J. Physiol. (1963), **165**, *pp.* 62–82
With 5 text-figures
Printed in Great Britain

THE EXCITATION OF LATERAL GENICULATE NEURONES BY QUATERNARY AMMONIUM DERIVATIVES

By D. R. CURTIS and R. DAVIS
From the Department of Physiology, Australian National University, Canberra, Australia

(*Received* 5 *March* 1962)

The excitation of neurones in the lateral geniculate nucleus by impulses in optic nerve fibres is depressed by certain indoles, particularly by 4-, 5-, and 7-hydroxytryptamine but also by some lysergic-acid derivatives (Curtis & Davis, 1962). The nature of this depression is such as to suggest a structural relationship between these depressants and the excitatory transmitter which is released by the optic nerve terminals. However, although a large series of indoles and tryptamine derivatives was applied electrophoretically to geniculate neurones, no excitant was found. The investigation was extended to include compounds active at other synapses and the finding that acetylcholine activated lateral geniculate neurones (Jung, 1959) was confirmed. This choline ester is unlikely to be the excitatory transmitter released by optic nerve fibres, since there is very little choline acetylase in the optic nerve (Hebb, 1957). Consequently, other choline esters and related quaternary ammonium compounds were applied electrophoretically to geniculate neurones. This method of application was essential because many of these agents fail to penetrate the blood–brain barrier (Curtis & Eccles, 1958*b*).

METHODS

The experiments were performed upon neurones located in the dorsal part of the lateral geniculate nucleus of the cat. The animals were anaesthetized with pentobarbital sodium and the neurones were activated synaptically, either by electrical stimulation of the contralateral optic nerve, or by a light flash applied to the intact ipsilateral eye. This eye was shielded from stray light and the room in which the experiments were performed was darkened. The cells were located stereotaxically and the methods were identical with those of the earlier investigation (Curtis & Davis, 1962). The spike responses of single neurones, and the focal potentials generated by the activity of many cells, were recorded by means of the centre barrel of five-barrel electrodes. These electrodes had a total tip diameter of 5–8 μ and the recording barrel contained 5 M-NaCl. The four outer barrels contained saturated aqueous solutions of salts of the compounds to be tested, from which the substances were passed as cations. The electrodes were filled from above and then centrifuged for 5 min with the tips outermost (1600 *g*). In this way complete filling of all five barrels was obtained and substances could be applied to neurones within 20 min of the preparation of a solution.

This method of filling was thus superior to the previously described diffusion technique in which filling occupied 48 hr (Curtis & Eccles, 1958*a*).

As in the earlier investigation, the spike responses of single neurones were counted by means of a rate-meter, the counts being displayed on an ink recorder. Routinely a counting period of 1 sec was used, a period of 0·2 sec being allowed between counts. When cells were activated by a light flash (400 msec in duration), the total number of spikes occurring in a fixed period after the onset of the flash could be displayed.

Electrophoretic currents are expressed as nA (10^{-9} A) and a cationic current is one which applies a cation from the electrode. Unless otherwise mentioned, a backing potential of 0·5 V was applied to each drug-containing barrel of these electrodes in order to control diffusion from the tips (Curtis & Eccles, 1958*a*). 5-Hydroxytryptamine was used as the complex with creatinine sulphate and the combined agent will be referred to as 5-HT. The compounds which were tested were obtained either commercially or as gifts from other investigators.

RESULTS

Throughout these experiments observations were made upon cells located in all layers of the dorsal part of the lateral geniculate nucleus, and, as with the depressant compounds, no difference was observed in the sensitivity to pharmacological agents of neurones fired monosynaptically by volleys in the low- or high-threshold contralateral optic nerve fibres or by light-evoked impulses from the ipsilateral eye (Curtis & Davis, 1962). Before presenting results obtained with the various compounds it will be convenient to discuss briefly the properties expected of depressants and excitants of geniculate neurones. The effects produced by these agents are somewhat complicated by the tendency of lateral geniculate neurones to fire several times when activated by just-threshold optic nerve volleys, whereas maximal optic nerve stimulation usually evokes but a single spike (Bishop & Davis, 1960). This is in contrast to spinal interneurones, which fire repetitively only in response to synaptic excitation above that necessary to produce a single spike.

5-Hydroxytryptamine (5-HT) depresses the orthodromic excitation of lateral geniculate neurones by optic nerve impulses, but is without effect upon the antidromic activation of the cells. Furthermore, the excitation of these neurones by L-glutamic acid is unaffected (Curtis & Davis, 1962). When applied electrophoretically to neurones excited by a maximal optic nerve volley, 5-HT usually caused changes identical with those observed when the strength of the nerve stimulus was reduced. During the first few seconds of application the nerve volley evoked two or three spikes, and this phase of repetitive firing was followed by complete suppression of the post-synaptic spike response. With very weak depressants such as morphine, complete suppression of spike responses was occasionally not obtained; the production of this post-stimulus repetitive firing could then mistakenly be considered a property of an excitant. Nevertheless, depressant compounds were easily detected because their action upon single

neurones was paralleled by a reduction in the amplitude of field potentials which were produced by the synaptic activation of many neurones.

When recording extracellularly, the depolarization produced by an excitant compound must exceed the threshold for firing before an excitatory action can be detected; an extracellular electrode cannot detect a steady membrane depolarization, but merely detects the presence or absence of spike potentials of a neurone. When the depolarization reaches threshold, continuous repetitive firing may occur, such as follows the application of acetylcholine to Renshaw cells (Curtis & Eccles, 1958*a*). However, if the drug-induced depolarization remains subthreshold for spike initiation, the excitatory action could be demonstrated only by showing that the agent facilitates firing evoked by other means. In the case of geniculate neurones it must be remembered that an increase in synaptic excitation may convert the two or three spikes that are evoked by a submaximal synaptic stimulus to a single response (Bishop & Davis, 1960). A convenient way to test the excitability of neurones was to apply L-glutamic acid from one of the barrels of the multibarrel electrode. This amino acid produces repetitive firing of lateral geniculate neurones which is not depressed by the simultaneous application of tryptamine derivatives (Curtis & Davis, 1962). Another way of detecting an excitant action was to study the effect of the agent upon focal potentials generated by the synaptic excitation of many cells. These potentials are readily reduced in amplitude by the electrophoretic application of 5-HT, and an excitant might be expected to increase their magnitude, not only by increasing the number of neurones in the vicinity of the electrode which produce spike potentials, but also by reducing the latency of firing of individual cells. In this type of experiment excessive depolarization may cause the blocking of neuronal responses and the reduction of field potentials, which would be misinterpreted as a depressant action (cf. Curtis, Phillis & Watkins, 1960).

Acetylcholine

The excitants investigated were not of high potency when compared with their actions upon cholinoceptive Renshaw cells (cf. Curtis & Eccles, 1958*a*; Curtis, Phillis & Watkins, 1961). Apart from carbachol, the agents usually failed to activate cells unless a background discharge was provided, either by the application of L-glutamic acid or by synaptic action. The features of excitatory action can be demonstrated in detail by considering the effect of acetylcholine (ACh). The effects of the other related compounds differ only in magnitude and time course. Acetylcholine was applied as a cation to over 60 neurones in 10 different preparations. Half the cells responded synaptically to a contralateral optic nerve volley and the others were activated by an ipsilateral light flash. Three cells were

activated by impulses in both optic nerves. The 'background' discharge rate of cells which could only be activated by light was usually higher than that of cells responding only to a contralateral optic nerve volley, and almost invariably an application of ACh increased this rate. This effect is illustrated in Fig. 1*A* where the actions of ACh and 5-HT are compared

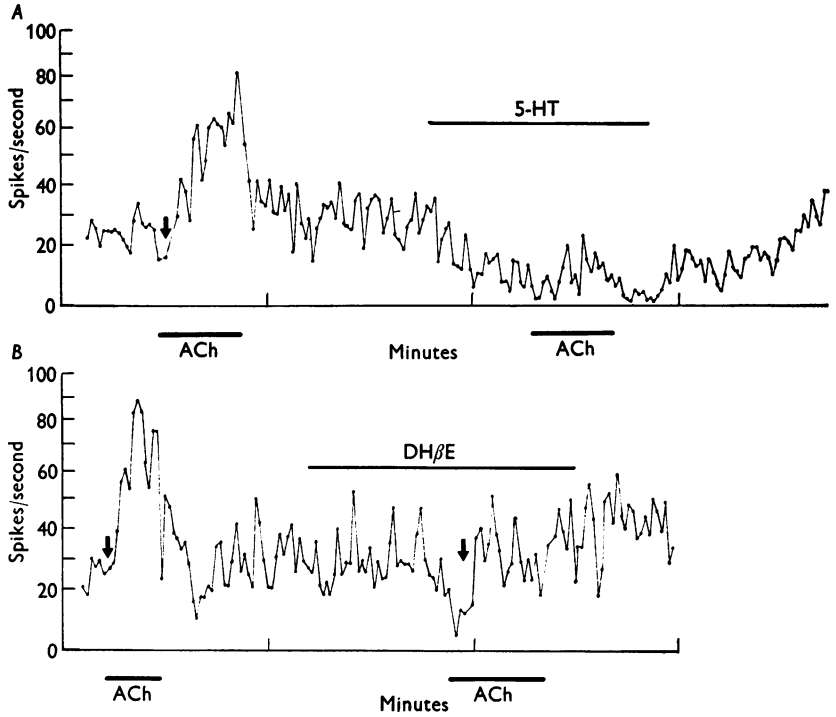


Fig. 1. The frequency of firing a lateral geniculate neurone is indicated by the black dots, which chart the number of spikes counted in the previous 1 sec. *B* is continuous with *A*; this neurone was firing spontaneously at a rate of 20–40 spikes/sec. The downward arrows show defects in the record due to overloading of an amplifier in the counting circuit, which occurred at the beginning of the ACh applications. ACh was applied, for the periods indicated by heavy black lines beneath each section of the recording, by means of a current of 100 nA. The application of 5-hydroxytryptamine (5-HT) by a current of 20 nA, and of dihydro-β-erythroidine (DHβE) by a current of 50 nA are indicated by the upper horizontal lines in *A* and *B*, respectively.

upon the 'spontaneous' discharge of a single neurone located in layer A_1 of the nucleus. The spike responses were recorded by means of the central barrel of a 5-barrel electrode and after suitable amplification were counted for periods of 1 sec, as mentioned in Methods. Throughout the series (I*A* and *B*) the background illumination of the ipsilateral eye was constant, the rate of discharge of the neurone being of the order of 20–30 spikes/sec.

After an initial control period, ACh was applied from one barrel of a 5-barrel electrode for 24 sec, by a cationic electrophoretic current of 100 nA. Over a period of about 8 sec the discharge rate of the neurone increased to 60/sec. Following the cessation of the ACh application the discharge rate fell. The actual recovery time was difficult to evaluate, owing to the inconstant rate of discharge, but exceeded 15 sec. Fifty-five seconds after the first dose of ACh, 5-HT was applied from another barrel of the electrode and the discharge rate subsided over the ensuing 2 min. After the termination of the application of 5-HT, the discharge rate gradually increased and, at the beginning of Fig. 1*B*, had recovered to the

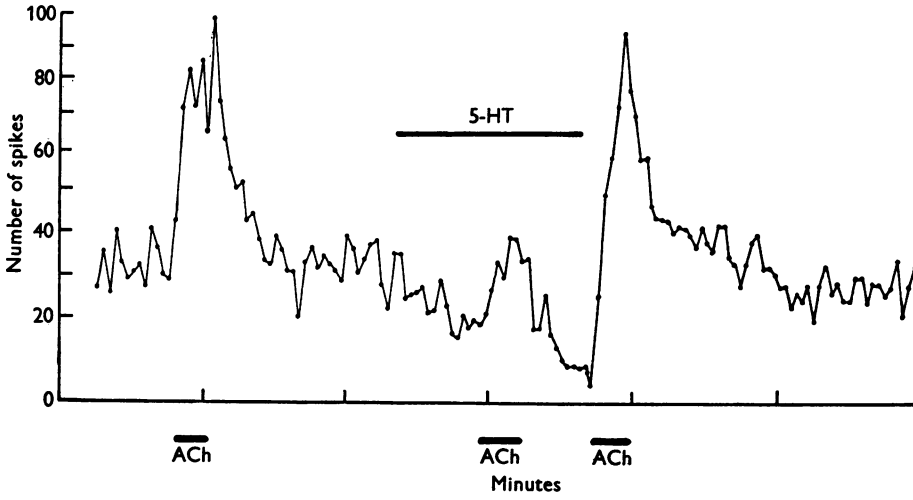


Fig. 2. The ordinate of this graph describes the number of spikes of a lateral geniculate neurone, counted in periods of 1·4 sec, during the first 0·4 sec of which a flash of light stimulated the ipsilateral eye. The stimulus was repeated every 2·5 sec throughout the record. Three applications of ACh were made with a current of 65 nA (horizontal lines beneath the recording) and 5-HT was applied with a current of 15 nA.

earlier value of 20–30/sec in 90 sec. During the administration of 5-HT a further electrophoretic application of ACh (100 nA) produced a slight increase in the rate of discharge, but this was not sustained. Following recovery from the depressant effect of 5-HT, another application of ACh was as effective as the first dose in increasing the discharge rate (Fig. 1*B*). The possible interaction between 5-HT and ACh will be discussed below.

Figure 2 illustrates the effect of ACh upon the number of spike responses evoked by a light flash, and recorded from a cell in layer A_1 of the lateral geniculate nucleus. A light flash of 400 msec duration was applied every 2·5 sec and the number of spikes counted for a period of 1·4 sec after the

start of the flash. The 'spontaneous' discharge rate of the cell was only 1–2/sec, and the light stimuli produced 25–40 spikes. Three applications of ACh were made by an electrophoretic current of 65 nA. The first and third applications were effective in increasing the number of light-evoked spikes for as long as 60 sec, whereas the second application, during the administration of 5-HT, was much less effective. As with the neurone illustrated in Fig. 1, 5-HT was an effective depressant of the light-evoked responses, and apparently reduced the excitant action of ACh.

The excitatory action of ACh upon neurones which were activated synaptically only by contralateral optic nerve volleys is shown in Figs. 3 and 4. In Fig. 3 the spike responses of one neurone are indicated by arrows, and in the control records (Fig. 3*A*) the spikes follow a focal potential generated by the excitation of nearby neurones. The electrophoretic application of 5-HT (20 nA) abolished the spike responses within 13 sec (Fig. 3*B*) and reduced the focal potential (Fig. 3*C*). Recovery was observed 29 sec after this application ceased (Fig. 3*D*). In contrast, although the repetitive firing evoked by a just-threshold optic nerve volley (*E*) was abolished by ACh (70 nA), the latency of the first spike was decreased (Fig. 3*F*, lower record) and the initial component of the recorded potential increased in magnitude, presumably because of the superimposition of the spike upon potentials generated by nearby cells which were also affected by the choline ester. This action of ACh was similar to the effect produced by increasing the strength of the optic nerve stimulus and can be ascribed to a membrane depolarization produced by the compound. The effect was reversible, and repetitive firing was observed 42 sec after the cessation of the ACh application (Fig. 3*H*). Larger concentrations of the choline ester, applied by means of electrophoretic currents of 150–200 nA, failed to suppress the firing of this and other neurones. Figure 4*A* shows the firing frequency of another neurone which had virtually no spontaneous discharge and which was activated only by volleys in the contralateral optic nerve. ACh failed to excite this neurone when applied alone by an electrophoretic current of 150–200 nA. The excitant action of ACh was, however, shown by using test applications of L-glutamic acid. The amino acid was applied as an anion from a solution of the sodium salt (pH 8·0) with an electrophoretic current of 100 nA and evoked firing at a frequency of 24–34/sec. These applications are indicated by the horizontal lines at the lower margin of the figure, and were of a duration such as to produce a plateau at which the firing rate was maximal. During the application of ACh (90 nA) the frequency of firing evoked by the amino acid was increased, the maximal increase in this case was observed after the ACh application was terminated. Thereafter recovery occurred in approximately 90 sec. This technique of testing neurone

excitability by means of comparatively brief applications of L-glutamate ion was invariably successful in detecting the excitant action of ACh and of closely related compounds upon lateral geniculate neurones. The possibility that this excitatory action of choline esters was due merely to

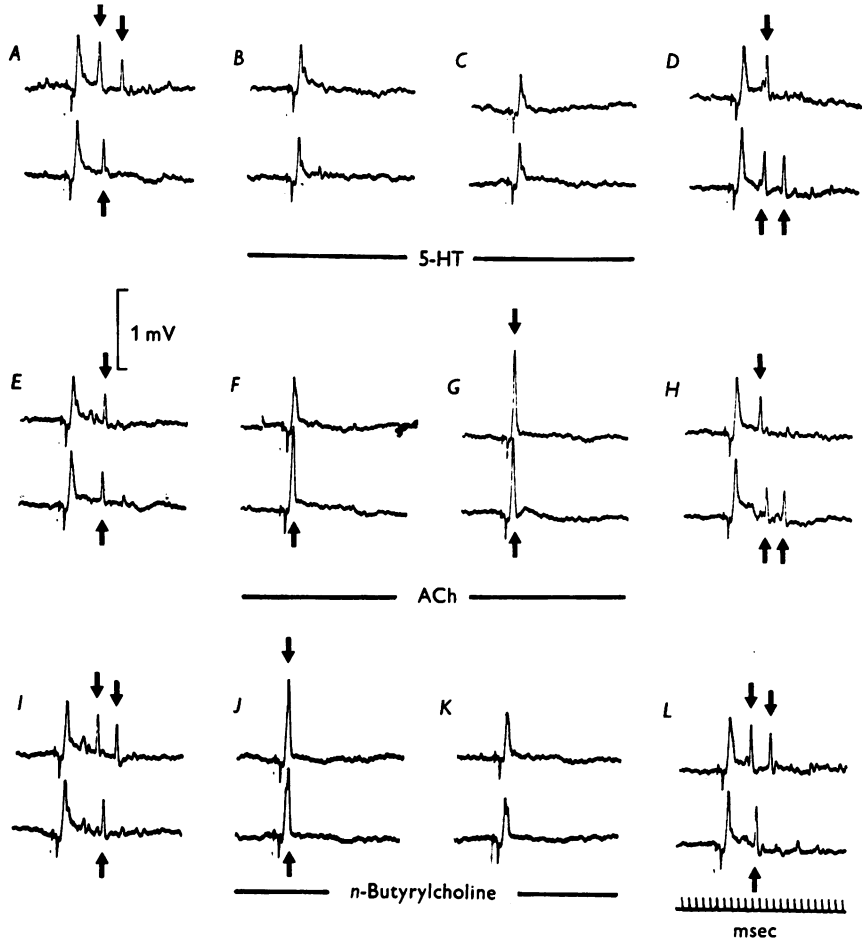


Fig. 3. Potentials recorded by the central barrel of a 5-barrel electrode in the vicinity of a single lateral geniculate neurone in response to submaximal optic nerve stimulation, the spikes being indicated by arrows. The two responses of each section were photographed at an interval of 1 sec, the upper preceding the lower. *A*, *E*, *I*, controls. *B*, *C*, 13 sec and 19 sec after a current of 20 nA began to apply 5-HT from one barrel of the electrode; *D*, 29 sec after this application was ended. *F*, *G*, 2 sec and 12 sec after a current of 70 nA began to apply the ACh from another barrel; *H*, 42 sec after the application ceased. *J*, *K*, 12 sec and 31 sec after a current of 80 nA began to apply *n*-butyrylcholine; *L*, 16 sec after this current was ended. Voltage calibration, 1 mV for all records. Time marker for all records, msec.

current flow was excluded by passing an inert cation, such as Na^+, into the environment of the neurones. When this was done no potentiation of the neuronal responses to L-glutamic acid was observed.

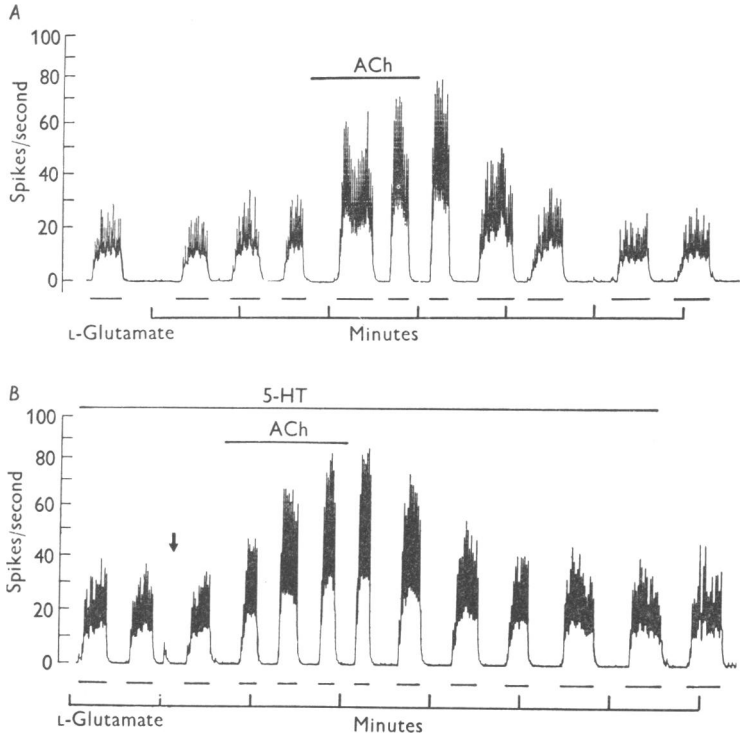


Fig. 4. The frequency of firing of a single lateral geniculate neurone evoked by applications of L-glutamate ion (100 nA) for the times indicated by the horizontal lines beneath the charts. The frequency is indicated, as in Fig. 5, by the peaks of the recordings which chart the number of spikes counted in the previous 1 sec. In both *A* and *B* ACh was applied with currents of 90 nA and in *B* a current of 20 nA applied 5-HT for the time shown by the upper horizontal line. The downward arrow indicates the time when the synaptically-evoked responses of the cell were blocked by 5-HT.

When ACh is applied to Renshaw cells the maximal frequency of firing is produced within 5–10 sec, and the firing invariably ceases within 1–3 sec after the termination of the electrophoretic current (Curtis & Eccles, 1958*a*). In contrast to this, the excitant action of ACh upon lateral geniculate neurones had a much slower onset (Figs. 1, 2, 4 and 5), and occasionally the maximal effect was not obtained for 60 sec. In addition, following the cessation of the electrophoretic current, recovery was rarely complete in 15 sec and occasionally took as long as $2\frac{1}{2}$ min.

Interaction of ACh and other pharmacologically active agents

The results presented above raise two questions concerning the importance of cholinoceptive receptors on geniculate neurones. Previous investigations have shown that the synaptic excitation of these cells by optic nerve volleys is depressed by certain tryptamine derivatives (Curtis & Davis, 1962). Consequently it was of interest to determine whether these compounds also depressed the excitation evoked by ACh. Secondly, the presence of receptors with which ACh can interact suggests the possibility that this substance, or a chemically related compound, may normally be a synaptic transmitter in this region of the central nervous system. Thus experiments were performed with both anticholinesterases and known blocking agents of ACh receptors. Tests with anticholinesterases suffered from the complication that both neostigmine and edrophonium excited lateral geniculate neurones in a manner similar to ACh.

The interaction between 5-HT and ACh has already been mentioned in reference to Figs. 1 and 2. In both cases the excitant action of the choline ester was depressed during the administration of 5-HT. However, in the case of the neurone illustrated in Fig. 1 a 'background' discharge rate of 20–30 spikes/sec was provided by impulses generated in the ipsilateral eye, and in Fig. 2 the excitant action of ACh was demonstrated by its effect upon the number of spikes which were evoked by a flash of light. The synaptic firing of both neurones was diminished by 5-HT and the apparent decrease in the ACh effect could be due merely to the reduction of the synaptic depolarization, since ACh by itself was unable to evoke firing. Although from structural considerations it is unlikely that 5-HT and ACh could react with the same receptor site, a direct antagonism between these compounds would only be detected if the tryptamine derivative reduced the effect of the excitant, under conditions where synaptic depolarization was virtually absent. These conditions were obtained by using neurones of layer A or B which failed to respond to an ipsilateral light flash and had no spontaneous discharge. The excitant action of ACh was demonstrated by testing with L-glutamic acid as in Fig. 4*A*.

Figure 5 illustrates an experiment performed upon a lateral geniculate neurone which responded to impulses only in high-threshold fibres of the contralateral optic nerve. Brief applications of L-glutamate (lower line) were made with a current of 63 nA, and the maximal rate of firing so produced was originally about 50/sec. The whole experiment lasted $10\frac{1}{2}$ min, and the frequency of firing evoked by L-glutamate alone fell slightly, so that towards the end of the series the maximal frequency was only about 42/sec. ACh was administered three times, as indicated in the figure (ACh), and one application, of slightly longer duration than the other two,

was made simultaneously with 5-HT. The tryptamine derivative abolished the synaptic excitation of the neurone when this was tested 40 sec after the start of the current (indicated by the downward arrow), and recovery was observed (upward arrow) 97 sec after the current applying 5-HT was terminated. Nevertheless, ACh still facilitated the firing evoked by L-glutamic acid, in spite of the presence of a compound which completely suppressed the synaptic excitation of the neurone.

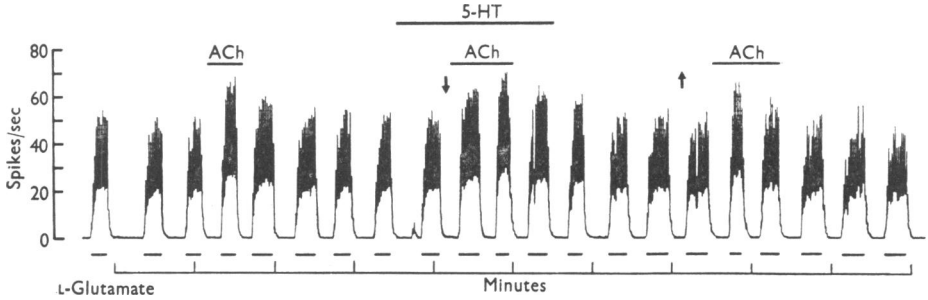


Fig. 5. The frequency of firing of a lateral geniculate neurone evoked by electrophoretic applications (63 nA) of L-glutamate ion, the duration of these being indicated by the lower line of the figure. Three applications of ACh were made (50 nA); the second was simultaneous with the administration of 5-HT (50 nA). The durations of these applications are shown by the upper horizontal lines. The downward and upward arrows signal the onset and recovery respectively of the suppression of the orthodromic responses of the neurone.

Conditions such as those shown in Fig. 5, in which the neuronal response to L-glutamic acid remained reasonably constant for a prolonged period, were rarely obtained, but many other neurones were observed in which only two applications of ACh were made, before and during the administration of 5-HT. Such an experiment is illustrated in Fig. 4, where ACh potentiated the firing evoked by L-glutamic acid (Fig. 4*A*). This action was virtually unaffected by the simultaneous application of 5-HT (Fig. 4*B*). 5-HT also failed to affect the glutamate responses but suppressed the orthodromic activation of the neurone (indicated by a downward arrow) shortly before ACh was applied.

The failure of 5-HT to influence the excitant action of ACh, as demonstrated by the glutamate test, was observed with five different neurones. In two, excitation by ACh was also unaffected by 4-hydroxytryptamine (4-HT), which is a more powerful depressant of the synaptic excitation than 5-HT (Curtis & Davis, 1962). In several other neurones simultaneous application of 5-HT reduced the excitatory action of ACh applied by weak currents (20–50 nA) but not that produced by stronger currents (100 nA). In these cases the excitatory action of ACh was revealed by testing the

neurone excitability with L-glutamic acid, and it is probable that in addition there was a background depolarization produced by random subthreshold synaptic activity. The reduction of this excitation by 5-HT would be sufficient to produce an apparent diminution in the excitatory action of ACh. In all these cases it was clear that, although 5-HT completely abolished the synaptic excitation of lateral geniculate neurones by optic nerve impulses, the excitatory action of ACh could always be demonstrated. This excitatory action was, however, of subthreshold intensity and required the simultaneous application of an excitant amino acid in order to be detected. It can be concluded, therefore, that ACh and 5-HT are unlikely to interact with the same receptor site.

When applied electrophoretically, dihydro-β-erythroidine (DHβE) was an effective depressant of the excitation of Renshaw cells by both ventral root volleys and electrophoretically applied ACh (Curtis & Eccles, 1958*b*; Curtis *et al.* 1961). When applied with electrophoretic currents of 40–100 nA, DHβE failed to depress the synaptic excitation of the 25 lateral geniculate neurones upon which it was tested. However, in tests on 9 of these neurones, the excitant action of ACh was diminished or abolished by DHβE. In Fig. 1*B* are shown the results from one cell which was firing spontaneously, presumably because of impingement of 'background' light upon the ipsilateral eye. This cell was activated by ACh (100 nA for 16 sec); but when an additional application was made during the administration of DHβE (50 nA) ACh failed to increase the firing rate. The blocking action of DHβE upon Renshaw cells was usually prolonged (Curtis & Eccles, 1958*b*), and, although definitive experiments were not carried out, the impression was gained that the depression of the ACh excitation of geniculate neurones by this compound was of comparatively short duration.

Tubocurarine was applied with currents of 30–50 nA for periods as long as 60 sec; it failed to depress the action of ACh and had neither an excitant nor a depressant action upon the cells. Gallamine triethiodide (Flaxedil) proved to be an excitant with an action similar to, but less potent than, that of ACh.

Other excitants and related compounds

Although possibly not related to the synaptic excitation of lateral geniculate neurones by optic nerve volleys, the excitatory effect of ACh was nevertheless of considerable interest, since experiments carried out upon spinal interneurones failed to demonstrate any excitatory activity of this and other choline esters (Curtis *et al.* 1961). Furthermore, these substances do not facilitate the firing of spinal interneurones by the excitant amino acids (D. R. Curtis, and J. C. Watkins, unpublished observations).

In order to determine the nature of the receptor with which ACh was

interacting, a number of compounds were tested for their possible excitatory effect upon geniculate neurones. The compounds were selected because of structural resemblances to ACh or because of known activities at cholinoceptive synapses. A full structure-activity comparison, with a determination of the potencies of the various compounds, was not attempted. Instead, an effort was made merely to gauge whether the various compounds were more or less active than ACh in order to ascertain the gross structural features which were important for combination with the receptor sites. The electrophoretic application of ACh to Renshaw cells with currents of 10–100 nA produced firing at rates of 50–120 spikes/sec (Curtis & Eccles, 1958*a*). However, with a similar application quiescent geniculate neurones were not excited even though currents of 100–150 nA were used. The excitant action of ACh and the other active agents was revealed by the potentiation of the firing evoked either by synaptic excitation or the application of L-glutamate ion. The potencies, relative to ACh, were assessed by comparing the electrophoretic currents which were necessary to produce approximately equal degrees of facilitation of neuronal responses. In addition, many of the agents were applied whilst recording the focal potentials generated by the synaptic excitation of many neurones. The active excitants increased these potentials by 10–30 %, and a rough estimate of potency was obtained by comparing currents necessary to produce a similar augmentation of focal potentials. The maximal electrophoretic current used was 150 nA.

The compounds of Table 1 are arranged according to their structures and the potencies are expressed relative to that of ACh. The number of symbols is not a measure of potency but merely indicates greater (+ + +) or less (+) activity than ACh (+ +). The enclosure of a potency in brackets in the table indicates that there is some doubt concerning its true value, but in these cases the order of the activity was such that further investigation was not warranted.

The activity of ACh was exceeded only by that of carbamoylcholine (Table 1*A*). In several instances applications of this choline ester fired quiescent lateral geniculate neurones, though the frequencies did not exceed 20 spikes/sec. The duration of the action of carbamoylcholine invariably exceeded that of ACh and with both compounds a β-methyl side-chain substituent reduced the excitant potency (Table 1*A*–acetyl-β-methylcholine and carbamoyl-β-methylcholine). Although both acetyl-β-methylcholine and *n*-butyryl-choline potentiated the excitant action of L-glutamic acid, the synaptic excitation of lateral geniculate neurones by optic nerve volleys was initially facilitated and then depressed by these compounds. This effect of the butyryl ester is shown in Fig. 3, where the repetitive firing of the neurone (Fig. 3*I*) was replaced by a single spike of

shorter latency (Fig. 3*J*), 12 sec after the application commenced. This effect is thus identical to that of ACh (Fig. 3*F* and *G*), but continued application of *n*-butyrylcholine depressed the neuronal responses (*K*) so that the action was then similar to that of 5-HT (Fig. 3*C*). Recovery from this apparently dual action of the choline ester was fairly rapid, the responses of Fig. 3*L* being recorded 16 sec after the electrophoretic current was terminated. In contrast to this facilitation of the amino-acid-evoked excitation, but depression of the orthodromically-evoked responses,

TABLE 1. Compounds tested as excitants of geniculate neurones

Group	Compound	Potency*
(*A*) Choline and esters	Choline chloride	(+)
	Acetylcholine bromide	++
	Acetyl-β-methylcholine chloride	+
	Propionylcholine *p*-toluenesulphonate	+ (+)
	n-Butyrylcholine iodide	+
	γ-Aminobutyrylcholine chloride hydrochloride	0†
	Crotonylcholine iodide	(+)
	β,β-Dimethylacrylylcholine iodide	(+)
	Urocanylcholine chloride hydrochloride	(+)
	Carbamoylcholine chloride	++++
	Carbamoyl-β-methylcholine chloride	+
	Succinylcholine bromide	(+)
(*B*) Betaines‡	β-Propiobetaine	0
	γ-Butyrobetaine	0
	(±)-Carnitine	0
	Acetyl-(±)-carnitine	0
(*C*) Simple quaternary ammoniums	Tetramethylammonium bromide	+ (+)
	Tetraethylammonium bromide	+
(*D*) Complex quaternary ammoniums	Phenyltrimethylammonium iodide	(+)
	3-Hydroxyphenyltrimethylammonium iodide	(+)
	3-Acetoxyphenyltrimethylammonium methyl-sulphate	(+)
	3-Hydroxyphenyldimethylethylammonium chloride	+
	Neostigmine bromide	+
(*E*) Bis-quaternary ammoniums	1,5-Pentane-bis(trimethylammonium) iodide	(+)
	1,6-Hexane-bis(trimethylammonium) iodide	(+)
	1,10-Decane-bis(trimethylammonium) iodide	+
(*F*) Miscellaneous	Arecoline hydrobromide	+
	Nicotine hydrochloride	(+)
	Gallamine triethiodide	+

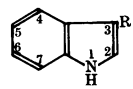
* Potency expressed in relation to that of ACh, ++ (see text). † See text. ‡ These substances were dissolved in hydrochloric acid to produce solutions of pH 3 from which they were passed electrophoretically as cations.

γ-aminobutyrylcholine depressed both the amino-acid and synaptic excitation of these neurones. The action of this choline ester was thus identical to that of γ-amino-*n*-butyric acid, which had the same effect upon geniculate neurones as upon spinal interneurones (Curtis, Phillis & Watkins, 1959). β-Propiobetaine, γ-butyrobetaine, DL-carnitine and acetyl-DL-carnitine (Table 1*B*) were all without action. Of the simple

quaternary ammonium derivatives (Table 1*C*) the potency of tetramethylammonium was similar to that of ACh, but tetraethylammonium was less effective. Neostigmine and 3-hydroxyphenyldimethylethylammonium (edrophonium) were both less active than ACh (Table 1*D*), as were other phenyltrimethylammonium derivatives. The action of neostigmine was of considerable duration. In the case of one neurone, which was activated by a flash of light to the ipsilateral eye, the potentiating action of ACh lasted approximately 60 sec after the electrophoretic current ceased, whereas the effect of neostigmine persisted for 11 min. With this cell neostigmine increased the number of spikes evoked by the light flash but did not prolong the duration of the response. The bis-quaternary ammonium ions (Table 1*E*) were weak excitants, 1,10-decane-bis (trimethylammonium iodide) (decamethonium) being the most active. Arecoline and nicotine were also of low activity and the excitant effect of gallamine (Table 1*F*) has been mentioned above.

TABLE 2. Depressants related to 5-HT

Structural feature of interest	Name of compound	Common name	Number	Ring substituent	Side chain, R	Potency*	Duration†
(*a*) ω-N-Alkylation	5-Hydroxytryptamine	5-HT	1	5-OH	$-CH_2.CH_2.NH_2$	12	1
	ω-N-Methyl-5-hydroxytryptamine	—	2	5-OH	$-CH_2.CH_2.NH(CH_3)$	9–12	7–11
	N,N-Dimethyl-5-hydroxytryptamine	Bufotenine	3	5-OH	$-CH_2.CH_2N(CH_3)_2$	6	20–40
	N,N-Dimethyl-5-hydroxytryptamine methiodide	Bufotenidine	4	5-OH	$-CH_2.CH_2.N^+(CH_3)_3I^-$	3–6	1–2
(*b*) Substitution of phenolic hydroxyl group	4-Hydroxytryptamine	—	5	4-OH	$-CH_2.CH_2.NH_2$	18–24	24–36
	4-Methyltryptamine	—	6	$4-CH_3$	$-CH_2.CH_2.NH_2$	1–2	Prolonged‡
	4-Chlorotryptamine	—	7	4-Cl	$-CH_2.CH_2.NH_2$	0	—
	7-Hydroxytryptamine	—	8	7-OH	$-CH_2.CH_2.NH_2$	12–18	6–10
	7-Methyltryptamine	—	9	$7-CH_3$	$-CH_2.CH_2.NH_2$	1	Prolonged‡



* Potency indicates ratios of activity as depressants of the orthodromic excitation of lateral geniculate neurones, relative to 5-HT = 12.
† Duration of depressant activity relative to that of 5-HT = 1. ‡ Actual values not measured but were greater than 4.

Depressants related to 5-HT

The compounds of Table 2 were of interest since, although the depressant activity of several has already been reported (Curtis & Davis, 1962), bufotenidine, which has a side chain with a terminal quaternary nitrogen atom, has not previously been applied electrophoretically to geniculate neurones. All the compounds were applied as cations and bufotenidine proved to be a depressant of the synaptic excitation of these cells by optic nerve volleys and had a lower potency and a slightly longer duration of action than 5-HT. The method of estimating the potency and the duration

of action of depressant compounds has been presented in detail (Curtis & Davis, 1962) and the values in Table 2 are expressed relative to arbitrary values of 12 (potency) and 1 (duration) for 5-HT. It is clear from a consideration of the N-methyltryptamine derivatives that substitution upon the terminal nitrogen atom of the side chain reduces activity to an extent which is roughly proportional to the degree of substitution. Several cells were tested with bufotenidine and L-glutamic acid in order to determine whether the indole derivative had an excitant action, which might be expected to be associated with its quaternary nitrogen atom. No potentiation of the action of the excitant amino acids was observed, and it is therefore improbable that bufotenidine interacts with the same receptor as does ACh.

The remaining compounds of Table 2 are related to the very potent depressants of the synaptic excitation of lateral geniculate neurones, 4- and 7-hydroxytryptamine. Substitution of methyl for the hydroxyl groups of both compounds reduced depressant activity, and in the case of 4-hydroxytryptamine, replacement of the 4-position hydroxyl group by chlorine completely abolished activity.

DISCUSSION

When applied electrophoretically, certain quaternary ammonium compounds have been shown to be excitants of neurones of the lateral geniculate nucleus. The features of this excitant action make it unlikely that there is a similarity between these compounds and the excitatory transmitter which is released by optic nerve terminals. In the first place, although the synaptic excitation was suppressed by 4- and 5-HT, the excitatory effect of ACh was unaffected by the indolealkylamines. If the site of action of these tryptamine derivatives is post-synaptic, preventing the access of excitatory transmitter to specific receptor sites (cf. Curtis & Davis, 1962), the present observations contra-indicate either an interaction between quaternary ammonium compounds and these specific sites, or the release of transmitter as a consequence of a direct action of the excitants upon the presynaptic terminals. On the other hand, the possibility has been considered that 4- and 5-HT, and structurally related depressants of the synaptic excitation of lateral geniculate neurones, may prevent the release of transmitter from optic nerve terminals (Curtis & Davis, 1962). If this is so, it would be reasonable to expect that these compounds would also depress any release of transmitter resulting from a presynaptic action of the excitants. A second reason for considering unlikely a similarity between the quaternary ammonium derivatives and the optic nerve transmitter was the failure of dihydro-β-erythroidine to affect the synaptic

excitation of neurones by optic nerve volleys; yet this blocking agent reduced the excitatory action of ACh. Thus it must be postulated that the excitant quaternary ammonium derivatives produce a membrane depolarization by interaction with some component of the post-synaptic membrane which is not directly associated with the action of the optic nerve excitatory transmitter. The action is not, however, a non-specific one, since no such effect could be demonstrated upon spinal interneurones, even by the application of large electrophoretic currents. Furthermore, the electrophoretic currents used to demonstrate the action of the excitant compounds upon geniculate neurones, and consequently the extracellular concentrations which were obtained, were of a similar order to those necessary to excite Renshaw cells, a group of neurones for which ACh is an excitatory transmitter (Eccles, Fatt & Koketsu, 1954; Curtis & Eccles, 1958*a*, *b*). It is therefore improbable that the excitant action of these agents is due to a disturbance of intra- or extracellular processes maintaining the resting level of membrane polarization, unless enzymes associated with such mechanisms differ in the geniculate nucleus from those in the spinal cord.

If the action of these compounds is restricted to particular membrane sites, which are not present upon spinal neurones, it is possible that these receptors lie beneath the excitatory synaptic terminals of fibres not originating in the optic nerves. Physiological evidence is not available concerning the origin of all nerve fibres which terminate upon lateral geniculate neurones. Many non-optic synapses have been observed (J. Szentagothai, personal communication), and W. R. Hayhow (personal communication) has confirmed histologically, by Nauta degeneration studies, the presence of striate cortico-geniculate connexions. This pathway has been suggested by the observation that stimulation of the optic radiation beneath the visual cortex occasionally evokes synaptic, and not antidromic firing of lateral geniculate neurones (Widen & Ajome-Marsan, 1960; Bishop, Burke & Davis, 1962; D. R. Curtis and R. Davis, unpublished observations). The post-synaptic responses of lateral geniculate neurones are also affected by stimulation of the mesencephalic reticular formation (Susuki & Taira, 1961). However, in any case, ACh is unlikely to be the transmitter operating at these non-optic synapses, since the characteristics of its excitant action differ in several respects from those observed with Renshaw cells. One explanation of the weak action, which requires additional depolarization to evoke spike discharges, is that this choline ester does not interact strongly with the active sites of the membrane receptors. On the other hand, the weak excitatory action may be due either to the paucity of appropriate synapses, or to the presence of diffusional barriers near the sites. When cholinomimetic excitants are applied

electrophoretically to Renshaw cells, in the absence of cholinesterase inhibitors, carbamoylcholine, nicotine and tetramethylammonium are much more effective excitants than ACh, whereas *n*-butyrylcholine and propionylcholine are less active than the acetyl ester (D. R. Curtis, unpublished observations). This contrasts with the order of the relative potencies of these agents as excitants of geniculate neurones, the comparatively weak actions of nicotine and tetramethylammonium being particularly noticeable (cf. Table 1). Furthermore, tetraethylammonium is a weak excitant of geniculate neurones but is a powerful depressant of both the synaptic and acetylcholine-evoked firing of Renshaw cells. It is therefore probable that the receptors with which these excitant compounds are interacting upon geniculate neurones differ from the cholinoceptive receptors of Renshaw cells. In addition, ACh also fails to satisfy several of the criteria necessary to establish the nature of a transmitter agent (cf. Paton, 1958; Curtis, 1961). There is very little choline acetylase in the lateral geniculate region (Hebb, 1957) and although choline esterases are present (Burgen & Chipman, 1951) the comparatively prolonged effect of ACh upon the neurones indicates the relative lack of importance of these enzymes in its removal after application.

Recent investigations have raised the possibility that ACh may be an excitatory transmitter upon neurones other than Renshaw cells. Jung (1958), Spehlmann & Kapp (1961) and Phillis & Krnjević (1961) have indicated that ACh excites neurones in the visual cortex, but insufficient details are available to conclude that the reported action of the choline ester is similar to its effect upon geniculate neurones or is, in fact, an excitatory transmitter. In particular, no studies of the action of blocking agents upon the synaptic activation of cortical neurones have been reported. Phillis & Krnjević (1961) have mentioned, however, that gallamine 'selectively' blocks ACh, whereas in the present experiments this compound proved to be an excitant, with similar properties to those of ACh. It is therefore possible that the pharmacology of the acetylcholine-like excitation of cortical neurones differs from that of the lateral geniculate nucleus. Curtis & Koizumi (1961) have reported the apparent excitatory action of ACh upon neurones located in the inferior colliculus. In this case, however, DHβE failed to influence the excitatory action of the choline ester. The present results indicate that sensitivity of particular neurones to ACh may not necessarily mean that this substance is an excitatory transmitter acting upon them. The depression of the synaptic excitation of the neurones by blocking agents, known to be active at cholinoceptive synapses, would be additional evidence, but if the actual transmitter is related structurally to the choline ester, difficulty may be experienced in selecting a suitable specific blocking agent. It is note-

worthy that in the present experiments d-tubocurarine did not prevent the action of ACh upon geniculate neurones, and Phillis & Krnjević (1961) report that the compound excited cortical neurones when administered electrophoretically. The use of cholinesterase inhibitors may facilitate the identification of a transmitter as ACh (Eccles *et al.* 1954; Curtis & Eccles, 1958*b*), but the lack of specificity of these agents may be a difficulty, since enzymes associated with the removal of acetylcholine-like transmitters may also be inhibited. (It is conceivable that such enzymes are at present included in the term 'pseudocholinesterase'.) In addition, since the structural requirements for an acetylcholinesterase inhibitor are often similar to those for a cholinomimetic excitant, it is possible, as in the present investigation, that cholinesterase inhibitors may prove to be excitant compounds. Again, it may be difficult to determine whether this is a direct excitant action or is merely associated with diminution of the enzymic destruction of 'spontaneously' released transmitter, particularly if synaptic activation by controlled afferent volleys is not possible and only the 'spontaneous' firing of the neurone can be observed.

The electrophoretic method of drug administration does not permit a close analysis to be made of the relative potencies of ejected agents, since the local concentrations which are achieved cannot be determined. Thus, one substance may be apparently a more powerful excitant than another, merely because it fails to be removed enzymically from its site of action. Nevertheless, structure–activity relationships, which were revealed in the present investigation, provide some evidence as to the nature of the sites associated with the excitant activity of the quaternary ammonium compounds. The most active excitants had a quaternary nitrogen atom, together with another active site separated by an unbranched chain. Both the chain length and composition were important, and the inactivity of γ-butyrobetaine, compared with the activity of acetylcholine, suggests that a terminal ester linkage may be necessary. The terminal quaternary nitrogen atom was preferably trimethyl substituted, since the excitant activity of tetramethylammonium was greater than that of tetraethylammonium. The requirements for this portion of the molecule are thus probably similar to those necessary for the combination of quaternary ammonium compounds with cholinoceptive receptors (Holton & Ing, 1949). With respect to the terminal acyl group, the greater activity of carbamoylcholine, compared with that of acetylcholine, suggests that hydrogen bonding at the carbonyl oxygen atom may be important. This observation might also indicate the importance of the ether oxygen atom (Hey, 1952). On the other hand, however, the lower potency of ACh might merely be due to its partial inactivation by enzymic processes. In this respect it has already been pointed out that the prolonged excitant

action of ACh, compared with its effect upon Renshaw cells, suggests that such processes may not be very active within the geniculate nucleus.

The substances of Table 2 confirm and extend the earlier observations (Curtis & Davis, 1962) concerning the nature of the receptor with which the tryptamine derivatives interact. The presence of a phenolic hydroxyl group and of a terminal unsubstituted amino group upon the tryptamine side chain appears necessary for interaction with the receptor. The depression of the synaptic firing of lateral geniculate neurones by *n*-butyrylcholine and acetyl-β-methylcholine was an unexpected finding. Possibly these compounds combine with the hydroxytryptamine receptor, in addition to interacting with the receptor with which acetylcholine combines. If this is so, ACh and the other structurally related excitants may also interact with both groups of receptors, but any resultant depression of synaptic transmission may be masked by the greater excitant effect. The depressant action of γ-aminobutyrylcholine was of different type from that of *n*-butyrylcholine and acetyl-β-methylcholine and was identical with that produced by γ-amino-*n*-butyric acid. It is, therefore, possible that this depressant amino acid was produced by hydrolysis of the choline ester in the neighbourhood of the neurones (cf. Curtis *et al.* 1961).

The observation that repetitive firing of lateral geniculate neurones could be evoked by a maximal optic nerve volley during the early phase of the depressant action of 5-HT provided confirmatory evidence that the repetitive firing is associated with synaptic excitation close to the threshold for post-synaptic discharge (cf. Bishop & Davis, 1960). This finding, together with the observation that excitants such as ACh suppress repetitive firing, indicate that the replacement of repetitive firing by a single spike, in the absence of drug action and as the size of the optic nerve volley is increased, is due to an increase in the synaptic drive to the cell, rather than to any special effect of impulses in higher-threshold nerve fibres. It is therefore probable that as the synaptic excitation of the neurone increases, repetitive firing is suppressed by the accentuation and prolongation of the membrane conductance change which is responsible for the excitatory post-synaptic potential.

SUMMARY

1. A series of quaternary ammonium derivatives, including some choline esters, have been applied electrophoretically to neurones of the lateral geniculate nucleus of the cat. The animals were anaesthetized with pentobarbitone.

2. Many of the compounds had an excitant action which was very weak and could be displayed only as a facilitation of either the synaptic or the excitant amino-acid activation of the neurones. Carbamoylcholine was the most active excitant tested.

3. 5-Hydroxytryptamine suppressed the synaptic excitation of lateral geniculate neurones by optic nerve volleys, but did not affect the excitatory action of acetylcholine. Conversely, dihydro-β-erythroidine did not affect the synaptic responses but prevented the action of the choline ester. It is therefore unlikely that the transmitter released from optic nerve terminals is closely related structurally to acetylcholine, but this choline ester and the other related excitants may be interacting with the subsynaptic receptor sites of other excitatory synapses.

4. Some of the molecular features necessary for interaction with this cholinoceptive-type of receptor are discussed.

5. Some additional tryptamine derivatives have been tested for their depressant action upon the synaptic excitation of these neurones by optic nerve volleys.

It is a pleasure to acknowledge discussions with Dr J. C. Watkins concerning the structure-activity relationships investigated. Very grateful acknowledgement is made to the following for gifts of substances: Dr J. A. Aeschlimann (Hoffmann–La Roche Inc.); Professor S. Friedman (Purdee University); Dr A. Hofmann (Sandoz Ltd.); Dr W. F. Riker (Cornell University Medical College); Dr M. E. Speeter (The Upjohn Co.); Dr J. C. Watkins (Canberra) and Dr V. P. Whittaker (Babraham).

REFERENCES

Bishop, P. O., Burke, W. & Davis, R. (1962). Single unit recording from antidromically activated optic radiation neurones. *J. Physiol.* **162**, 409–431.

Bishop, P. O. & Davis, R. (1960). Synaptic potentials, after-potentials and slow rhythms of lateral geniculate neurones. *J. Physiol.* **154**, 514–546.

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

Curtis, D. R. (1961). The identification of mammalian inhibitory transmitters. In *Nervous Inhibition*, ed. Florey, E. New York: Pergamon Press.

Curtis, D. R. & Davis, R. (1962). Pharmacological studies upon neurones of the lateral geniculate nucleus of the cat. *Brit. J. Pharmacol.* (in the Press).

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

Curtis, D. R. & Eccles, R. M. (1958*b*). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol.* **141**, 446–463.

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

Curtis, D. R., Phillis, J. W. & Watkins, J. C. (1959). The depression of spinal neurones by γ-amino-*n*-butyric acid and β-alanine. *J. Physiol.* **146**, 185–203.

Curtis, D. R., Phillis, J. W. & Watkins, J. C. (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *J. Physiol.* **150**, 656–682.

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

Eccles, J. C., Fatt, P. & Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

Hebb, C. O. (1957). Biochemical evidence for the neural function of acetylcholine. *Physiol. Rev.* **37**, 196–220.

Hey, P. (1952). On relationships between structure and nicotine-like stimulant activity in choline esters and ethers. *Brit. J. Pharmacol.* **7**, 117–129.

Holton, P. & Ing, H. R. (1949). The specificity of the trimethylammonium group in acetylcholine. *Brit. J. Pharmacol.* **4**, 190–196.

JUNG, R. (1958). Neuropharmakologie: Zentrale Wirkungsmechanismen Chemischer Substanzen und ihre Neurophysiologischen Grundlagen. *Klin. Wschr.* **36**, 1153–1167.

JUNG, R. (1959). Discussion. In *Neuropsychopharmacology*, pp. 133–138, ed. BRADLEY, P. B., DENIKER, P. and RADOUCO-THOMAS, C. Amsterdam: Elsevier.

PATON, W. D. M. (1958). Central and synaptic transmission in the nervous system (pharmacological aspects). *Annu. rev. Physiol.* **20**, 431–470.

PHILLIS, J. W. & KRNJEVIĆ, K. (1961). Sensitivity of cortical neurones to acetylcholine. *Experientia*, **17**, 469.

SPEHLAMNN, R. & KAPP, H. (1961). Die Wirkung localer Microelectrophorese von Acetylcholine auf einzelne Neurone des visuellen Cortex. *Pflüg. Arch. ges. Physiol.* **274**, 37–38.

SUSUKI, H. & TAIRA, N. (1961). Effect of reticular stimulation upon synaptic transmission in cat's lateral geniculate body. *Jap. J. Physiol.* **11**, 641–655.

WIDEN, L. & AJMONE-MARSAN, C. (1960). Effects of corticopetal and corticofugal impulses upon single elements of the dorsolateral geniculate nucleus. *Exp. Neurol.* **2**, 468–502.