Brit. J. Pharmacol. (1966), **26**, 87–107.

THE PHARMACOLOGY OF NEURONES IN THE PYRIFORM CORTEX

BY

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(*Received April* 26, 1965)

The response of neocortical cells to various drugs applied into their environment by microelectrophoresis has been widely studied in recent years (Krnjević & Phillis, 1963a; Crawford & Curtis, 1964). Here an attempt has been made to study the chemical sensitivity of individual palaeocortical cells to acetylcholine, 5-hydroxytryptamine and the catechol amines, noradrenaline and dopamine (3,4-dihydroxyphenylethylamine). There is some evidence that acetylcholine could be a neurotransmitter in the pyriform cortex though it is, of course, incomplete. Choline acetylase (Feldberg & Vogt, 1948; Hebb & Silver, 1956) and cholinesterase (Burgen & Chipman, 1951) are known to be present in this region. Further, MacLean & Delgado (1953) have shown that when acetylcholine is applied locally to the pyriform area of unanaesthetized cats it produces changes in affective behaviour, similar in type to those produced by local electrical stimulation.

The pyriform cortex contains relatively large amounts of 5-hydroxytryptamine and moderate amounts of noradrenaline compared with the neocortex, as well as some of the enzyme systems necessary for their synthesis and destruction (Amin, Crawford & Gaddum, 1954; Vogt, 1954; Bogdanski, Weissbach & Udenfriend, 1957; Paasonen, MacLean & Giarman, 1957; Kuntzmann, Shore, Bogdanski & Brodie, 1961). Such evidence provides some grounds for the postulate that these substances are central neurotransmitters. This idea is strengthened by recent histochemical investigations which have shown a general correlation between the content of 5-hydroxytryptamine and noradrenaline and their presence in fine nerve terminals of the hypothalamus, brain stem and spinal cord (Carlsson, Falck, Hillarp & Torp, 1962; Dahlström & Fuxe, 1964; Carlsson, Falck, Fuxe & Hillarp, 1964).

In this paper it is shown that acetylcholine excites single cells in the pyriform cortex and that these cholinoceptive cells have a fairly uniform distribution in depth. Their sensitivity to various cholinomimetic and cholinolytic substances has been determined. In contrast, 5-hydroxytryptamine, noradrenaline and dopamine have fairly strong depressant actions on the firing of single cells. A preliminary report of some of these findings has been given (Randić & Straughan, 1965).

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METHODS

Twenty adult cats of 1.6 to 2.6 kg weight were used. They were mostly anaesthetized with Dial Compound (Ciba), containing diallylbarbituric acid (0.1 g/ml.) and urethane (0.4 g/ml.), injected intraperitoneally (0.7 ml./kg). In a few experiments a mixture of chloralose (50 mg/kg; Hopkin & Williams) and urethane (500 mg/kg; Hopkin & Williams) was given intravenously. The temperature of the preparation was maintained at 37 $\pm 1°$ C by means of an automatically controlled heating pad under its abdomen (Krnjević & Mitchell, 1961).

The procedure for exposing the pyriform cortex was based on that described by Fox, McKinley & Magoun (1944). The zygoma, mandibular ramus and coronoid process with associated muscles, and the contents of the left orbit were removed. The anterolateral area of the pyriform cortex (corresponding to the stereotaxic co-ordinates F +9 to F +16 (Jasper & Ajmone-Marsan, 1960), was exposed by chipping away the overlying portions of the petrous temporal and the sphenoid bone. The exposure of the more medial parts of the pyriform cortex was improved by removal of the lateral portion of the hard palate and by retraction of the mandible.

After opening the dura mater the cortex was covered with 3% agar (Difco Laboratories) in Ringer-Locke solution at 38° C. This procedure prevented drying of the cortex and substantially reduced pulsations so that units could be held for longer periods (sometimes up to 15 min).

When afferent volleys were required, the ipselateral olfactory bulb was exposed in the floor of the frontal sinus and paired stainless steel stimulating electrodes were inserted. The lateral olfactory tract was also exposed and stimulated through paired platinum electrodes placed on its surface.

The construction and filling of the five-barrelled glass micropipettes used have been described by Krnjević & Phillis (1963a). The technique and theory of iontophoresis in its present application has been elaborated in detail in a recent review by Curtis (1964). Routinely, one of the outer barrels of the micropipette contained 1 M-sodium L-glutamate (*p*H 8.5), from which the active anion could be expelled by passing inward currents (tip negative). It was necessary to fill another outer barrel with 2.7 M-sodium chloride as a control for the usual exciting effect of inward (tip negative) current or the depressing effect of outward (tip positive) current (Curtis & Koizumi, 1961; Krnjević & Phillis, 1963a). The remaining outer barrels were filled with aqueous solutions of the various salt complexes to be tested. These solutions were kept at an acid *p*H (4.5 to 5.5) to improve their stability. The lysergic acid derivatives, the catechol amines and the adrenergic blocking agents were prepared just before use, and the electrode tips were filled by centrifugation. The other drugs were placed in their respective barrels 48 hr before each experiment, and the tips were allowed to fill by diffusion. After filling, all micropipettes were kept in the dark at 4° C.

Extracellular spike potentials from single units were recorded through the central barrel of the micropipette. This barrel was filled with 2.7 M-sodium chloride (a.c. resistance 4.5 to 9 mΩ) and was connected to the grid of a cathode-follower valve by a short chlorided-silver wire. After suitable amplification the spike responses were displayed on an oscilloscope face with Z-axis modulation (Pokrovsky, 1960) to improve their intensity. When required, photographic records were taken with the beam stationary and the film moving at 5 mm/sec. In all experiments the amplified spike responses were fed into a ratemeter (Ekco N 522 C) with a variable discriminator, allowing the selection of large spikes from small spikes and noise. The constant voltage output pulses from the discriminator were monitored on the second beam of the oscilloscope, and were integrated electrically with a time constant of 0.2 or 1 sec. To provide a continuous record of cell firing the output of the ratemeter was led through a d.c. preamplifier and displayed on a pen writer with a curvilinear write-out.

The drugs used, with their usual concentrations, are arranged in their order of use, and were as follows: sodium L-glutamate, 1.0 M (B.D.H.); γ-aminobutyric acid, 0.2 and 1.0 M (Cal Biochem.); acetylcholine chloride, 1.0 M (B.D.H.); acetyl-β-methylcholine chloride, 1.0 M (Koch-Light Laboratories); carbachol chloride, 1.0 M (B.D.H.); (±)-muscarine iodide, 0.4 M (Geigy*); nicotine hydrogen tartrate, 0.2 M (B.D.H.) atropine sulphate, 0.2 M (B.D.H.); dihydro-β-erythroidine hydrobromide, 0.3 M (Merck*); 5-hydroxytryptamine creatinine sulphate, 0.05 M (May & Baker); 5-hydroxytryptamine bimaleinate, 0.2 M (Koch-Light); lysergide (D-lysergic acid diethylamide hydrogen tartrate), 0.07 M; methysergide hydrogen maleate (UML 491), 0.03 M; 2-bromo-D-lysergic acid diethylamide hydrogen tartrate (BOL 148), 0.05 M (Sandoz*); 3,4-

dihydroxyphenylethylamine (dopamine), 0.5 M; (—)-noradrenaline bitartrate, 0.5 M; dibenamine hydrochloride, 0.1 M (Koch-Light); phentolamine methane sulphonate, 0.1 M (Ciba*); and dichloroisoprenaline hydrochloride, 0.25 M (Lilly). The firms indicated with an asterisk supplied their drugs as free gifts, and their generosity is gratefully acknowledged.

RESULTS

General features

Units were chosen for study either because they were discharging spontaneously (about 10% of those studied) or because they could be excited by L-glutamate ejected into their vicinity by iontophoresis. Even with our relatively wide-tipped microelectrodes (external diameter 5 to 10 μ) we have regularly recorded extracellular spikes of the order of 300 to 600 μV in amplitude. It was usually possible to select for recording either single units (Figs. 1 and 14) or contrasting large and small units (Figs. 9 and 11).

Amino acid sensitivity

L-Glutamate, expelled as an anion with currents ranging from 4 to 50 nA, proved to be a potent excitant of all the pyriform cortical cells tested, as shown in Figs. 1, 9, 11 and 14. Characteristically the latency of onset was short (about 200 msec) and firing stopped within 400 msec of the end of the L-glutamate application. γ-Aminobutyric acid (5 to 20 nA) was a very potent inhibitor of all unit firing, whether it was spontaneous or had been evoked by L-glutamate. The time course of this inhibitory action was rapid (in both onset and recovery) and comparable with the excitatory action of L-glutamate. Thus, the actions of L-glutamate and γ-aminobutyric acid did not differ from those described for neocortical cells by Krnjević & Phillis (1963a) or from those described elsewhere in the brain and spinal cord.

Excitation by acetylcholine

Acetylcholine was expelled from the micropipette as a cation (tip of the pipette positive) with currents ranging from 40 to 150 nA, for periods up to 2 min. Of the 286 units tested with this substance in fourteen cats, 77 units (26.9%) were excited. The proportion of cholinoceptive units found in a single cat, however, varied greatly between animals. In one animal nine out of the eleven units tested were excited by acetylcholine while in another only one out of thirteen units was excited. In some cells acetylcholine produced sufficient depolarization to fire directly (as Fig. 1), while in other cells the depolarization was insufficient to fire the cell directly but was readily revealed by the increase in firing when small doses of L-glutamate were superimposed (as shown for carbachol in Fig. 3).

Our usual experience was that the response to acetylcholine was increased if a second application was made within a few minutes of the first. Thereafter acetylcholine-firing remained reasonably constant with successive periods of ejection. This is in general agreement with the findings of Krnjević & Phillis (1963b) for neocortical cells, and is in contrast to the fall in acetylcholine sensitivity with successive applications seen with thalamic cells by Andersen & Curtis (1964a). Another interesting feature was that many cells which had been completely quiescent initially would continue to discharge spontaneously at a slow rate after excitation by acetylcholine, but not after excitation by L-glutamate.

A typical excitatory response to acetylcholine is illustrated in Fig. 1. When acetylcholine was applied with a current of 150 nA there was a latent period of 28 sec before regular

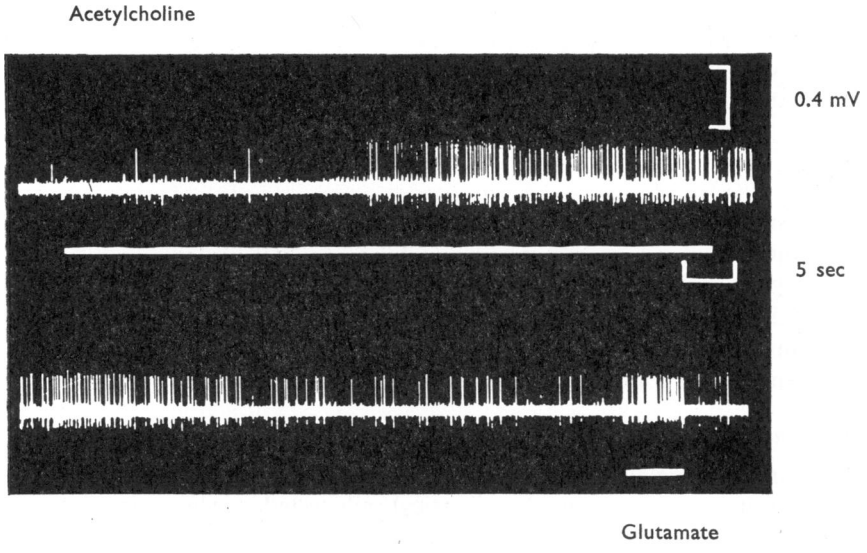

Fig. 1. Oscilloscope record of extracellular spike potentials from a single unit at 0.9 mm depth. The top and bottom records are continuous. The horizontal bars below the record indicate the duration of iontophoretic application of acetylcholine (top record, 150 nA) and L-glutamate (bottom record, 40 nA). Note the long delay before the cell fired with acetylcholine and the slow recovery, compared with the rapid action of L-glutamate. Calibrations, 0.4 mV and 5 sec.

firing occurred. On stopping the current, the firing gradually declined over the next minute. This is quite different from the almost immediate onset of firing in the same unit when L-glutamate was applied with a current of 40 nA, and the cessation within 300 msec of stopping the expelling current. The long latency in the onset of acetylcholine-excitation and the slow recovery were very generally observed, and are similar to the behaviour described for neocortical neurones by Krnjević & Phillis (1963b) and for hippocampal neurones by Biscoe & Straughan (1965).

Depth distribution of acetylcholine-sensitive cells

The depth of every cell tested was recorded by means of a helical potentiometer attached to the micromanipulator. The most probable source of error in these estimates of depth was the difficulty of ensuring that the microelectrode was moving exactly perpendicular to the surface of the cortex, particularly as the contours of the pyriform cortex change very sharply in this region. The general effect of this was that cells would be recorded at an apparently greater depth than that at which they actually lay.

In Fig. 2 the graph shows the superimposed histograms of the depth distribution of the ninety-one cholinoceptive cells found in ten preparations and of all the cells excited by L-glutamate and found at random in the same experiments. To make the histograms dimensionally comparable the depth distributions of the 375 cells excited by L-glutamate were recalculated on a sample size of 91 and the total was used in a χ^2 test, with the L-glutamate-excited cells as the expected distribution. The differences were not significant ($\chi^2=1.46$, n=9, $P>0.99$). Thus it would seem that there is no selective depth distribution

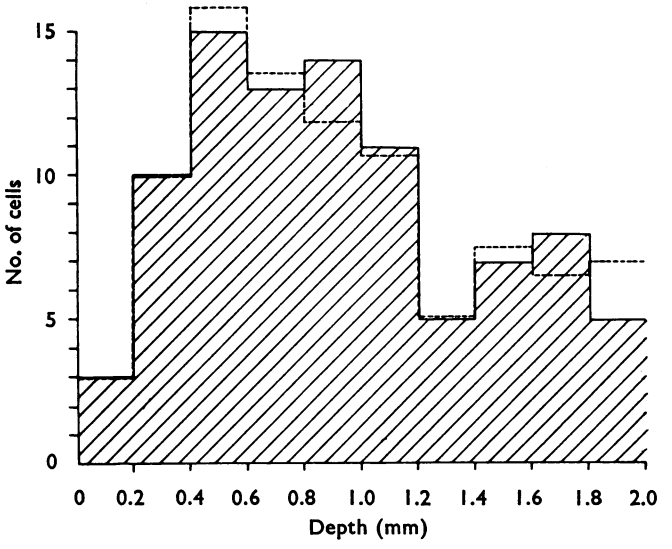

Fig. 2. Superimposed histograms of the depth distribution of 91 cholinoceptive cells found in the pyriform cortex of ten cats (continuous line, crosshatched area); and of the 375 cells found at random in the same experiments (broken line, unshaded area). To make the histograms dimensionally comparable the distribution of these 375 cells excited by L-glutamate was recalculated on a sample size of 91. The distributions were not significantly different. $\chi_2 = 1.46$, n = 9, $P > 0.99$.

of cholinoceptive cells, as has been noted in the neocortex by Krnjević & Phillis (1963b) and in the hippocampus by Biscoe & Straughan (1965). One feature of the distribution of cholinoceptive cells is apparent from this histogram and that is the predominance of cells below 0.4 mm of depth. This agrees with the localization of larger cells below 0.4 mm of depth in frozen sections of the pyriform cortex. Presumably the relatively coarse tips of our microelectrodes (5 to 10 μ) tend to select these larger cells at the expense of smaller ones.

Depression by acetylcholine

Thirteen out of the 286 units (4.5%) tested were depressed by acetylcholine. The depression was seen either as a reduction in the rate of spontaneous firing or as a reduction in the firing induced by superimposed brief applications of L-glutamate. This proportion of acetylcholine-depressed units is similar to that noted for the neocortex by Randić, Siminoff & Straughan (1964). These units were not investigated further.

Cholinomimetic agents

Carbachol. This drug excited twenty-three out of the thirty-six units tested in four cats. As with acetylcholine, the depolarization was either sufficient to fire the unit directly or could be revealed by the increase in firing when L-glutamate was superimposed. The latter procedure is illustrated in Fig. 3. In this cell L-glutamate (40 nA) applied at regular intervals gave reproducible firing but without maximal depolarization. A background current delivering carbachol (80 nA) was now applied, and after about 45 sec there was an obvious facilitation of the firing induced by L-glutamate. This carbachol-excitation persisted for about 1 min after stopping the current.

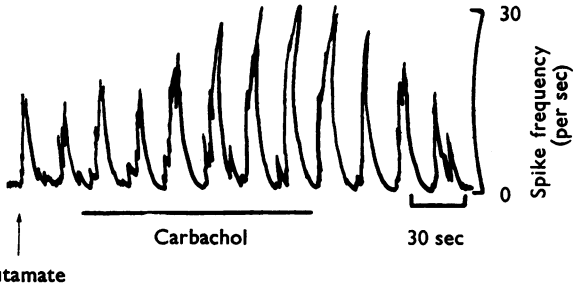

Fig. 3. Ratemeter record. Quiescent unit at 0.2 mm depth excited by the regular application of L-glutamate (40 nA) for 5 sec in every 15 sec. First application of L-glutamate is shown by the arrow; subsequent applications are not arrowed. The horizontal bar below the record indicates the duration of application of carbachol (80 nA). Note the delayed onset of facilitation and slow recovery after stopping the delivery of carbachol. The scale shows the number of spikes counted per second by the ratemeter with a time constant of 1 sec.

Twelve units were held long enough to compare the excitation produced by carbachol with that produced by equal currents releasing acetylcholine. The results were somewhat inconclusive, since carbachol was more potent than acetylcholine in seven cells and less potent in four others. Further, the duration of excitation by carbachol after termination of the current exceeded the values for acetylcholine by 10 to 60 sec, when both were applied for an equal period of 1 min. Similar findings were reported for ventrobasal thalamic neurones by Andersen & Curtis (1964b) and for neocortical neurones by Krnjević (1965).

Acetyl-β-methylcholine. This excited fifteen out of twenty-three units tested in four cats. In four of these units, the relative potency of acetyl-β-methylcholine against acetylcholine was determined, and on two units the former drug was more potent while on two others they were equipotent. The number of units tested with acetylcholine and acetyl-β-methylcholine is too small to allow any definite conclusion regarding their relative potencies in the palaeocortex. In eleven cells the excitation produced by carbachol was compared with that produced by equal amounts of acetyl-β-methylcholine. In the majority of these (nine cells) carbachol appeared to be slightly more potent than acetyl-β-methylcholine.

Nicotine. This was tested on twenty-four units in five preparations. In nine units after a long latency of 20 to 40 sec there was a small increase in the firing rate of the same negative spike which was fired by L-glutamate or by acetyl-β-methylcholine. The cells continued to fire for a time after stopping the expelling of nicotine before their regaining a normal level of excitability.

(±)-*Muscarine.* This drug has not been studied in great detail. In one preparation, five units were tested, and two of these were excited by both muscarine and acetylcholine. In each case muscarine was the more potent excitant. Muscarine did not excite the three units which were not excited by acetylcholine.

Cholinolytic agents

Atropine was expelled as a cation from an 0.2 M solution of atropine sulphate with currents ranging between 40 and 100 nA for periods of 30 to 90 sec. Nine cholinoceptive units were tested with atropine in five preparations; in eight of these atropine selectively

blocked the excitatory response to acetylcholine (Fig. 4). The specificity of this block was demonstrated by the failure of atropine to diminish the firing produced by the applications of L-glutamate. Complete recovery of normal excitation by acetylcholine took anything from 3 to 20 min. On several occasions we observed only a partial recovery, because of deterioration in, or loss of, the unit. A complete loss of the response to acetylcholine and L-glutamate with atropine was only seen in one unit, which had received a comparatively large amount of atropine (110 nA for 8 min).

This nonspecific depressant effect of atropine has been observed more frequently in neocortical (Krnjević & Phillis, 1963a), thalamic (Andersen & Curtis, 1964b) and spinal Renshaw cells (Curtis & Phillis, 1960) and is presumably due to a local anaesthetic type of action of atropine upon the spike mechanism of these cells (Curtis & Phillis, 1960; Krnjević & Phillis, 1963a,c). However, this local anaesthetic action of atropine, as measured by the reduction in spike amplitude and reduced excitation by L-glutamate, was of short duration and small extent. Thus, soon after turning off the current expelling atropine the excitatory response of cells to L-glutamate was fully recovered when excitation by acetylcholine was still selectively depressed.

Seven of the cholinoceptive units blocked by atropine were also tested with dihydro-β-erythroidine. In one unit dihydro-β-erythroidine (130 nA) had no effect until it had been applied for 7 min, when complete block of the excitation produced by acetylcholine occurred. The response to acetylcholine recovered fully within 2 min of turning off the expelling current. In three units dihydro-β-erythroidine (60 to 110 nA applied for 1.5 to 3 min) caused a partial reduction of the response to acetylcholine (30 to 50%). In the remaining three units, dihydro-β-erythroidine-delivering currents of 60, 80 and 120 nA were passed for 1.5, 2 and 7 min respectively, without any effect. One of these dihydro-β-erythroidine-insensitive cells is shown in Fig. 4. In this cell dihydro-β-erythroidine (80 nA)

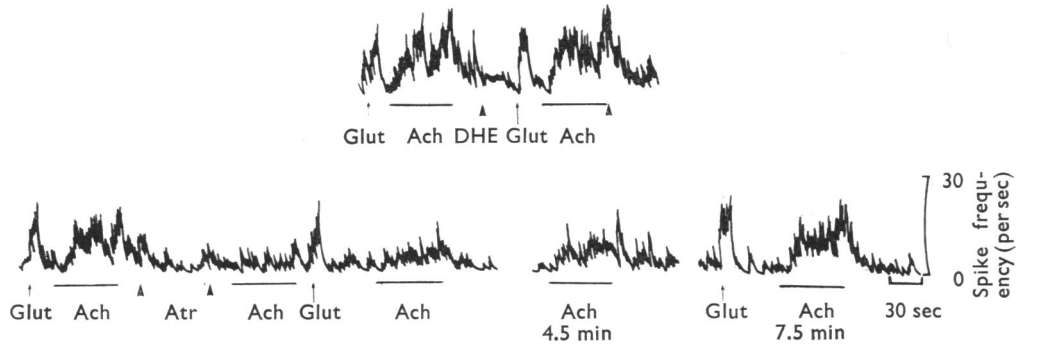

Fig. 4. Ratemeter record. Quiescent unit at 0.8 mm depth excited by a 10 sec application of L-glutamate (20 nA, Glut) when indicated by the arrows. The unit was also excited by acetylcholine (100 nA, Ach) applied for a period of 45 sec when indicated by the horizontal bars below the records. In the top record dihydro-β-erythroidine (80 nA, DHE) was applied for 2 min between the filled triangles; this did not block the excitant action of acetylcholine. The record was continued (lower line) when atropine (80 nA, Atr) applied for 1 min only between the triangles blocked the excitant action of a subsequent application of acetylcholine but not that of L-glutamate; on the right is shown the partial recovery of the excitation by acetylcholine at 4.5 min after, and the complete recovery at 7.5 min after stopping the delivery of atropine. The scale shows the number of spikes counted per second by the ratemeter.

applied for 2 min did not block excitation by acetylcholine, though the same amount of atropine applied for 1 min completely abolished the excitant action of acetylcholine, leaving the response to L-glutamate unaffected. Full recovery of the acetylcholine-effect occurred 7.5 min after turning off the current expelling atropine.

In all, thirteen cholinoceptive cells were tested with dihydro-β-erythroidine in seven preparations. In most of these cells a direct excitant effect of the drug was noted similar to that described for medullary (Salmoiraghi & Steiner, 1963) and ventrobasal thalamic neurones (Andersen & Curtis, 1964b). This direct excitant action of dihydro-β-erythroidine is illustrated in Fig. 5; here, after 1 min of dihydro-β-erythroidine (100 nA) the addition of L-glutamate (40 nA) was sufficient to trigger maintained firing.

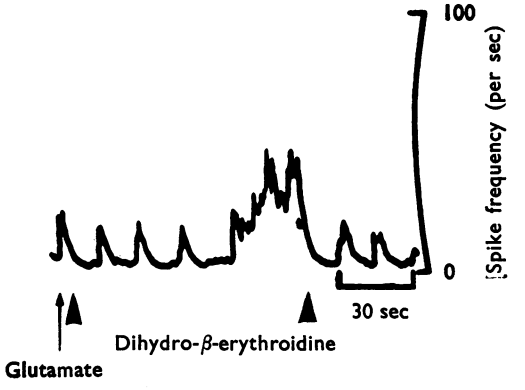

Fig. 5. Ratemeter record. Quiescent unit at 0.4 mm depth excited by the regular application of L-glutamate (40 nA) for 5 sec. The arrow shows the first application; subsequent applications are not arrowed. Dihydro-β-erythroidine (100 nA) was applied for 1 min 40 sec, between the filled triangles. After 1 min this increased the excitability of the unit sufficiently for the next application of L-glutamate to trigger maintained firing. When the delivery of dihydro-β-erythroidine was turned off the unit recovered its normal excitability. The scale shows the number of spikes per second.

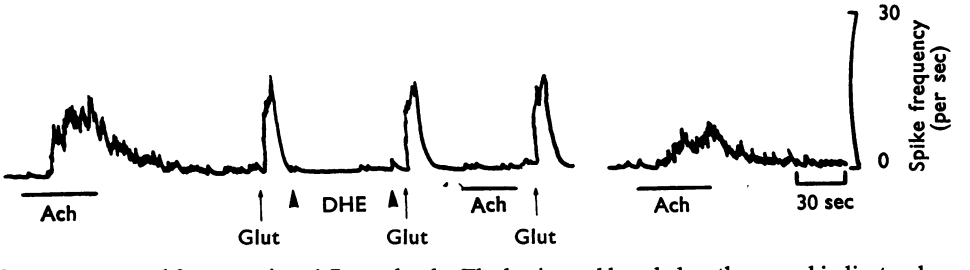

Fig. 6. Ratemeter record from a unit at 1.7 mm depth. The horizontal bars below the record indicate when acetylcholine (100 nA, Ach) was applied for 45 sec. Note the delay before the unit fired (about 18 sec), and also that firing continued for about 1 min after stopping the expelling current. The unit was also excited by L-glutamate (40 nA, Glut, for 10 sec) at times indicated by the arrow. Between the filled triangles dihydro-β-erythroidine (100 nA, DHE) was applied for 1 min. The subsequent application of L-glutamate caused normal firing, but the excitant action of acetylcholine was completely blocked. In the last section of the record, taken 3.5 min after stopping the delivery of dihydro-β-erythroidine, partial recovery of excitation by acetylcholine has occurred. Full recovery cannot be shown as the cell was lost.

Seven units were held long enough for their acetylcholine-response to be tested after atropine; the results have been discussed above. Of the remaining six units tested, in two the acetylcholine-excitation was completely blocked by dihydro-β-erythroidine (100 nA applied for 1 to 1.5 min), in two others it was partially blocked (80 to 100 nA applied for a period of 1.5 to 3 min), while in the last two units acetylcholine-induced excitation was unaffected (90 to 110 nA for 1.5 to 5 min). The selective blocking action of dihydro-β-erythroidine on one cholinoceptive unit is illustrated in Fig. 6. Here, dihydro-β-erythroidine (100 nA) applied for 1 min caused a complete block of the excitatory response to acetylcholine though the excitability of the cell to L-glutamate remained unimpaired. Owing to movement of the microelectrode and consequent loss of the cell, only partial recovery of the acetylcholine-response was observed, occurring 3.5 min after stopping the dihydro-β-erythroidine-releasing current. Normally, full recovery of acetylcholine-sensitivity after dihydro-β-erythroidine took about 5 to 6 min.

5-Hydroxytryptamine

In all, 116 units were studied in ten preparations. The 5-hydroxytryptamine was applied as a cation with currents between 20 and 90 nA for periods of 30 to 90 sec, and the rate of spontaneous or L-glutamate-induced firing was depressed in sixty-four of these units. Though the frequency of firing was reduced, the size of the extracellular spike potential was unaffected; thus a local anaesthetic type of action on the postsynaptic membrane seems unlikely. Maximal depression was usually seen within 10 to 30 sec, though the latency varied considerably with different units. After stopping the current expelling 5-hydroxytryptamine, full recovery of the firing rate did not usually occur for about 2 min.

The unit illustrated in Fig. 7 was excited by L-glutamate (6 nA) applied for 5-sec periods; a continuous application of 5-hydroxytryptamine (40 nA for 2 min) caused a gradual

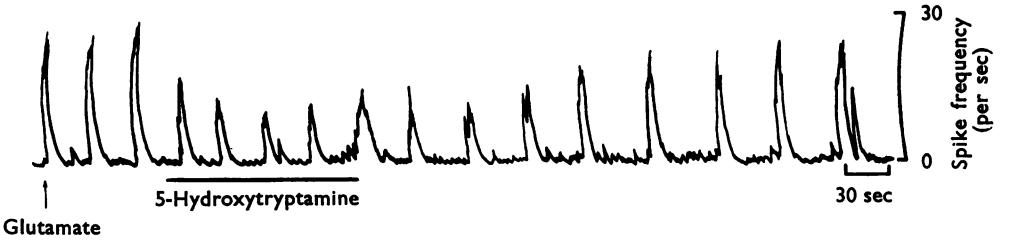

Fig. 7. Ratemeter record from unit at 0.5 mm depth. Excitability tested by the regular applications of L-glutamate (6 nA) for 5 sec. The first application is indicated by the arrow; subsequent applications are not arrowed. Background application of 5-hydroxtryptamine (40 nA), applied continuously for 2.2 min, is indicated by the horizontal bar. Unit excitability was depressed by 5-hydroxytryptamine and took 5.0 min to recover. The scale shows the number of spikes per second.

depression of its excitability. On turning off the current expelling 5-hydroxytryptamine, the normal firing rate was not regained for about 5 min. An even more persistent depression produced by 5-hydroxytryptamine is shown in Fig. 8 where full recovery of the firing rate was not achieved for 5 min. This figure also shows that, when a larger amount of L-glutamate was applied (double arrow sign) during the recovery phase, it was able to excite the unit above the control frequency rate. This means that the persistent depression

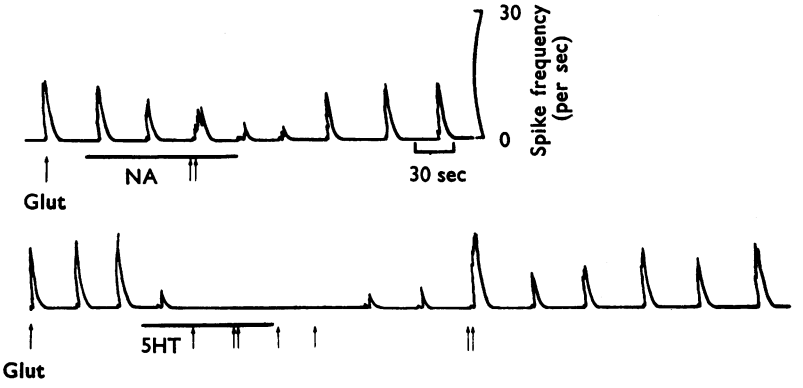

Fig. 8. Ratemeter record from cell at 2.3 mm depth. This cell was quiescent, so L-glutamate (20 nA, Glut) was applied for 5 sec in every 15 sec, to give regular bursts of firing. The first application was at the first arrow; subsequent applications are not arrowed unless stated below. In the upper record a background current of noradrenaline (40 nA, NA) was applied for 2 min (indicated by the horizontal bar), causing a gradual depression of L-glutamate-induced firing. Doubling the time for which L-glutamate was applied (two close arrows) did not fire the cell at control levels. The control level of firing was not recovered for 2 min. In the bottom record 5-hydroxytryptamine (40 nA, 5 HT) was applied to the same cell for 2 min from another barrel of the micropipette (indicated by the horizontal bar). Within 15 sec firing was completely abolished (L-glutamate deliveries at arrows) and even doubling the time for which L-glutamate was applied (two close arrows) did not fire the cell. Cell excitability slowly recovered within 5 min of stopping the delivery of 5-hydroxytryptamine. Doubling the amount of L-glutamate applied during this recovery phase (two close arrows) caused an increase in the frequency of firing although the normal firing from 5 sec of L-glutamate had not recovered. The scale shows the number of spikes per second.

obtained with 5-hydroxytryptamine in this unit cannot be attributed to over-depolarization. However, in some units the time course of the depressant action of 5-hydroxytryptamine was much more rapid, as shown in Fig. 9, and this effect is much more comparable with that seen in the feline neocortex (Krnjević & Phillis, 1963d).

5-Hydroxytryptamine is also able to block firing in cells which have been synaptically activated by stimulation of the lateral olfactory tract. This is illustrated in Fig. 10; when 5-hydroxytryptamine (60 nA) was applied for 20 sec, synaptic activation of the unit was gradually blocked (b, c) ; full recovery took place immediately on stopping the iontophoretic current (*d*). When this unit was excited with L-glutamate (not illustrated) the same amount of 5-hydroxytryptamine produced a similar block.

When possible, 5-hydroxytryptamine-depressed cells were tested with other potential neurotransmitter substances. No particular correlation, positive or negative, was noted between 5-hydroxytryptamine-sensitivity and the response to acetylcholine, noradrenaline or dopamine. For example, out of eleven cells depressed by 5-hydroxytryptamine which were tested with noradrenaline, six were depressed. This is only just greater than the proportion of cells found to be depressed by noradrenaline on random testing. A record from one of these cells is shown in Fig. 8. Though 5-hydroxytryptamine (as creatinine sulphate) has a lower transport number than noradrenaline (Krnjević, Laverty & Sharman, 1963) it can be seen that 5-hydroxytryptamine produced a more potent and longer-lasting depression than noradrenaline.

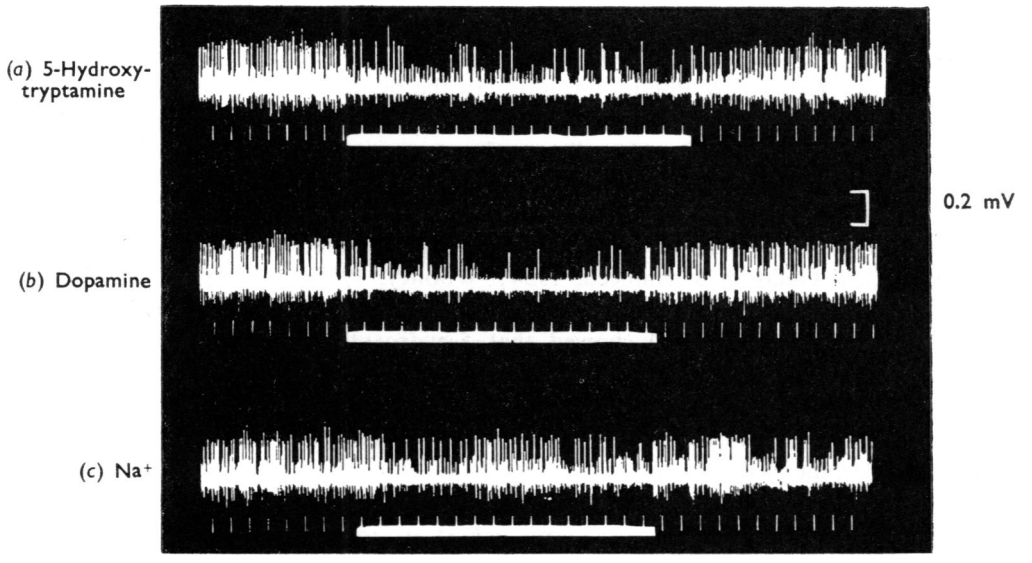

Fig. 9. Oscilloscope record of extracellular spikes from two units, one large and one small, at 0.5 mm depth. The units were firing continuously with a background application of L-glutamate (8 nA). (*a*) 5-Hydroxytryptamine (60 nA) was applied for 18 sec when indicated by the white line beneath the record. The firing rate of the larger unit is depressed before the smaller unit, and the spike sizes are unaltered. (*b*) Dopamine (60 nA) was applied for 16 sec when indicated by the white line beneath record. Both large and small units are fairly rapidly depressed. (*c*) A control current of 100 nA was passed through the barrel containing sodium chloride when indicated by the white line, and there is some current depression of the large unit but the small unit is comparatively unaffected. Calibrations, 0.2 mV and 1 sec.

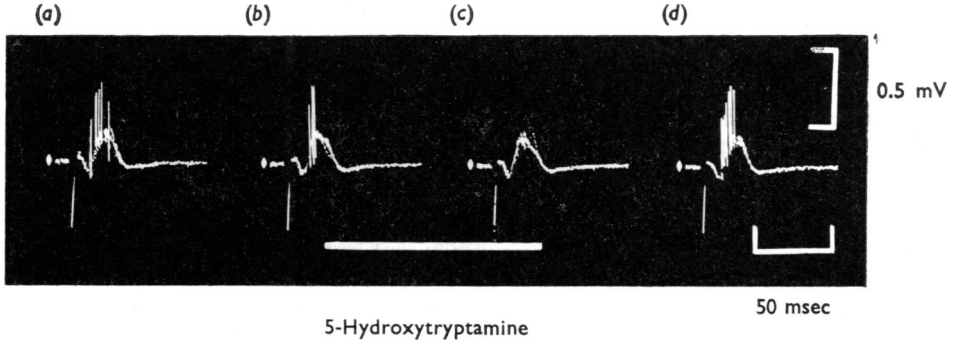

Fig. 10. In (*a*), a unit was evoked with an average latency of 17 msec (range 12.5 to 22.5 msec) by stimulation of the lateral olfactory tract with 13-V and 0.1-msec duration pulses. In (*b*), stimulation continued with 5-hydroxytryptamine (60 nA) applied to the unit over 10 sec from an adjacent barrel of the micropipette (during white line). In (*c*), application of 5-hydroxytryptamine continued for a further 10 sec. The synaptically evoked unit response is now blocked. In (*d*), immediately on stopping the current expelling 5-hydroxytryptamine, the synaptically evoked unit fires again. Each record consists of superimposed frames of responses to ten successive stimuli at 1 per sec. The spikes are retouched. Calibrations, 0·5 mV and 50 msec.

Occasional cells in the hippocampal cortex are excited by small deliveries of 5-hydroxytryptamine (Biscoe & Straughan, 1965), while large doses are needed to produce excitation in the neocortex (Krnjević & Phillis, 1963a,d). In the pyriform cortex, however, whether small or large deliveries (up to 240 nA) of 5-hydroxytryptamine were used, no definite excitation was seen in any of the 116 cells tested.

Lysergic acid derivatives

Several lysergic acid derivatives antagonize the peripheral effects of 5-hydroxytryptamine (Gaddum, 1953; Gaddum & Hameed, 1954; Woolley, 1958). Though several studies have shown that lysergide does not antagonize the effects of 5-hydroxytryptamine in many areas of the brain, Olds (1958) showed that 5-hydroxytryptamine and lysergide had antagonistic actions on self-stimulation responses in the rat prepyriform cortex. Also, Vogt, Gunn & Sawyer (1957) showed that lysergide given intraventricularly increased the electrical activity of the cat amygdala and could briefly antagonize the depressant effect of 5-hydroxytryptamine in that area.

More recently it has been shown that lysergide can antagonize the effect of 5-hydroxytryptamine on the transcallosal response (Bond & Guth, 1964). Thus it seemed of interest to see if lysergide or its congeners (bromolysergic acid diethylamide and methysergide) could antagonize the depression produced by the iontophoretic application of 5-hydroxytryptamine in the pyriform cortex. But, even though these three substances were applied for several minutes with large iontophoretic currents, the depressant effect of 5-hydroxytryptamine on the frequency of cell firing did not appear to be impaired. Lysergide had a mild progressive depressant action of its own, but bromolysergic acid diethylamide and methysergide had a more profound depressant effect so that full recovery of cell excitability could take up to 7 min. Because of these long-lasting depressant effects it was difficult to demonstrate the additional depression induced by 5-hydroxytryptamine.

One of the 5-hydroxytryptamine-depressed cells tested with lysergide is shown in Fig. 11. At (*a*), 5-hydroxytryptamine (40 nA) caused a very marked depression of cell firing compared with the minor depressant effect of sodium ions (50 nA) at (*b*). When lysergide was applied at (*c*) the rate of cell firing was depressed. The immediate fall was probably due to anodal depression. The smaller progressive fall in firing rate which followed this is probably a direct effect of the lysergide. When the lysergide had been applied for 2.5 min at (*d*), the application of 5-hydroxytryptamine still produced a characteristic depression.

The action of these lysergic acid derivatives is generally similar to that observed in the lateral geniculate nucleus (Curtis & Davis, 1962) and in the neocortex (Krnjević & Phillis, 1963d).

Catechol amines

Noradrenaline was tested on 117 cells in twelve cats with currents from 20 to 120 nA for periods of 60 to 90 sec. In forty-eight of these cells, noradrenaline had a depressant effect, seen as a reduction in the rate of spontaneous or L-glutamate-induced firing. Full recovery usually occurred within 30 sec. No change in spike amplitude was observed during this depression. A typical unit depressed by noradrenaline is shown in Fig. 12. This cell was quiescent, but was easily excited by L-glutamate (20 nA). An outward current passed through the control barrel had little effect on cell firing, but a slightly smaller current of

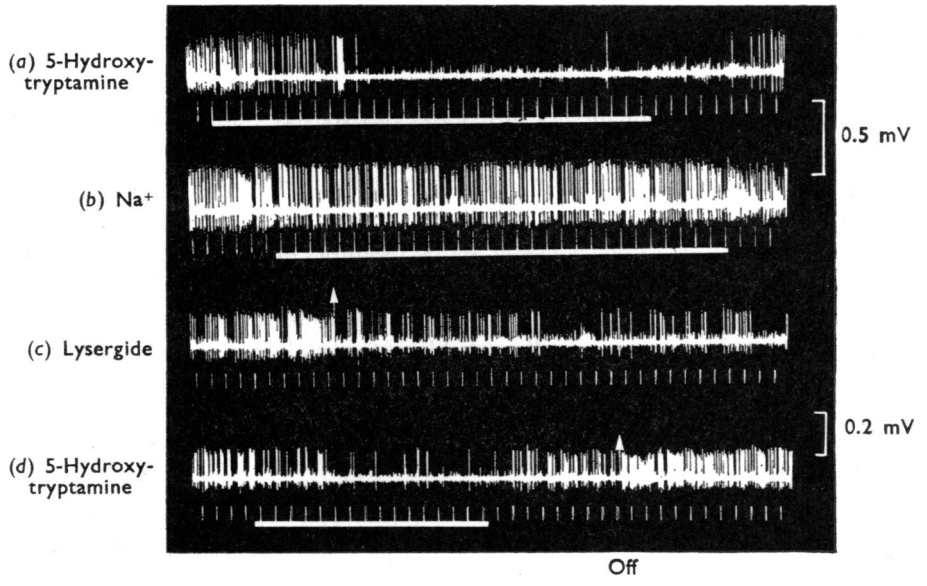

Fig. 11. Oscilloscope record of extracellular spike responses from a unit at 0.45 mm depth. Background application of L-glutamate (16 nA) was continued throughout to give steady firing. In (*a*), when 5-hydroxytryptamine (40 nA) was applied for 30 sec (indicated by the white bar below the record), unit firing was completely depressed in 10 sec. Full recovery occurred 8 sec after stopping the expelling current of 5-hydroxytryptamine. Note the spike size was not reduced by 5-hydroxytryptamine and there was no evidence of excitation. In (*b*), a very slight current depression occurred when a tip-positive current of 50 nA was applied through the barrel containing sodium chloride. In (*c*), lysergide (100 nA) was applied at the arrow. The immediate depression of cell firing observed is probably mostly "anodal depression," but there is some slow progressive depression due to lysergide. In (*d*), after 2.5 min of lysergide, 5-hydroxytryptamine (40 nA) was superimposed, when indicated by the white bar below the record. The characteristic depression of cell firing still occurred. When delivery of lysergide was stopped (at the arrow) there was an immediate postanodal increase in cell firing. Note that amplifier gain was reduced between (*b*) and (*c*).

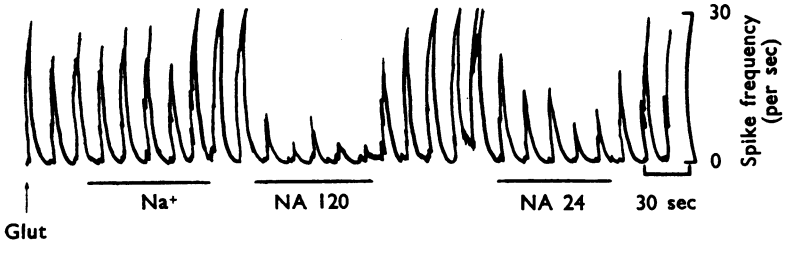

Fig. 12. Ratemeter record from a unit at 0.8 mm depth. The unit was quiescent so L-glutamate (20 nA, Glut) was applied for 5 sec in every 15 sec to give a regular level of firing. The first application is indicated by the arrow, but subsequent applications are not arrowed. Tip-positive current of 140 nA was passed through the control barrel containing sodium chloride for 1.5 min (indicated by the black bar) and had little effect on unit excitability. Noradrenaline (120 nA, NA 120) was applied for 1.5 min, and caused a rapidly developing powerful depression, which persisted for 45 sec after stopping the expelling current. Even noradrenaline, 24 nA (NA 24), caused significant depression. The scale shows the number of spikes per second.

120 nA passed through the barrel containing noradrenaline rapidly caused depression. This unit regained its control level of firing within 30 sec of stopping the expelling current of noradrenaline.

Several cells excited by L-glutamate and presynaptically by stimulation of the ipselateral olfactory bulb were tested with noradrenaline. Noradrenaline caused a similar depression of excitability in each instance. There is thus no evidence for a presynaptic blocking action for noradrenaline.

In an effort to determine if specific catechol amine receptors are involved in this depression of palaeocortical neurones by noradrenaline, we have investigated the action of various α- and β-receptor blocking agents. Dibenamine, phenoxybenzamine and phentolamine (α-receptor blocking agents) as well as dichloroisoprenaline (a β-receptor blocking agent), applied iontophoretically for several minutes, consistently failed to modify the depressant effect of noradrenaline. This is shown in Fig. 13, where, after the application of dibenamine (110 nA) for 5 min, the depression caused by noradrenaline (120 nA) remained unaffected.

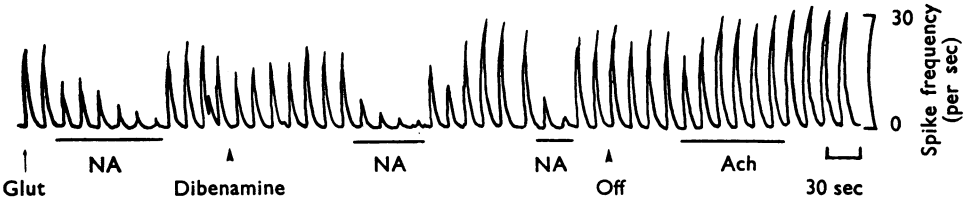

Fig. 13. Ratemeter record from a unit at 0.3 mm depth. The unit was quiescent so L-glutamate (40 nA, Glut) was applied for 5 sec in every 15 sec to give regular bursts of firing. The first application is shown by the arrow; subsequent applications are not arrowed. A background application of noradrenaline (120 nA, NA) for 1.5 min (indicated by the black bar) depressed cell firing in a characteristic fashion. Between the filled triangles a background current of dibenamine (110 nA) was applied for 5.5 min, but when noradrenaline was superimposed the depression of cell firing still occurred. The application of acetylcholine (120 nA, Ach) for 1.5 min to this cell (extreme right of the record) had no effect. The scale shows the number of spikes per second.

As with 5-hydroxytryptamine, we were unable to obtain any evidence of neuronal excitation, despite the delivery of noradrenaline by currents of the order of 150 nA. On several occasions, however, there was an increase in the amount of synaptic noise—suggesting excitation of distant units—but we were unable to isolate any definite cell thus excited.

Fifty-three cells in seven cats were tested with dopamine (20 to 120 nA). In twenty-seven cells the rate of firing, whether spontaneous or induced by L-glutamate, was depressed. As with 5-hydroxytryptamine and noradrenaline there was no evidence of a presynaptic action, or of a local anaesthetic or depolarizing type of blockade. A typical response to dopamine is illustrated in Fig. 9. Here, two characteristic features of the action of dopamine are apparent. Firstly, it is slightly more potent as a depressant than 5-hydroxytryptamine, and secondly the depressant effect develops and recovers more quickly than that due to 5-hydroxytryptamine. The apparently greater depressant potency of dopamine could be due to the fact that equal currents pass relatively more dopamine than 5-hydroxytryptamine. In this connexion it is interesting to note that Krnjević *et al.* (1963) observed that 5-hydroxytryptamine had a lower transport number than either noradrenaline or adrenaline.

In three cats, seventeen cells depressed by dopamine were also tested with noradrenaline but only three of them were depressed. In each instance dopamine was more potent than noradrenaline. Since roughly half the cells tested at random with noradrenaline were depressed, these figures would suggest that the cells in the pyriform cortex have receptors more selective for dopamine than for noradrenaline. These results are generally similar to those noted for the neocortex (Krnjević & Phillis, 1963d).

Inhibition of palaeocortical neurones

We have tried to evoke units synaptically by single and repetitive volleys in the lateral olfactory tract, since it is known that the fibres of this tract terminate mainly in the superficial layers of the pyriform area (Clark, 1947). However, it usually proved very difficult to evoke unit responses in this way, probably because of the strong inhibitory effect of this form of stimulation. This point is illustrated in Fig. 14, where a unit at a depth of 1.5 mm

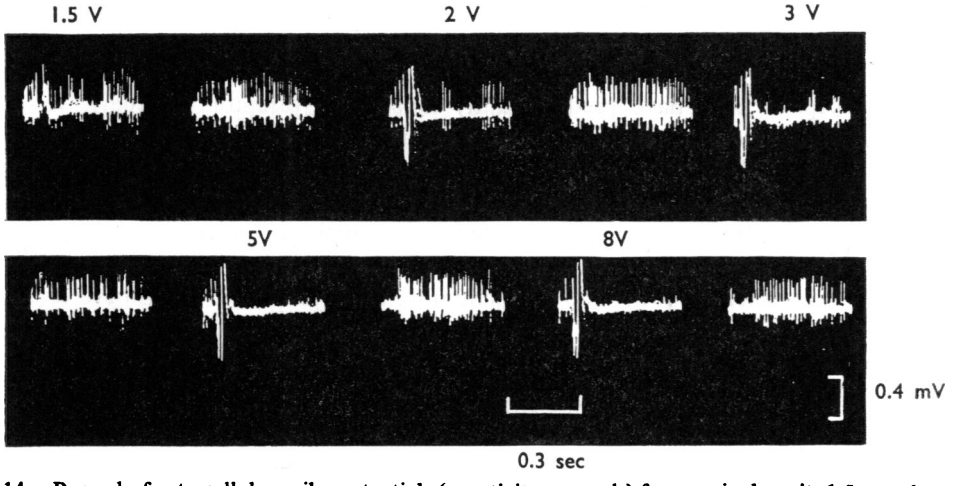

Fig. 14. Record of extracellular spike potentials (negativity upwards) from a single unit, 1.5 mm deep, in the pyriform cortex. Each frame consists of five superimposed traces taken at a frequency of 1 per sec. The effect of increasing the intensity of lateral olfactory tract stimuli (100 μsec pulse duration) is shown with intervening control records. Note that, since this unit was quiescent, L-glutamate (24 nA) was applied continuously to produce steady firing. There is good inhibition of cell firing at 3 V, becoming complete at 5 V.

was firing continuously with a background application of L-glutamate (24 nA). Stimulation of the lateral olfactory tract with 1.5-V pulses of 0.1 msec duration had no definite inhibitory effect but, when the intensity of stimulation was increased to 5 V, complete inhibition of cell firing occurred, which persisted for 400 msec after the stimulus. A similar inhibitory period was observed on stimulation of the ipselateral olfactory bulb, though it was often preceded by either a single spike or a burst of spikes.

DISCUSSION

From the results presented in this paper it is apparent that many palaeocortical cells can be excited by acetylcholine. The actual proportion of acetylcholine-sensitive cells (27%) of the total tested is similar to that reported for neocortical cells by Krnjević & Phillis

(1963b) and Randić *et al.* (1964). However, palaeocortical cholinoceptive cells are fairly uniformly distributed throughout all layers, and not concentrated in the deeper layers as in the neocortex. This uniform depth distribution of acetylcholine-sensitive cells in the pyriform cortex is in good agreement with the even distribution of acetylcholinesterase in this region (Krnjević & Silver, 1965).

Cholinoceptive cells in the pyriform cortex are excited relatively effectively by muscarine and several choline esters. This excitation can be blocked specifically by atropine. However, some palaeocortical cells are weakly excited by nicotine, and in half the cells examined dihydro-β-erythroidine caused a partial or complete selective block of acetylcholine-excitation. Now, acetylcholine receptors can be classified into " muscarinic " or " nicotinic " types by the excitatory actions of muscarine and nicotine (Dale, 1914). Also, muscarinic receptors are blocked fairly selectively by atropine, and nicotinic receptors by nicotine, tubocurarine and dihydro-β-erythroidine. Thus, the results suggest that the receptors on the cholinoceptive cells in the pyriform cortex have mixed muscarinic and nicotinic properties, a pattern which is seen in other limbic cortical cells—cingulate gyrus (Krnjević, 1965) and hippocampal cortex (Biscoe & Straughan, 1965). However, in the feline neocortex the cholinoceptive cells have predominantly muscarinic receptors (Krnjević & Phillis, 1963c), though in the monkey neocortex there were a few cells in which the excitation by acetylcholine was slightly antagonized by dihydro-β-erythroidine. Cholinoceptive cells with mixed muscarinic and nicotinic receptors also occur in other parts of the brain, for example the ventrobasal complex of the thalamus (Curtis & Andersen, 1962; Andersen & Curtis, 1964b), lateral geniculate nucleus (Curtis & Davis, 1963), brain stem (Bradley, Dhawan & Wolstencroft, 1964) and cerebellum (Crawford, Curtis, Voorhoeve & Wilson, 1963; McCance & Phillis, 1964). In the Renshaw cell system, Curtis & Ryall's (1964) results suggest that the same cell possesses two different types of receptor but each receptor type mediates a different response.

The functional significance of these mixed acetylcholine receptors on palaeocortical cholinoceptive cells is uncertain at present. On the basis of histochemical studies of acetyl- and pseudocholinesterase distribution in the rat forebrain and brain stem, Shute & Lewis (1963) proposed the existence of two types of cholinergic neurone. It is possible, though as yet still entirely speculative, that a cell receiving two types of cholinergic fibre might have receptors of mixed sensitivity, or even two different types of acetylcholine receptor.

Apart from the presence of cholinesterase and choline acetylase in the pyriform cortex, discussed previously, the evidence for the presence of cholinergic fibres in the pyriform cortex is still uncertain. However, MacLean, Horwitz & Robinson (1952) have observed that atropine given intravenously to rabbits prevented the development of olfactory-induced rhythmically recurring potentials in the pyriform cortex. These potentials had been described earlier by Adrian (1942, 1950). Future work must be directed towards seeing if the units evoked by olfactory stimulation are cholinoceptive and if atropine will selectively antagonize their synaptic activation and direct excitation by acetylcholine.

Our interest in the actions of 5-hydroxytryptamine on palaeocortical cells stems from the fact that, as mentioned previously, 5-hydroxytryptamine and several enzymes involved in its metabolism are concentrated in this region; and from the self-stimulation studies in the prepyriform region of rats, which have shown that lysergide has strong inhibitory effects which can be antagonized by prior administration of 5-hydroxytryptamine (Olds,

1958). This suggested a possible transmitter function for 5-hydroxytryptamine in this region, since lysergide is known to be a powerful antagonist of 5-hydroxytryptamine in the periphery (Gaddum, 1953; Gaddum & Hameed, 1954). Further, lysergide has remarkable psychotropic effects (Hofman, 1959), and stimulation of the pyriform cortex, electrically or chemically, or surgical lesions there, produce very marked changes in the affective behaviour of experimental animals (MacLean *et al.*, 1952; Schreiner & Kling, 1953; MacLean & Delgado, 1953; Green, Clemente & de Groot, 1957). Thus, elucidation of the actions of 5-hydroxytryptamine and lysergide in this region could have far-reaching implications.

The present studies show that 5-hydroxytryptamine has a relatively strong depressant action on the cells in the pyriform cortex, whether they are firing spontaneously, as a result of afferent nervous stimulation, or as a result of L-glutamate. This suggests that 5-hydroxytryptamine acts directly on the postsynaptic membrane and not presynaptically as in the lateral geniculate nucleus (Curtis & Davis, 1962). These results are in keeping with the general observations that, in the neocortex and archicortex, the effect of 5-hydroxytryptamine is predominantly one of depression (Krnjević & Phillis, 1963a,d; Biscoe & Straughan, 1965). The predominant action of 5-hydroxytryptamine is also depressant in the cerebellum (Phillis, 1965), hypothalamus (Bloom, Oliver & Salmoiraghi, 1963), olfactory bulb (Baumgarten, Bloom, Oliver & Salmoiraghi, 1963) and ventrobasal thalamus (Andersen & Curtis, 1964b). By contrast, nearly half of the cells tested in the brain stem are excited by 5-hydroxytryptamine (Bradley & Wolstencroft, 1965).

One curious feature of the pyriform cortex is the absence of any excitation by 5-hydroxytryptamine, compared with the occasional excitation seen in the neocortex, archicortex and hypothalamus. Andersen & Curtis (1964b) attributed the absence of excitation by 5-hydroxytryptamine in the thalamus to the small currents they used. This cannot be the explanation for the present observations since large currents up to 240 nA were used for several minutes.

Though 5-hydroxytryptamine has been stated to have no effect on motoneurones, interneurones or Renshaw cells in the spinal cord (Curtis, Phillis & Watkins, 1961; Curtis, 1962), it may be that the use of pentobarbitone in these experiments caused insensitivity to tryptamine (Marley & Vane, 1963). It will be of interest to determine the extent to which anaesthetics modify the response of palaeocortical neurones not only to 5-hydroxytryptamine but to other potential neurotransmitters.

As in the neocortex (Krnjević & Phillis, 1963d), depression was the only effect obtained with noradrenaline and dopamine in the pyriform cortex. Since both drugs reduced excitation of cells produced by L-glutamate, the depression appears to be postsynaptic. Other central neurones differ considerably in their responses to these substances. Thus the predominant action of noradrenaline is depression in the hypothalamus, the olfactory bulb and the caudate nucleus, but definite excitation was also noted (Bloom *et al.*, 1963; Baumgarten *et al.*, 1963; Bloom, Costa, Oliver & Salmoiraghi, 1964). However, in the brain stem it has been found that the majority of the units tested are excited by noradrenaline (Bradley & Wolstencroft, 1962, 1965). Though dopamine is a depressant in the lateral geniculate nucleus (Curtis & Davis, 1962), excitatory and depressant effects have been noted in the caudate nucleus (Bloom *et al.*, 1964).

It has been shown that in the olfactory bulb the inhibition of mitral cells produced by lateral olfactory tract stimulation (Green, Mancia & Baumgarten, 1962; Phillips, Powell & Shepherd, 1963) may be noradrenergic in nature, since it is significantly reduced by applications of peripheral adrenergic blocking agents or after chronic depletion of noradrenaline with α-methylmetatyrosine (Salmoiraghi, Bloom, Oliver & Costa, 1964; Bloom, Costa & Salmoiraghi, 1964). Thus, though it is possible that the depressant actions of noradrenaline, dopamine and 5-hydroxytryptamine in the pyriform cortex could be non-specific (Krnjević & Phillis, 1963d), these substances might act as inhibitory transmitters or be chemically related to the inhibitory transmitter. Our failure to antagonize the effects of 5-hydroxytryptamine or noradrenaline by their specific blocking agents does not entirely rule out the possibility that specific receptors are involved, for in the rabbit olfactory bulb a specific blockade of noradrenaline-depression sometimes required large currents and prolonged application (up to 15 min) of the dibenamine or phentolamine (Bloom *et al.*, 1964). It is possible that because of the difficulty in holding units for this length of time such an antagonism was overlooked. However, it is worth noting that attempts to block the action of noradrenaline in the brain stem (Bradley & Wolstencroft, 1965) and of 5-hydroxytryptamine in the neocortex (Krnjević & Phillis, 1963d) with specific peripheral blocking agents have so far been unsuccessful.

SUMMARY

1. The chemical sensitivity of cells in the cat pyriform cortex was studied using the microelectrophoretic method.

2. All the cells tested were strongly excited by L-glutamate, while γ-aminobutyric acid was a potent depressant of cell firing. These amino acids produced their effect within several hundred msec of starting the expelling current, and recovery was equally rapid when the current was stopped.

3. Acetylcholine excited 27% of the cells tested. These cholinoceptive cells appeared to be uniformly distributed in depth. Several other cholinomimetic substances: acetyl-β-methylcholine, carbachol and muscarine were potent excitants, while nicotine was a weak excitant of these cells. Atropine produced a selective block of acetylcholine-excitation in nearly every cell tested, while dihydro-β-erythroidine was less potent and caused partial or complete block of acetylcholine-excitation in only half the cells tested. Several cells depressed by acetylcholine were noted.

4. 5-Hydroxytryptamine depressed 55% of cells tested. The depression appeared to be postsynaptic and was usually maximal within 10 to 30 sec of starting the expelling current. Recovery of the normal firing rate after it had been depressed by 5-hydroxytryptamine often took several minutes. No cells excited by 5-hydroxytryptamine were seen.

5. The depressant effect of 5-hydroxytryptamine did not appear to be blocked by lysergide, bromolysergic acid diethylamide or methysergide. These lysergic acid derivatives depressed cell firing and their effect persisted longer than that of 5-hydroxytryptamine.

6. Noradrenaline depressed 41% and dopamine depressed 51% of the cells to which they were applied. Dopamine was more potent than noradrenaline. The depression appeared to be postsynaptic, and its time course though prolonged was shorter than that seen with 5-hydroxytryptamine. Noradrenaline-induced depression could not be antagonized by the

prior iontophoretic release of several adrenergic blocking agents. No excitation was seen with noradrenaline or dopamine.

7. The most likely explanation for our failure to modify the effects of 5-hydroxytryptamine and noradrenaline with their respective blocking agents is that specific receptors for 5-hydroxytryptamine and noradrenaline, similar to those in the periphery, are not present in pyriform cortical neurones.

The authors wish to thank Miss C. O. Hebb and Dr K. Krnjević for their helpful comments on this paper. Mrs T. E. Hartridge and Miss J. Aldred provided valuable secretarial assistence. M. R. was supported by a grant from the Medical Research Council. D. W. S. is a Rothschild Fellow.

REFERENCES

ADRIAN, E. D. (1942). Olfactory reactions in the brain of the hedgehog. *J. Physiol. (Lond.)*, **100**, 459–473.

ADRIAN, E. D. (1950). The electrical activity of the mammalian olfactory bulb. *Electroenceph. clin. Neurophysiol.*, **2**, 377–388.

AMIN, A. H., CRAWFORD, T. B. B. & GADDUM, J. H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol. (Lond.)*, **126**, 596–618.

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

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

BAUMGARTEN, R. VON, BLOOM, F. E., OLIVER, A. P. & SALMOIRAGHI, G. C. (1963). Response of individual olfactory nerve cells to microelectrophoretically administered chemical substances. *Pflügers Arch. ges. Physiol.*, **277**, 125–140.

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

BLOOM, F. E., COSTA, E., OLIVER, A. P. & SALMOIRAGHI, G. C. (1964). Caudate nucleus neurons: their responsiveness to iontophoretically administered amines and the effects of anesthetic agents. *Fed. Proc.*, **23**, 249.

BLOOM, F. E., COSTA, E. & SALMOIRAGHI, G. C. (1964). Analysis of individual rabbit olfactory bulb neuron responses to the microelectrophoresis of acetylcholine norepinephrine and serotonin synergists and antagonists. *J. Pharmacol. exp. Ther.*, **146**, 16–23.

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

BOGDANSKI, D. F., WEISSBACH, H. & UDENFRIEND, S. (1957). The distribution of serotonin, 5-hydroxytryptophan decarboxylase, and monoamine oxidase in brain. *J. Neurochem.*, **1**, 272–278.

BOND, H. W. & GUTH, P. S. (1964). Interaction of 5-hydroxytryptamine and lysergic acid diethylamide in the central nervous system. *Pharmacologist*, **6**, 171.

BRADLEY, P. B., DHAWAN, B. N. & WOLSTENCROFT, J. H. (1964). Some pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. *J. Physiol. (Lond.)*, **170**, 59–60*P*.

BRADLEY, P. B. & WOLSTENCROFT, J. H. (1962). Excitation and inhibition of brain-stem neurones by noradrenaline and acetylcholine. *Nature (Lond.)*, **196**, 840, 873.

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

BURGEN, A. S. V. & CHIPMAN, L. M. (1951). Cholinesterase and succinic dehydrogenase in the central nervous system of the dog. *J. Physiol. (Lond.)*, **114**, 296–305.

CARLSSON, A., FALCK, B., FUXE, K. & HILLARP, N-Å. (1964). Cellular localization of monoamines in the spinal cord. *Acta physiol. scand.*, **60**, 112–119.

CARLSSON, A., FALCK, B., HILLARP, N.-Å. & TORP, A. (1962). Histochemical localization at the cellular level of hypothalamic noradrenaline. *Acta physiol. scand.*, **54**, 385–386.

CLARK, W. E. LE GROS (1947). The terminal connexions of the olfactory tract in the rabbit. *Brain*, **70**, 304–328.

CRAWFORD, J. M. & CURTIS, D. R. (1964). The excitation and depression of mammalian cortical neurones by amino acids. *Brit. J. Pharmacol.*, **23**, 313–329.

CRAWFORD, J. M., CURTIS, D. R., VOORHOEVE, P. E. & WILSON, V. J. (1963). Excitation of cerebellar neurones by acetylcholine. *Nature (Lond.)*, **200**, 579–580.

CURTIS, D. R. (1962). Action of 3-hydroxytyramine and some tryptamine derivatives on spinal neurones. *Nature (Lond.)*, **194**, 292.

CURTIS, D. R. (1964). Microelectrophoresis. In *Physical Techniques in Biological Research*, ed. NASTUK, W. L., vol. 5, part A, pp. 144–190. London: Academic Press.

CURTIS, D. R. & ANDERSEN, P. (1962). Acetylcholine: a central transmitter? *Nature* (*Lond.*), **195**, 1105–1106.

CURTIS, D. R. & DAVIS, R. (1962). Pharmacological studies upon neurones of the lateral geniculate nucleus of the cat. *Brit. J. Pharmacol.*, **18**, 217–246.

CURTIS, D. R. & DAVIS, R. (1963). The excitation of lateral geniculate neurones by quaternary ammonium derivatives. *J. Physiol.* (*Lond.*), **165**, 62–82.

CURTIS, D. R. & KOIZUMI, K. (1961). Chemical transmitter substances in brain stem of cat. *J. Neurophysiol.*, **24**, 80–90.

CURTIS, D. R. & PHILLIS, J. W. (1960). The action of procaine and atropine on spinal neurones. *J. Physiol.* (*Lond.*), **153**, 17–34.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1961). Cholinergic and non-cholinergic transmission in the mammalian spinal cord. *J. Physiol.* (*Lond.*), **158**, 296–323.

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

DAHLSTRÖM, A. & FUXE, K. (1964). Localisation of monoamines in the lower brain stem. *Experientia* (*Basel*), **20**, 398–399.

DALE, H. H. (1914). The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharmacol. exp. Ther.*, **6**, 147–190.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol.* (*Lond.*), **107**, 372–381.

FOX, C. A., MCKINLEY, W. A. & MAGOUN, H. W. (1944). An oscillographic study of olfactory system of cats. *J. Neurophysiol.*, **7**, 1–16.

GADDUM, J. H. (1953). Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. *J. Physiol.* (*Lond.*), **121**, 15*P*.

GADDUM, J. H. & HAMEED, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **9**, 240–248.

GREEN, J. D., CLEMENTE, C. D. & DE GROOT, J. (1957). Rhinencephalic lesions and behaviour in cats. *J. comp. Neurol.*, **108**, 505–545.

GREEN, J. D., MANCIA, M. & BAUMGARTEN, R. VON (1962). Recurrent inhibition in the olfactory bulb. I. Effects of antidromic stimulation of the lateral olfactory tract. *J. Neurophysiol.*, **25**, 467–488.

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

HOFMANN, A. (1959). Psychotomimetic drugs, chemical and pharmacological aspects. *Acta physiol. pharm. neerl.*, **8**, 240–258.

JASPER, H. H. & AJMONE-MARSAN, C. (1960). *A Stereotaxic Atlas of the Diencephalon of the Cat.* Ottawa: National Research Council.

KRNJEVIĆ, K. (1965). Actions of drugs on single neurones in the cerebral cortex. *Brit. med. Bull.*, **21**, 10–14.

KRNJEVIĆ, K. LAVERTY, R. & SHARMAN, D. F. (1963). Iontophoretic release of adrenaline, noradrenaline and 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **20**, 491–496.

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

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

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

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

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

KRNJEVIĆ, K. & SILVER, A. (1965). A histochemical study of cholinergic fibres in the cerebral cortex. *J. Anat.*, **99**, 711–760.

KUNTZMAN, R., SHORE, P. A., BOGDANSKI, D. & BRODIE, B. B. (1961). Microanalytical procedures for fluorometric assay of brain DOPA-5HTP decarboxylase, norepinephrine and serotonin, and a detailed mapping of decarboxylase activity in brain. *J. Neurochem.*, **6**, 226–232.

MACLEAN, P. D. & DELGADO, J. M. R. (1953). Electrical and chemical stimulation of frontotemporal portion of limbic system in the waking animal. *Electroenceph. clin. Neurophysiol.*, **5**, 91–100.

MACLEAN, P. D., HORWITZ, N. H. & ROBINSON, F. (1952). Olfactory-like responses in pyriform area to non-olfactory stimulation. *Yale J. Biol. Med.*, **25**, 159–172.

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

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

OLDS, J. (1958). Self-stimulation of the brain. *Science*, **127**, 315–324.

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.

PHILLIPS, C. G., POWELL, T. P. S. & SHEPHERD, G. M. (1963). Responses of mitral cells to stimulation of the lateral olfactory tract in the rabbit. *J. Physiol. (Lond.)*, **168**, 65–88.

PHILLIS, J. W. (1965). Cholinergic mechanisms in the cerebellum. *Brit. med. Bull.*, **21**, 26–29.

POKROVSKY, M. V. (1960). In *Medical Electronics*, ed. SMYTH, C. N., pp. 85–86. London: Iliffe & Sons.

RANDIĆ, M., SIMINOFF, R. & STRAUGHAN, D. W. (1964). Acetylcholine depression of cortical neurons. *Exp. Neurol.*, **9**, 236–242.

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

SALMOIRAGHI, G. C., BLOOM, F. E., OLIVER, A. P. & COSTA, E. (1964). Adrenergic inhibition in rabbit olfactory bulb. *Fed. Proc.*, **23**, 152.

SALMOIRAGHI, G. C. & STEINER, F. A. (1963). Acetylcholine sensitivity of cat's medullary neurons. *J. Neurophysiol.*, **26**, 581–597.

SCHREINER, L. & KLING, A. (1953). Behavioural changes following rhinencephalic injury in cat. *J. Neurophysiol.*, **16**, 643–659.

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

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol. (Lond.)*, **123**, 451–481.

VOGT, M., GUNN, C. G. & SAWYER, C. H. (1957). Electroencephalographic effects of intraventricular 5HT and LSD in the cat. *Neurology (Minneap.)*, **7**, 559–566.

WOOLLEY, D. W. (1958). Neurologic and psychiatric changes related to serotonin. In *Progress in Neurobiology III: Psychopharmacology*, ed. PENNES, H. H., pp. 152–170. New York: Hoeber-Harper.