also L-glutamate usefully distinguishes between pre- and post-synaptic cell depression.

Over half the hippocampal units tested were excited by ACh applied into their environment by micro-electrophoresis. The time course of this effect was similar to that seen in the cerebral and pyriform cortex (Krnjević & Phillis, 1963*b*; Legge *et al.* 1965). However, Stefanis (1964) reported that the time course of ACh effects in the hippocampus was different from that seen in the cortex, while Herz & Nacimiento (1965) found that the majority of cells tested were unaffected by ACh, MeCh or carbamoylcholine. These discrepancies cannot at present be explained in the absence of further experimental details.

The distribution of the ACh-sensitive cells in these present experiments shown in Text-fig. 7 is different from that predicted from the excitation seen with L-glutamate. The cholinoceptive cells are concentrated in two main groups, one superficial and the other deep. This distribution correlates quite well with the distribution of acetylcholinesterase in cat brain, shown in Pl. 1 A and B, though there are differences between this and the enzyme distribution in the rat hippocampus (Shute & Lewis, 1961, 1963; Mathisen & Blackstad, 1964). There are large amounts of choline-acetylase in the hippocampus (Hebb & Silver, 1956); and in the rat hippocampus; there is a close relation between choline-acetylase and acetylcholinesterase distribution (Lewis, Shute & Silver, 1964). Thus it is possible that ACh is a transmitter substance in this region. But this can be no more than a suggestion at present, since more precise identification of these cholinergic units is needed. Also cholinergic and cholinolytic drugs must be shown to bring about selective changes in the synaptic activation of these ACh-sensitive units. In this connexion it is interesting to note that eserine facilitated the response of single hippocampal units to septal stimulation (Brücke *et al.* 1963). This could be due to a direct action of eserine in the hippocampus, although indirect increases in hippocampal excitability are by no means excluded.

Atropine selectively blocked ACh or MeCh excitation in all the hippocampal units in which it was tested, while DHβE was without effect. This would suggest that the receptors on hippocampal cholinoceptive cells are predominantly muscarinic as in the neocortex (Krnjević & Phillis, 1963*b*, *c*). This differs from the palaeocortex where DHβE caused partial or complete block in half the units in which it was tested (Legge *et al.* 1965). However, two hippocampal units were seen in which DMTC blocked ACh and MeCh excitation, suggesting that the receptors of some hippocampal cells at least are not exclusively muscarinic. In this connexion MacLean's (1957) observations are of interest. He noted that the action of MeCh applied directly into the cat hippocampus differed from that of ACh (with eserine) and carbamoylcholine, in that MeCh induced a slow θ-rhythm with superimposed fast activity but without the high voltage spikes seen with

carbamoylcholine. He attributed this to a lack of 'nicotinic' properties in MeCh compared with carbamoylcholine. It is not clear whether there is a common ACh receptor with mixed sensitivity, or whether the same cell shares different receptors as do Renshaw cells (Curtis & Ryall, 1964). This problem of the division of ACh responses into muscarinic and nicotinic has been discussed in some detail by Andersen & Curtis (1964*b*) and by Curtis, Ryall & Watkins (1965).

It has been known for many years that curare has excitatory effects in the C.N.S. e.g. Tillie (1890). In more recent years Chang (1953), Rech & Domino (1960) and Morlock & Ward (1961) have reported excitatory responses in the cortex to curare applied topically and intracisternally. Curare also causes excitation in the hippocampus (Feldberg & Fleischhauer, 1963). It is difficult to see how in either of these areas curare could cause excitation by blocking a cholinergic excitatory system. Further, curare applied micro-electrophoretically excites both cholinoceptive and non-cholinoceptive cells in the hippocampal cortex, as in the neo-cortex (Krnjević & Phillis, 1963*a*). Thus, it would seem that this curare excitation of cortical tissues, though apparently a direct one, is not 'specific' in terms of ACh receptor theory.

The relatively high concentration of 5-HT in the hippocampal cortex (Paasonen, MacLean & Giarman, 1957), and its probable localization within the nerve terminals (Dahlström & Fuxe, 1964) makes it a potential neurotransmitter substance. Udenfriend, Weissbach & Bogdanski (1957) showed that 5-hydroxytryptophan (5-HTP) readily passed the blood–brain barrier and was then decarboxylated to 5-HT. The effect of 5-HT in the hippocampus following the administration of 5-HTP is by no means clear: intra-carotid injections yield a late arousal reaction and θ-rhythm (Costa, Pscheidt, van Meter & Himwich, 1960), while intravenous injections caused a disappearance of the θ-rhythm and spike discharges (Costa & Rinaldi, 1958; Monnier & Tissot, 1958). In general the effects of 5-HT (and also dopamine) applied micro-electrophoretically were depressant as in the neocortex (Krnjević & Phillis, 1963*a*, *d*). However, in several cells relatively small concentrations of 5-HT caused excitation. This is of interest, since when 5-HT excitation was seen in the neocortex (Krnjević & Phillis, 1963*a*, *d*) it required much larger currents of the order of 100 nA and was not considered to be a specific effect. In the hypothalamus Bloom, Oliver & Salmoiraghi (1963) observed 5-HT excitation in two units, but gave no account of the magnitude of the ejecting current. Both Stefanis (1964) and Herz & Nacimiento (1965) described depressant effects with a slow time course with 5 HT applied micro-electrophoretically in the hippocampus; they did not describe excitatory effects, but no details regarding the anaesthetic were given. It could be that in the hippocampus the normal

effect of 5-HT is predominantly one of excitation if animals are unanaesthetized, for Marley & Vane (1963) have shown that barbiturates cause tryptamine insensitivity in spinal cord neurones. It is interesting to note that a considerable number of 5-HT excited cells have been seen only in the brain stem of the decerebrate cat when the effects of anaesthesia could be excluded (Bradley & Wolstencroft, 1965). The possibility that anaesthetics may modify the effects of monoamines applied by microelectrophoresis merits closer investigation.

The authors are indebted to Dr D. R. Curtis and Dr K. Krnjević for reading the manuscript and for their valuable criticism. The skilled technical assistance of Mr K. F. Legge and Mr J. O. Yates is gratefully acknowledged. Merck, Sharpe & Dohme Ltd. kindly supplied us with dihydro-β-erythroidine hydrobromide.

REFERENCES

ANDERSEN, P. (1959). Interhippocampal impulses. I. Origin, course and distribution in cat, rabbit and rat. *Acta physiol. scand.* **47**, 63–90.

ANDERSEN, P. (1960). Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. *Acta physiol. scand.* **48**, 178–208.

ANDERSEN, P. & CURTIS, D. R. (1964*a*). The excitation of thalamic neurones by acetylcholine. *Acta physiol. scand.* **61**, 85–99.

ANDERSEN, P. & CURTIS, D. R. (1964*b*). The pharmacology of the synaptic and acetylcholine induced excitation of ventrobasal thalamic neurones. *Acta physiol. scand.* **61**, 100–120.

ANDERSEN, P., ECCLES, J. C. & LØYNING, Y. (1964*a*). Location of postsynaptic inhibitory synapses on hippocampal pyramids. *J. Neurophysiol.* **27**, 592–607.

ANDERSEN, P., ECCLES, J. C. & LØYNING, Y. (1964*b*). Pathway of postsynaptic inhibition in the hippocampus. *J. Neurophysiol.* **27**, 608–619.

BISCOE, T. J. & STRAUGHAN, D. W. (1965). The pharmacology of hippocampal neurones. *J. Pharm. Pharmac.* **17**, 60–61.

BLOOM, F. E., OLIVER, A. P. & SALMOIRAGHI, G. C. (1963). The responsiveness of individual hypothalamic neurons to microelectrophoretically administered endogenous amines. *Int. J. Neuropharmac.* **2**, 181–193.

BRADLEY, P. B. & NICHOLSON, A. N. (1962). The effects of some drugs on hippocampal arousal. *Electroen. Neurophysiol.* **14**, 824–834.

BRADLEY, P. B. & WOLSTENCROFT, J. H. (1965). Actions of drugs on single neurones in the brain-stem. *Br. med. Bull.* **21**, 15–18.

BROOKS, D. C. (1962). Rhinencephalic spikes and beta waves in the cat under pentobarbital. *Am. J. Physiol.* **202**, 1221–1229.

BRÜCKE, F., GOGOLÁK, G. & STUMPF, C. (1963). Mikroelektrodenuntersuchung der Reizantwort und der Zelltätigkeit im Hippocampus bei Septumreizung. *Pflügers Arch. ges. Physiol.* **276**, 456–470.

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

COSTA, E., PSCHEIDT, G. R., VAN METER, W. G. & HIMWICH, H. E. (1960). Brain concentrations of biogenic amines and e.e.g. patterns of rabbits. *J. pharmac. exp. Ther.* **130**, 81–88.

COSTA, E. & RINALDI, F. (1958). Biochemical and electroencephalographic changes in the brain of rabbits injected with 5-hydroxytryptophan (influence of chlorpromazine premedication). *Am. J. Physiol.* **194**, 214–220.

CRAWFORD, J. M. & CURTIS, D. R. (1964). The excitation and depression of mammalian cortical neurones by amino acids. *Br. J. Pharmac. Chemother.* **23**, 313–29.

CRAWFORD, J. M., CURTIS, D. R., VOORHOEVE, P. E. & WILSON, V. J. (1963). Strychnine-resistant central inhibition: strychnine and cortical inhibition. *Nature, Lond.*, **200**, 845–846.

CURTIS, D. R. & ECCLES, R. M. (1958). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol.* **141**, 435–445.

CURTIS, D. R. & RYALL, R. W. (1964). Nicotinic and muscarinic receptors of Renshaw cells, *Nature, Lond.*, **203**, 652–653.

CURTIS, D. R., RYALL, R. W. & WATKINS, J. C. (1965). Cholinergic transmission in the mammalian central nervous system. In *Pharmacology of Cholinergic and Adrenergic Transmission*, ed. KOELLE, G. B., DOUGLAS, W. W. & CARLSSON, A., vol. 3, pp. 137–143. Oxford: Pergamon.

DAHLSTRÖM, A. & FUXE, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta. physiol. scand.* **62**, suppl. 232.

FELDBERG, W. & FLEISCHHAUER, K. (1963). Site of action of tubocurarine reaching the brain via the cerebral ventricles. In *Progress in Brain Research*, vol. 3. *The rhinencephalon and related structures*, ed. BARGMANN, W. & SCHADÉ, J. P., pp. 1–19. Amsterdam: Elsevier.

FELDBERG, W. & MALCOLM, J. L. (1959). Experiments on the site of action of tubocurarine when applied via the cerebral ventricles. *J. Physiol.* **149**, 58–77.

GREEN, J. D. (1964). The hippocampus. *Physiol. Rev.* **44**, 561–608.

GREEN, J. D. & ARDUINI, A. (1954). Hippocampal electrical activity in arousal. *J. Neurophysiol.* **17**, 533–557.

HEBB, C. O. & SILVER, A. (1956). Choline acetylase in the central nervous system of man and some other mammals. *J. Physiol.* **134**, 718–728.

HERZ, A. & NACIMIENTO, A. (1965). Über die Wirkung von Pharmaka auf Neurone des Hippocampus nach mikroelektrophoretischer Verabfolgung. *Arch. exp. Path. Pharmak.* **250**, 258–259.

KANDEL, E. R. & SPENCER, W. A. (1961). Electrophysiology of hippocampal neurons. II. After-potentials and repetitive firing. *J. Neurophysiol.* **24**, 243–259.

KANDEL, E. R., SPENCER, W. A. & BRINLEY, F. J. JNR. (1961). Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. *J. Neurophysiol.* **24**, 225–242.

KRNJEVIĆ, K. & MITCHELL, J. F. (1961). A simple and reliable device utilizing transistors for the maintenance of a constant body temperature. *J. Physiol.* **158**, 6–8*P*.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*a*). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.* **165**, 274–304.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*b*). Acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol.* **166**, 296–327.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*c*). Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol.* **166**, 328–350.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*d*). Actions of certain amines on cerebral cortical neurones. *Br. J. Pharmac. Chemother.* **20**, 471–490.

KRNJEVIĆ, K., RANDIĆ, M. & STRAUGHAN, D. W. (1964). Cortical inhibition. *Nature, Lond.*, **201**, 1294–1296.

KRNJEVIĆ, K. & SILVER, A. (1965). A histochemical study of cholinergic fibres in the cerebral cortex. *J. Anat.* **99**, 711–759.

LEGGE, K. F., RANDIĆ, M. & STRAUGHAN, D. W. (1965). The pharmacology of neurones in the pyriform cortex. *Br. J. Pharmac. Chemother.* (In the Press.)

LEWIS, P. R., SHUTE, C. C. D. & SILVER, A. (1964). Confirmation from choline acetylase analysis of a massive cholinergic innervation to the hippocampus. *J. Physiol.* **172**, 9–10*P*.

LORENTE DE NÓ, R. (1934). Studies on the structure of the cerebral cortex II. Continuation of the study of the ammonic system. *J. Psychol. Neurol., Lpz.*, **46**, 113–177.

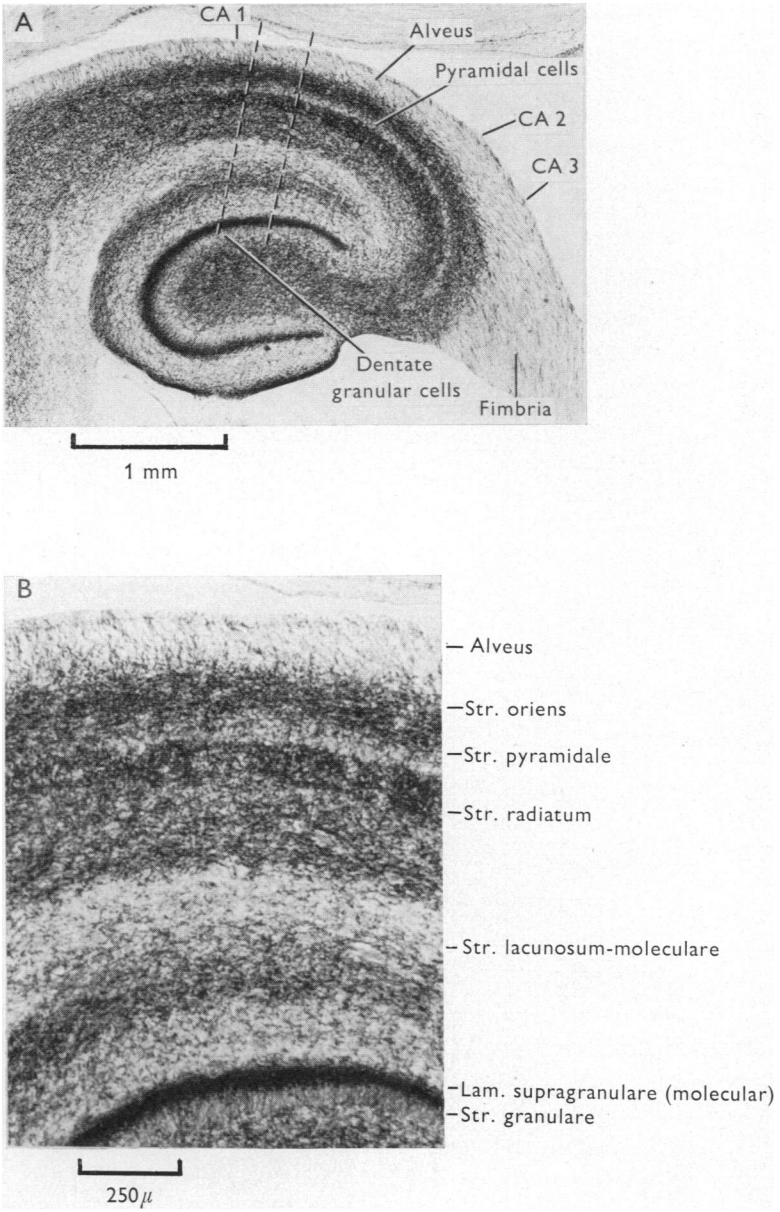
MACLEAN, P. D. (1957). Chemical and electrical stimulation of hippocampus in unrestrained animals. *Archs. Neurol. Psychiat., Chicago*, **78**, 113–127.

MCCANCE, I. & PHILLIS, J. W. (1964). The action of acetylcholine on cells in cat cerebellar cortex. *Experientia*, **20**, 217–218.

MARLEY, E. & VANE, J. R. (1963). Tryptamine receptors in the central nervous system: effects of anaesthetics. *Nature, Lond.*, **198**, 441–444.

MATHISEN, J. S. & BLACKSTAD, T. W. (1964). Cholinesterase in the hippocampal region. Distribution and relation to architectonics and afferent systems. *Acta anat.* **56**, 216–253.

MONNIER, M. & TISSOT, R. (1958). Action de la réserpine et de ses médiateurs (5-hydroxytryptophan-sérotonine et dopa-noradrenaline) sur le comportement et le cerveau du lapin. *Helv. physiol. pharmac. Acta*, **16**, 255–267.



T. J. BISCOE AND D. W. STRAUGHAN

(*Facing p.* 359)

MORLOCK, N. & WARD, A. A. (1961). The effects of curare on cortical activity. *Electroen. Neurophysiol.* **13**, 60–67.

PAASONEN, M. K., MACLEAN, P. D. & GIARMAN, N. J. (1957). 5-Hydroxytryptamine (serotonin, enteramine) content of structures of the limbic system. *J. Neurochem.* **1**, 326–333.

POKOVSKY, M. V. (1960). Appareil à transistors destiné à rendre la luminance du tracé sur l'écran d'un oscilloscope indépendante de la vitesse du déplacement vertical. In *Medical Electronics*, ed. SMYTH, C. N., pp. 85–86. London: Iliffe and Sons.

RANDIĆ, M. & STRAUGHAN, D. W. (1965). Iontophoretic study of palaeocortical neurones. *J. Physiol.* **177**, 67–68*P*.

RECH, R. H. & DOMINO, E. F. (1960). Effects of various drugs on activity of the neuronally isolated cerebral cortex. *Expl Neurol.* **2**, 364–378.

SHUTE, C. C. D. & LEWIS, P. R. (1961). The use of cholinesterase techniques combined with operative procedures to follow nervous pathways in the brain. *Biblthca Anat.* **2**, 34–49.

SHUTE, C. C. D. & LEWIS, P. R. (1963). Cholinesterase-containing systems of the brain of the rat. *Nature, Lond.*, **199**, 1160–1164.

STEFANIS, C. (1964). Hippocampal neurons: their responsiveness to microelectrophoretically administered endogenous amines. *Pharmacologist.* **6**, 171.

STUMPF, C. (1965). Drug action on the electrical activity of the hippocampus. *Int. Rev. Neurobiol.* **8**, 77–138.

STUMPF, C. PETSCHE, H. & GOGOLÁK, G. (1962). The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. *Electroen. Neurophysiol.* **14**, 212–219.

TILLIE, J. A. (1890). A contribution to the pharmacology of curare and its alkaloids. *J. Anat.* **24**, 379–406.

UDENFRIEND, S., WEISSBACH, H. & BOGDANSKI, D. F. (1957). Biochemical findings relating to the action of serotonin. *Ann. N.Y. Acad. Sci.* **66**, 602–608.

WINSBURY, G. J. (1956). Machine for the production of micro-electrodes. *Rev. scient. Instrum.* **27**, 514–516.

EXPLANATION OF PLATE

Transverse section of the cat hippocampus stained for acetylcholinesterase by the method of Krnjević & Silver (1965). A: Low power view. Area between dotted lines shown in greater detail in B. B: High power view from the alveus to the dentate granular layer (section stained and lent to us by Dr K. Krnjević and Dr Ann Silver). Scale for A 1 mm and for B 250 μ.