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A Re-Evaluation of the Mode of Action of 5-Hydroxytryptamine on Lateral Geniculate Neurones: Comparison with Catecholamines and LSD

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Summary. A reinvestigation was made of the effects of microelectrophoretically administered 5-HT, NA, DA and related compounds on LGN neurones in unanaesthetized (decerebrate) and barbiturate-anaesthetized cats. In both preparations 5-HT had two types of depressant action which were dose-dependent. With low ejecting currents 5-HT readily depressed the orthodromic firing evoked by light and optic nerve stimulation, and "spontaneous" activity, but had little or no action on the excitation evoked chemically by glutamate, aspartate, DLH or ACh, or on antidromic invasion of LGN neurones. With higher ejecting currents 5-HT also depressed chemically evoked firing and sometimes blocked antidromic invasion. 4-HT and LSD had actions similar to those of 5-HT, but of a longer time course. It is concluded that the tryptamine derivatives and LSD in low concentrations either block the release of the excitatory transmitter from optic nerve terminals, or block its access to receptors on LGN neurones. In higher concentrations, 5-HT, 4-HT and LSD also have postsynaptic depressant actions, as observed in other areas of the central nervous system. In contrast, DA and NA did not have a selective depressant action on synaptic activity in low quantities, indicating only postsynaptic effects. Low intravenous doses (2—7 mg/kg) of pentobarbitone sodium reduced or blocked the excitant action of ACh, but not that of glutamate, on LGN neurones.

Key words: Lateral geniculate neurones — 5-Hydroxytryptamine — Catecholamines — LSD — Optic nerve transmitter

Introduction

Two apparently conflicting conclusions have been reported concerning the effects of electrophoretically administered 5-hydroxytryptamine (5-HT) and related substances on neurones of the lateral geniculate nucleus (LGN). Curtis and Davis (1962) observed in cats anaesthetized with pentobarbitone sodium that 5-HT, a number of other tryptamine and lysergic acid derivatives, as well as catecholamines, depressed the synaptic firing of LGN neurones evoked by optic nerve impulses without affecting either antidromic invasion or the excitation evoked chemically by L-glutamate. The proposal that 5-HT (ejected with currents of 10—40 nA) did not have a postsynaptic action on LGN neurones, but either blocked the release of the excitatory transmitter from optic nerve terminals, or

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In contrast, Phillis, Tebēcis and York (1967a) showed that in cats anaesthetized with halothane or methoxyflurane and nitrous oxide, 5-HT NA and DA (administered with currents of up to 100 nA) depressed the synaptic responses evoked by optic nerve and light stimulation, as well as glutamate-induced firing and antidromic invasion of LGN neurones. Moreover, acetylcholine (ACh) fired LGN neurones (cf. Curtis and Davis, 1963), and this excitation was also depressed by the monoamines (Phillis *et al.*, 1967a, b). It was concluded that 5-HT and catecholamines probably have postsynaptic depressant effects on neurones in the LGN, comparable to their actions in other structures of the central nervous system (see review by Curtis and Crawford, 1969). Similarly, Satinsky (1967) observed that in unanaesthetized cats 5-HT depressed and ACh facilitated "spontaneous" firing of LGN neurones.

Since both 5-HT- and NA-containing nerve terminals have been demonstrated in the LGN by means of a sensitive (fluorescence) histochemical technique (Fuxe, 1965), it is possible that the effects of 5-HT, NA and related compounds are associated with a synaptic function for 5-HT and NA in this nucleus (Phillis *et al.*, 1967a). It was therefore of interest to reinvestigate the actions of tryptamines and catecholamines on LGN neurones in both anaesthetized and unanaesthetized cats. The results explain the apparent discrepancies, and suggest that 5-HT, 4-hydroxytryptamine (4-HT) and LSD, but not NA and DA, have two types of depressant action which are dose-dependent.

Methods

Results were obtained from neurones within the dorsal LGN of 22 adult cats (2.6—4.7 kg body weight). The majority (18) of the animals were decerebrated by coagulation of the brain stem at the level of the inferior colliculi (sub-tentorial) during halothane anaesthesia, which was afterwards discontinued (Crawford and Curtis, 1966). Four cats were anaesthetized with intraperitoneal pentobarbitone sodium (35 mg/kg), which was subsequently administered intravenously when required during the experiment. The general methods were similar to those described by Curtis and Davis (1962). In most cases the cerebral cortex was left intact, although in the early experiments a portion of the cortex overlying the hippocampus was removed. In about half the experiments the contralateral optic nerve was dissected free and mounted on stimulating electrodes (Bishop, Jeremy and Lance, 1953), and the ipsilateral eye was stimulated with flashes of light (50—60 msec duration) by a small lamp mounted in front of the eye-ball. In all other preparations both eyes were stimulated by light flashes. In all but the first few cats the optic radiation (beneath the ipsilateral marginal gyrus) was stimulated by six pairs of stainless steel needles mounted in two parallel rows 2 mm apart and inserted 4—5 mm below the surface of the striate cortex.

Action potentials of single neurones of the dorsal LGN were recorded between the stereotaxic coordinates A6—8.5 and L8—11, using the atlas of Reinoso-Suárez (1961). Neurones were accepted as being of the LGN only when they could be activated by optic nerve or light stimulation, and the position of the electrode tip was between the appropriate stereotaxic coordinates. The cells were further identified as geniculo-cortical relay neurones when they

were also activated antidromically by stimulation of the optic radiation. Action potentials were considered antidromically evoked when they were of short (usually less than 1 msec) and constant latency, and followed trains of repetitive stimuli at high frequencies (up to 625/sec). Stimulation of the optic radiation sometimes evoked action potentials of relatively short (about 2 msec) but variable latency, identified as synaptic excitation. "Spontaneous" activity, or background firing in the absence of stimulation of the optic radiation, optic nerve and eyes with light flashes, was considered to be largely due to impulses from optic nerve fibres, as it could be modified by changing the background illumination in the recording room, and the effects of drugs on such spontaneous firing were invariably similar to those on the orthodromic responses evoked by optic nerve or light stimulation. During recording the illumination in the room was maintained at a constant low level.

Action potentials were recorded extracellularly through the 4 M NaCl-containing central barrel of seven-barrel glass micropipettes, and the substances to be tested were expelled electrophoretically from the outer barrels (Curtis, 1964). L-Glutamate (British Drug Houses; 0.5 M pH 7.4 with NaOH), L-aspartate (Sigma; 0.5 M, pH 7.5 with NaOH) and DL-homocysteate (Calbiochem; 0.2 M, pH 8.0 with NaOH) were ejected with anionic (—) currents. 5-Hydroxytryptamine creatinine sulphate (Calbiochem; 0.02 M, pH 3.5), 4-hydroxytryptamine oxalate (Sandoz; saturated, less than 0.02 M, pH 5), L-noradrenaline bitartrate (Calbiochem; 0.2 M pH 3.5), 3-hydroxytyramine (dopamine) hydrochloride (Calbiochem, 0.2 M, pH 5.3), lysergic acid diethylamide bitartrate (Sandoz; 10 mM in 165 mM NaCl, pH 5.0), γ-aminobutyric acid (Calbiochem; 0.5 M, pH 3.0 with HCl) and acetylcholine chloride (Calbiochem; 0.5 M, pH 3.2) were ejected with cationic (+) currents. Electrodes were filled by centrifugation and stored in the dark in nitrogen at room temperature.

Action and field potentials of neurones were photographed from a cathode ray oscilloscope, and the firing frequencies were plotted on paper using an amplitude discriminator, a rate-meter and a chart recorder. The number of spikes evoked by optic nerve or light stimulation during a controlled interval were counted and averaged by means of a gated digital counter. It was thus possible to compare the effects of substances on orthodromic responses and the firing evoked chemically by excitant substances, simultaneously and alternately.

Exposed brain tissue was either continuously irrigated with a mammalian Ringer solution at 37°C, or covered with a 4% Agar (Difco) in Ringer solution. Femoral arterial blood pressure and expired CO_2 levels were monitored continuously. In some animals gallamine triethiodide (Flaxedil, 2—4 mg/kg administered intravenously at half-hourly intervals) was used to prevent sporadic reflex movements. These animals were respired artificially, the end tidal CO_2 levels being maintained at 3.8—4.5%. The animals were maintained at 36—38°C by heating pads.

Results

5-Hydroxytryptamine

The effects of electrophoretically administered 5-HT were studied on 103 LGN neurones from which records were obtained for 1/2—3 hr in order to compare different values of ejecting current on both synaptically and chemically evoked firing, and to compare 5-HT with other depressant compounds. 5-HT depressed the firing of more than 90% of the neurones tested, including all (35) cells identified as geniculo-cortical relay neurones. Orthodromic firing of LGN neurones was depressed more readily by 5-HT than the excitation by amino acids. Spontaneous firing, action and field potentials evoked by optic nerve and light stimulation were readily depressed and sometimes blocked by 5-HT, using ejecting currents of as low as 2—8 nA (with retaining currents of the order of —3 to —7 nA).

This depressant effect of 5-HT was of a slow time course (approximately 0.5—3 min for both maximal depression and for recovery), and could be repeated without diminution. The repetitive synaptic response evoked by a flash of light to the eyes was often completely suppressed by 5-HT at a concentration which had little or no action on the firing evoked by DL-homocysteate (DLH), glutamate or

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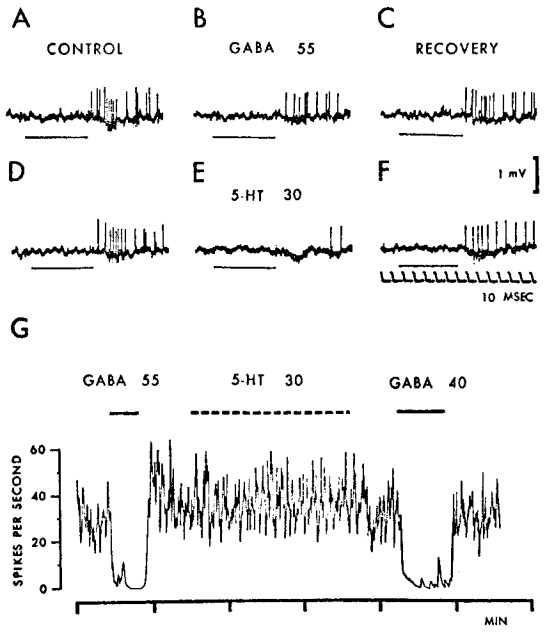


Fig. 1. *Comparison of the depressant actions of 5-HT and GABA on synaptically and chemically evoked excitation of a geniculo-cortical relay neurone.* A—F: repetitive discharges evoked by light stimulation (once every 3 sec, duration indicated by horizontal bars) of the ipsilateral eye. A, D: control responses. B: 1 min after the beginning of the ejection of GABA (55 nA), which was continued for a further 30 sec. C: 40 sec after the current ejecting GABA has been terminated. E: 2 min after the beginning of the ejection of 5-HT (30 nA). F: approximately 2.5 min after the current ejecting 5-HT had been terminated. G: recording of the firing frequency of the same neurone illustrating the actions of GABA (55 and 40 nA) and 5-HT (30 nA) on firing evoked by L-glutamate (—90 nA). *Ordinate*, spikes per second, *Abscissa*, time in minutes. *Calibration*: 1 mV and 10 msec for A—F

aspartate, even when the currents to eject the amino acids were selected to produce relatively slow firing frequencies (10—20/sec). Similarly, 5-HT invariably depressed spontaneous firing to a greater extent than the firing produced by intermittent pulses of glutamate or DLH "superimposed" on the background activity. Moreover, when the number of action potentials evoked by light stimulation were counted (usually over a period of 100 msec) and averaged, 5-HT ejected with low currents reduced the average number of spikes without decreasing the firing by either glutamate or DLH. In some instances, however, it was difficult to differentiate the depression by 5-HT into two different types, as low ejecting currents (15—20 nA) markedly depressed the amino acid evoked firing of these cells. For this reason, a more effective means of studying the depression by 5-HT was to compare its action with that of γ-aminobutyric acid (GABA) on both orthodromically and chemically evoked firing of the same neurone. Results from one experiment are illustrated in Fig. 1.

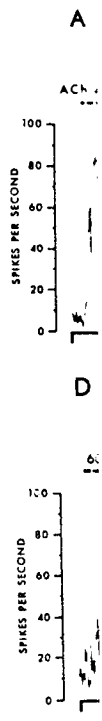
Stimulation of the ipsilateral eye with a flash of light (approximately 55 msec duration) evoked a response consisting of 12—15 spikes (Fig. 1A). GABA (55 nA) only slightly reduced the number of spikes evoked per stimulus: maximal depres-

sion is shown in Fig. 1B, recorded 1 min after beginning the ejection. Recovery was complete within 40 sec of the termination of the current ejecting GABA (Fig. 1C). In contrast, 5-HT administered with a lower current (30 nA) almost totally suppressed the synaptic discharge within 2 min (Fig. 1E), and recovery took more than 2 min (Fig. 1F). This neurone was not firing spontaneously, but was fired at a frequency of approximately 40 spikes/sec by glutamate (—90 nA) administered continuously (Fig. 1G). The chart recording illustrates that GABA (55 and 40 nA) completely blocked, whereas 5-HT (30 nA) had no action on the firing of the same neurone by glutamate. This firing, however, was slightly depressed by 5-HT administered with a current of 50 nA (not illustrated).

The observation that 5-HT was more effective than GABA (on the basis of a comparison of electrophoretic currents) as a depressant of synaptic responses evoked by light or optic nerve stimulation, whereas GABA was a more effective depressant of amino acid evoked firing of the same neurone, was confirmed on many neurones in several cats. With some cells GABA was a more effective depressant of synaptic firing than 5-HT ejected with equal currents. In all these cases, however, GABA was considerably more effective than 5-HT when the two substances were compared as depressants of chemical excitation of the same neurone.

The firing of LGN neurones by excitant amino acids was usually depressed by 5-HT when it was administered with higher currents than those required to depress synaptic excitation. Concentrations of 5-HT produced by ejecting currents of 40—60 nA were usually required to reduce the firing induced by glutamate, aspartate or DLH by at least 30%, although 5-HT ejected with currents of 15—30 nA sometimes clearly depressed chemically evoked excitation. The possibility that aspartate could be the excitatory transmitter released from optic nerve terminals (see Discussion) prompted a comparison of the effects of 5-HT on the excitation of neurones by glutamate and aspartate. When ejecting currents were selected to fire a cell at similar rates by the two amino acids, 5-HT depressed the effects of both to the same extent on all but one neurone (studied on 12 neurones in 4 cats). Excitation by glutamate of one cell was reduced to a greater extent than that by aspartate. Antidromic action potentials of approximately half the neurones tested were also blocked by 5-HT, using ejecting currents of 80—100 nA. The depressant action of 5-HT on excitant amino acid-induced firing and antidromic invasion was of a more rapid time course (several sec for both maximal depression and recovery) than the depression of synaptic activity, and was also comparable on repeated tests.

The spontaneous or DLH-induced firing of only 7 (7%) of the 103 LGN neurones tested was enhanced by 5-HT (10—40 nA); with 2 cells this excitation was preceded by a depression. These neurones could not be activated antidromically by stimulation of the optic radiation. No obvious differences in the effects of 5-HT were observed between unanaesthetized (decerebrate) cats and the four animals anaesthetized with pentobarbitone sodium. The possibility that barbiturate anaesthesia may affect the depressant action of 5-HT was studied in one unanaesthetized cat. Pentobarbitone sodium (2.5 mg/kg intravenously) had no effect on the depression by 5-HT, DA or GABA, of the spontaneous and DLH-induced firing of the neurone tested. Such doses of barbiturate were sufficient to reduce the excitant action of ACh.



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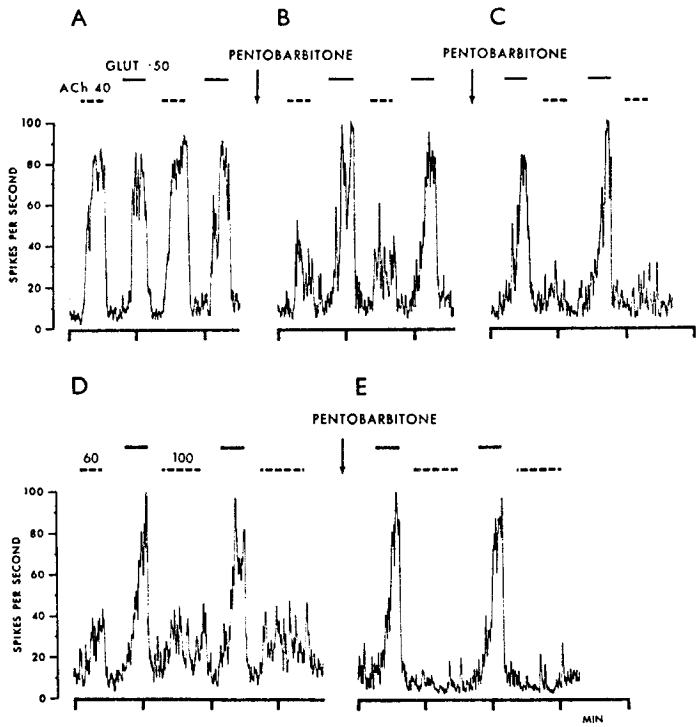

Fig. 2. *Effect of intravenously administered pentobarbitone sodium on the excitation of a geniculo-cortical relay neurone by ACh and L-glutamate.* ACh (40 nA, broken horizontal line) and glutamate (—50 nA), solid horizontal line) were administered at regular intervals and the firing frequency of the neurone was plotted continuously. A: control. B: approximately 3 min after pentobarbitone sodium (2.1 mg/kg, infused over a period of about 70 sec). C: approximately 3 min after another dose of pentobarbitone sodium (2.1 mg/kg). D: approximately 8 min after C, ACh was ejected for longer periods with currents of 60 and 100 nA. E: approximately 1 min after a final dose of pentobarbitone sodium (2.1 mg/kg). *Ordinates:* spikes per second. *Abscissae:* time in minutes

It was also of interest to compare the action of 5-HT on orthodromic firing and the excitant action of ACh. Acetylcholine rarely (3/15 neurones) fired LGN neurones in the four cats anaesthetized with pentobarbitone sodium, but facilitated amino acid and orthodromically evoked firing when relatively high ejecting currents (60—100 nA) were used (cf. Curtis and Davis, 1963). In unanaesthetized cats, however, ACh readily excited 74 of the 83 LGN neurones tested, including all 42 geniculo-cortical relay neurones, the time course of action being similar to that of the excitant amino acids (cf. Phillis *et al.*, 1967 b). In three unanaesthetized cats, small intravenous doses (2—7 mg/kg) of pentobarbitone sodium depressed or blocked the excitant action of ACh, but had little effect on that of glutamate. One example of this type of experiment is illustrated in Fig. 2.

The maximum firing rate of this neurone attained by constant pulses of ACh (40 nA for 20 sec) was approximately equal to that produced by glutamate (—50 nA for 20 sec) before the injection of pentobarbitone sodium (Fig. 2A). Sodium pentobarbitone was injected intravenously (2.1 mg/kg), and the excitant action

of ACh (40 nA), but not that of glutamate (—50 nA), was reduced within 1 min. The records in Fig. 2B were obtained a little over 3 min after the barbiturate had been administered. Another injection of pentobarbitone (2.1 mg/kg) almost completely blocked the excitant action of ACh (40 nA), but again had little effect on the action of glutamate (Fig. 2C). When the current ejecting ACh was increased to 60 and 100 nA, some excitation was observed (Fig. 2D), but a further injection of pentobarbitone (2.1 mg/kg) blocked this excitant action of ACh (100 nA) without affecting that of glutamate (Fig. 2E).

A study of 9 cells in unanaesthetized cats revealed that 5-HT depressed orthodromic firing more readily than firing produced by ACh. Ejecting currents of 40—50 nA of 5-HT were required to depress excitation evoked by ACh by at least 30%, whereas currents of 10—20 nA readily depressed synaptic activity of the same neurones.

4-Hydroxytryptamine

4-Hydroxytryptamine depressed the firing of 13 of 17 LGN neurones tested, including all (8) geniculo-cortical relay cells, and excited 2 neurones which were also excited by 5-HT. The remaining 2 neurones were unaffected by 4-HT (10 nA), but observations were not made using higher ejecting currents. The qualitative effects of 4-HT were identical to those of 5-HT in all respects (see Curtis and Davis, 1962). Thus, 4-HT readily depressed orthodromic firing in amounts (as low as 5 nA) which had little or no action on the firing produced by glutamate, DLH or ACh, but sometimes depressed chemically evoked excitation when higher amounts (10—30 nA) were ejected. Although the time to produce maximal depression by 4-HT was similar to that by 5-HT, the time course of recovery was invariably longer for 4-HT (see Curtis and Davis, 1962). Recovery periods of 5 min or more were not uncommon after 4-HT (10 nA) had been administered for 0.5—1 min.

The approximate relative potencies of the two tryptamine derivatives were estimated by comparing the ejecting currents required to produce equal degrees of depression (ignoring recovery times), or by comparing the effects of equal ejecting currents. Allowance was made for the presence of creatinine in the 5-HT solution (Curtis and Davis, 1962). Such estimates in 5 animals revealed that 4-HT was usually more effective than 5-HT as a depressant of LGN neurones (7 cells), although in some cases the two substances were approximately equipotent (3 cells) or 5-HT was slightly more effective (3 cells).

Catecholamines

The catecholamines were tested only in unanaesthetized cats. Dopamine depressed the firing of 39 of 41 LGN neurones, including all 26 geniculo-cortical relay cells tested, and had no action on 2 others (using ejecting currents of 80—100 nA). Dopamine was invariably less effective as a depressant than 5-HT tested on the same neurone. The depression of orthodromic firing by DA was always less than that by 5-HT ejected with lower currents, and DA often had to be expelled with currents of 60—80 nA before these responses were depressed.

Dopamine appeared to be as effective a depressant of orthodromic firing as of chemically evoked excitation. A comparison of the depression by DA and GABA indicated that the relative potencies of the two substances were the same on both

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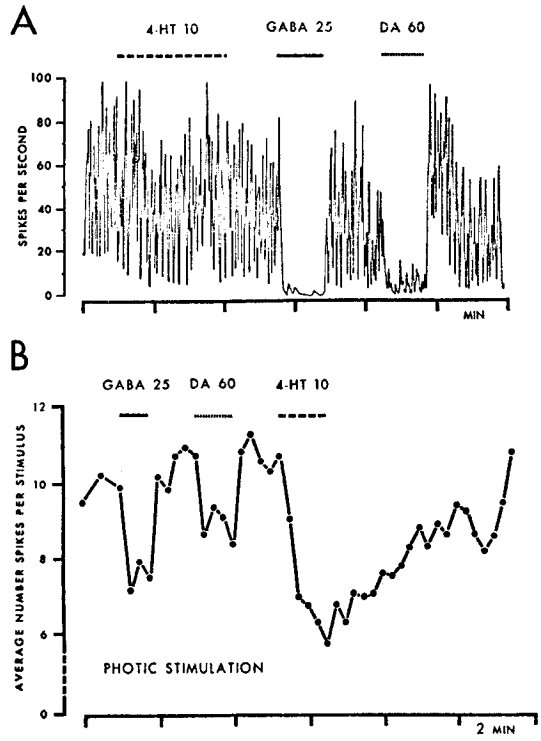

Fig. 3. *Comparison of the effects of 4-HT, GABA and DA on the firing evoked by DLH and stimulation of the eyes with light flashes.* A: chart recording showing the effects of 4-HT (10 nA), GABA (25 nA) and DA (60 nA) on the frequency of firing (*ordinate*) evoked by a continuous ejection of DLH (—9 nA) *Abscissa*, time in minutes. B: the three substances, administered with the same currents as in A, were compared as depressants of the action potentials evoked by stimulation of both eyes with light (60 msec duration, once every 3 sec). *Ordinate:* average number of spikes evoked per test (10 tests). *Abscissa:* time in 2 min intervals

types of firing of any one neurone, thus differing from the tryptamines which depressed synaptic activity more readily. An example of this is illustrated in Fig. 3.

The otherwise-quiescent neurone shown in Fig. 3A fired irregularly in bursts when DLH (—9 nA) was ejected continuously. The effects of 4-HT, GABA and DA were compared, using different ejecting currents. 4-Hydroxytryptamine (10 nA) had little effect on the firing evoked by DLH, but GABA (25 nA) and DA (60 nA) depressed the firing, GABA being slightly more effective than DA with these currents (Fig. 3A). The effects of these substances were subsequently tested on the synaptic excitation evoked by light stimulation. Both eyes were stimulated with 60 msec flashes of light every 3 sec, and the number of action potentials evoked were counted during the 100 msec period following the stimulus. The points plotted in Fig. 3B represent the average number of action potentials evoked per stimulus, counted over 10 tests. GABA (25 nA) was slightly more effective as a depressant than DA (60 nA), as was observed on the firing by DLH, but both were considerably less effective than 4-HT (10 nA) which depressed the synaptic

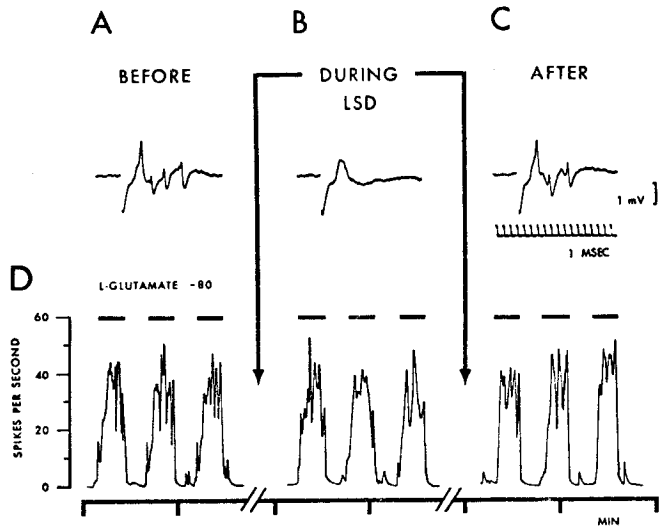

Fig. 4. *Selective depressant action of LSD on the synaptic excitation of a geniculo-cortical relay neurone.* A: synaptic response evoked by electrical stimulation of the contralateral optic nerve (0.1 msec duration, once every 2 sec). B: response at the end of an ejection of LSD (20—100 nA) for 11 min. C: approximately 3 min after the current ejecting LSD had been terminated. D: chart recording showing the responses of the same neurone to glutamate (—80 nA) before, during and after the ejection of LSD. *Ordinate:* spikes per second. *Abscissa,* time in minutes. *Calibrations:* 1 mV and 1 msec for A—C

responses for several minutes. Similar observations were made on 10 other neurones, using both 5-HT and 4-HT.

Noradrenaline had only a weak depressant effect on 9 of 15 LGN neurones tested, including most geniculo-cortical relay cells. The other neurones tested were either excited (slow time course) or initially depressed and then excited. The depressant action of NA was almost always less than that of DA administered with equal currents, whether tested on orthodromic or chemical excitation. Since NA often had to be ejected with currents of 80 nA or higher to produce a clear depression, and since firing was sometimes facilitated, extensive studies on the mode of depressant action of NA were not possible. However, NA resembled DA in the type of depression produced, and did not appear to selectively reduce orthodromic firing. In particular, the depression of 2 neurones by NA paralleled that by GABA on both the excitation by glutamate and the synaptic responses evoked by light, whereas 5-HT was a more effective depressant of the synaptic activity than of the amino acid evoked firing. The observations suggested that the depression of LGN neurones by NA is similar to that by DA, except that NA is usually less effective.

Lysergic Acid Diethylamide

The effects of LSD were similar to those of 5-HT (Curtis and Davis, 1962; Phillis *et al.*, 1967a). When ejected with currents of up to 100 nA from a 10 mM solution in 165 mM NaCl, LSD readily depressed spontaneous firing as well as the synaptic responses evoked by light and optic nerve stimulation. The substance sometimes also depressed the firing evoked by glutamate and ACh, but only when

high ejecting currents (80—120 nA) were used. As with 5-HT, it was possible to demonstrate a depression of orthodromic responses by LSD at a dose which had little or no effect on the firing by glutamate or ACh (4 neurones). An example is shown in Fig. 4.

Glutamate (—80 nA) was administered at regular intervals, and the firing frequency of the neurone was plotted continuously (Fig. 4D). Stimulation of the contralateral optic nerve evoked a synaptic response of 3—4 spikes superimposed on a field potential (Fig. 4D). During the ejection of LSD with a current of 20 nA, which was slowly increased to 40, 60, 80 and finally, 100 nA for a total duration of 11 min, the excitation by glutamate remained unchanged (Fig. 4D). The synaptic response was unaffected by LSD until the ejecting current was increased to 100 nA, when the spikes were blocked leaving a field potential (Fig. 4B). Increasing or decreasing the stimulus strength did not elicit action potentials at this stage. Recovery of the synaptic response occurred within 3 min of the termination of the current ejecting LSD (Fig. 4C).

With two neurones the firing evoked by glutamate and ACh was slightly depressed by LSD (100 nA), but the synaptic response to light stimulation was blocked before this depression of chemical sensitivity was apparent. An unusual finding was that in both cases LSD reduced the excitation by ACh to a greater extent than that by glutamate.

Discussion

If it is assumed that ACh, GABA and the excitant amino acids have predominantly postsynaptic effects on LGN neurones, and since a depression of antidromic invasion presumably indicates a postsynaptic action of administered drugs, the present results suggest that 5-HT has two types of depressant action on these cells, which are dose-dependent. With relatively low concentrations, 5-HT readily depressed spontaneous firing and the synaptic responses evoked by light and optic nerve stimulation, but had little or no action on antidromic excitation and that evoked chemically by excitant amino acids and ACh. In higher quantitites, 5-HT also reduced chemically evoked excitation and sometimes blocked antidromic invasion. This was especially evident from the observation that 5-HT was usually a more effective depressant of synaptic excitation than GABA, which was more effective as a depressant of chemical excitation of the same neurone. The apparent discrepancies between the reports of Curtis and Davis (1962) and Phillis *et al.* (1967a) cannot be explained in terms of different anaesthetics used, as the depressant actions of 5-HT did not differ between unanaesthetized cats and those anaesthetized with pentobarbitone sodium. The different findings more likely resulted from the use of different amounts of 5-HT. Curtis and Davis (1962) probably observed no action of 5-HT on the firing evoked by glutamate and antidromic stimulation because only low ejecting currents (10—40 nA) were used. Phillis *et al.* (1967a) ejected 5-HT with currents of up to 100 nA, and thus depressed all forms of excitation without distinguishing between the selective, dose-dependent actions of 5-HT.

The depression of orthodromic responses of LGN neurones by low concentrations of 5-HT is best interpreted as a block of the excitatory action of the transmitter released from optic nerve terminals on to LGN neurones (cf. Bishop *et al.*,

1959; Curtis and Davis, 1962), or as a block of release of this excitatory transmitter from the presynaptic terminals (Curtis and Davis, 1962). At higher concentrations, 5-HT probably also has a direct depressant action on the postsynaptic membrane of LGN neurones, as is thought to occur in other areas of the central nervous system, and which may be indicative of an inhibitory transmitter role for this monoamine (Phillis *et al.*, 1967a). In the ventrobasal complex of the thalamus, 5-HT in low concentrations did not have a selective depressant action on synaptic activity (Andersen and Curtis, 1964).

The similarities between the effects of 5-HT, 4-HT and LSD suggest that 4-HT and LSD also have these two types of depressant action on LGN neurones, which are dose-dependent, and presumably mediated by 5-HT receptors. In contrast, the catecholamines, DA and NA, did not appear to have a selective depressant action on orthodromic responses. These substances were weaker depressants than 5-HT of all forms of excitation, and the various observations suggest that DA and NA have purely postsynaptic actions on LGN neurones, as proposed earlier (Phillis *et al.*, 1967a).

The observation that 5-HT rarely excites LGN neurones, and depresses all geniculo-cortical relay neurones, even in unanaesthetized cats (Satinsky, 1967; present study) supports the view that the excitatory transmitter released from optic nerve terminals is unlikely to be 5-HT (Bogdanski, Weissbach and Udenfriend, 1957; Curtis and Davis, 1962). The release of 5-HT from slices of the superior colliculus in response to stimulation of the optic tract need not necessarily indicate a release from optic tract axons (Kawai, 1970), but may equally well be explained by assuming a release from 5-HT-containing terminals which were stimulated (directly or via interneurones) by optic nerve impulses. Histochemical evidence for 5-HT-containing nerve terminals which originate in the brain stem and project to the LGN in rats (Fuxe, 1965) suggests that this may be the course of 5-hydroxytryptaminergic afferents to the LGN in the cat, as 5-HT-containing cell bodies have been observed only in the brain stem. Stimulation of the mesencephalic reticular formation can inhibit LGN neurones (Suzuki and Taira, 1961), and the depression of various forms of excitation of most LGN neurones by 5-HT is compatible with the hypothesis that 5-HT could be the inhibitory transmitter involved in this pathway. It is also feasible that synaptically released 5-HT modulates the effect of optic nerve impulses by blocking the action of the excitatory transmitter. Alternatively, if 5-HT prevents the release of the transmitter from optic nerve terminals, this action is unlikely to be mediated by axo-axonic contacts, since optic nerve terminals always appear to be presynaptic at axo-axonic synapses in the LGN (Szentágothai, Hámori and Tömböl, 1966; Szentágothai, 1968; Jones and Powell, 1969).

It is of interest to compare studies made in the superior colliculus, where 5-HT may also be an inhibitory transmitter (Fuxe, 1965; Kawai and Yamamoto, 1969; Beleslin and Myers, 1970; Straschill and Perwein, 1971), as the excitatory transmitter released from optic nerve fibres in the LGN is possibly the same as that released in the superior colliculus. Electrophoretically administered 5-HT depressed both synaptically and chemically evoked excitation of neurones in the cat superior colliculus (Straschill and Perwein, 1971). In isolated slices of guinea pig brain, however, 5-HT in low concentrations, but not NA or DA, depressed the synaptic responses of superior collicular neurones to optic tract stimulation without affecting glutamate evoked or spontaneous firing (Kawai and Yamamoto, 1968, 1969). However, as in

the LGN, it is possible that 5-HT has two types of depressant action in the superior colliculus, which are dose-dependent. Straschill and Perwein (1971) did not compare the action of 5-HT on synaptic and chemical excitation of the same neurone, and although the depression of glutamate evoked firing by 5-HT is indicative of a postsynaptic action of the amine, the long time course of depression of the spontaneous firing of superior collicular neurones by 5-HT ejected with low currents resembles findings on LGN neurones, and may indicate that 5-HT selectively depresses orthodromic firing in low concentrations. Similarly, it is possible that Kawai and Yamamoto (1968, 1969) would also have observed an action of 5-HT on glutamate evoked or spontaneous firing had they used higher concentrations of the substance. The catecholamines, NA and DA, apparently had only postsynaptic depressant actions on superior collicular neurones (Kawai and Yamamoto, 1969; Straschill and Perwein, 1971).

The function of catecholamines in the LGN is unclear. The excitatory transmitter released from optic nerve terminals is unlikely to be NA because there is little NA in the optic nerve (Vogt, 1954; Cobbin, Leeder and Pollard, 1965), and no NA-containing cell bodies in the retina (Häggendal and Malmfors, 1963). Furthermore, most geniculo-cortical relay neurones are depressed by NA (Curtis and Davis, 1962; Phillis *et al.*, 1967a; present study). However, the presence of NA- (but not DA-) containing nerve terminals which originate in the brain stem and project to the LGN (Fuxe, 1965) suggests that NA, but not DA, may be synaptically released on to LGN neurones.

The finding that low intravenous doses of pentobarbitone sodium depressed the sensitivity of LGN neurones to ACh, but not to glutamate, is similar to the observations of Phillis and Tebēcis (1967) on various thalamic neurones, but differs from those of Andersen and Curtis (1964) made on neurones of the ventrobasal complex. The use of barbiturate anaesthesia most probably accounts for the weak excitant action of ACh on LGN neurones observed previously (Curtis and Davis, 1963; cf. Phillis *et al.*, 1967b; Satinsky, 1967; Steiner, 1968). In unanaesthetized cats ACh invariably excites geniculo-cortical relay cells. However, ACh is unlikely to be the transmitter released from optic nerve terminals as there is little ACh or choline acetyltransferase in the optic nerve (MacIntosh, 1941; Feldberg and Vogt, 1948; Hebb and Silver, 1956; Cobbin *et al.*, 1965), and antagonists of ACh have little action on the synaptic responses evoked by light and optic nerve stimulation (Curtis and Davis, 1963; Phillis *et al.*, 1967b). Cholinergic afferents to the LGN probably originate in the brain stem (Phillis *et al.*, 1967b; Shute and Lewis, 1967; Deffenu, Bertaccini and Pepeu, 1967), and suppression of this "background" cholinergic activity may account for the apparent depressant effects of systemically administered dihydro-β-erythroidine and atropine on the firing evoked by optic nerve impulses (David, Murayama, Machne and Unna, 1963).

The possibility that LSD antagonizes the excitant action of glutamate, but not that of DLH, under certain conditions (Boakes, Bradley, Briggs and Dray, 1970), and the observation that LSD readily blocks orthodromic firing of LGN neurones (Evarts *et al.*, 1955; Bishop *et al.*, 1958, 1959; Curtis and Davis, 1962), raised the possibility that the optic nerve transmitter could be glutamic acid. However, the optic nerve contains extremely low levels of glutamate (Johnson and Aprison, 1971). Furthermore, LSD and other tryptamines in concentrations which blocked synaptic activity had little or no action on the firing of LGN neurones by glutamate, aspartate or DLH (Curtis and Davis, 1962; present report), suggesting that these amino acids are unlikely to be the transmitter. However, if 5-HT acts by

depressing the *release* of the optic nerve transmitter, this transmitter could nevertheless be an excitant amino acid.

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