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THE INHIBITORY ACTION OF MONOAMINES ON LATERAL GENICULATE NEURONES

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SUMMARY

1. L-Noradrenaline (NA), dopamine, 5-hydroxytryptamine (5-HT) and lysergide were administered iontophoretically to neurones in the dorsal lateral geniculate nucleus of the cat and the responses to these drugs recorded.

2. Many neurones were depressed by the monoamines and lysergide.

3. This depression was manifested by a reduction or abolition of the effects of optic nerve or visual stimulation, by a failure in some instances of an antidromically propagating spike to invade the cell soma, and by a depression of the excitant effects of L-glutamate and acetylcholine.

4. Although there was considerable variation in the magnitude of the depressant effects of the monoamines, dopamine was found to be slightly more potent than 5-HT and NA. Neurones which were inhibited by catecholamines did not always respond to 5-HT and lysergide and vice versa.

5. The inhibitory actions of monoamines on lateral geniculate neurones are comparable with those that have been recorded in other structures of the central nervous system.

6. Lysergide did not antagonize the action of 5-HT.

7. Catecholamines and 5-HT are present in nerve terminals in the lateral geniculate nucleus and the findings of this study suggest that they act as inhibitory transmitters.

INTRODUCTION

With the recent development of histochemical techniques for the localization of monoamines, it has been possible to demonstrate the presence of catecholamine- and 5-HT-containing nerve cells and axons in many areas of the central nervous system (Fuxe, 1965; Andén, Dahlström, Fuxe, Larsson, Olson, & Ungerstedt, 1966). Histochemical information obtained in this manner can be utilized in conjunction with the results of pharmacological investigations in order to make an assessment of the 'transmitter potentiality' of monoamines in the nervous system.

Neurones that respond to iontophoretically applied catecholamines and 5-HT are widely distributed throughout the central nervous system, the responses ranging from pronounced depression to excitation. With the notable exception of neurones in the lateral geniculate nucleus (LGN) (Curtis & Davis, 1962, 1963) the inhibitory actions of monoamines have been demonstrated on a variety of different modes of excitation, including synaptic activation, spontaneous discharges, antidromic invasion and the firing induced by iontophoretically applied amino acids and cholinergic compounds. The mechanism of action of the monoamines has yet to be elucidated, but the effects are comparable with those that might be expected of an inhibitory transmitter acting on post-synaptic receptors and producing an increase in membrane conductance (Krnjević & Phillis, 1963*a*; Engberg & Ryall, 1966).

5-HT and related compounds had a different type of action on neurones in the dorsal lateral geniculate nucleus (Curtis & Davis, 1962, 1963), since synaptic activation of cells by volleys in the optic nerves was depressed without either a corresponding reduction in the excitant action of glutamic acid or a failure of antidromic invasion. It was concluded that 5-HT did not alter the excitability of cells by changing the conductance of the post-synaptic membrane but was either preventing the release of excitatory transmitter from presynaptic terminals or forming a complex with sub-synaptic receptors which prevented the access of excitatory transmitter.

A further peculiarity of the lateral geniculate cells was their insensitivity to phenylethylamine derivatives, which often have a comparable inhibitory potency to 5-HT on cells elsewhere in the central nervous system (Krnjević & Phillis, 1963*a*; Salmoiraghi & Stefanis, 1965; Legge, Randić & Straughan, 1966; Engberg & Ryall, 1966).

The optic nerves, optic tracts and lateral geniculate nuclei of several species contain barely detectable or undetectable amounts of catecholamine and 5-HT (Amin, Crawford & Gaddum, 1954; Vogt, 1954; Bogdanski, Weissbach & Udenfriend, 1957; Cobbin, Leeder & Pollard, 1965). Fluorescence microscopy, however, has revealed that both catecholamine- and 5-HT-containing nerve terminals are present in the dorsal lateral geniculate nucleus of the rat (Fuxe, 1965) and these may be a part of the monoaminergic projection system arising in the lower brain stem that has recently been postulated by Andén *et al.* (1966) and Shute & Lewis (1966). On the basis of the histochemical and pharmacological findings Fuxe (1965) has suggested that 5-HT may act as an inhibitory transmitter in the geniculate nucleus. This paper describes the results obtained in a study of the actions of various monoamines and related compounds on neurones in the dorsal lateral geniculate nucleus.

Barbiturates appear to depress the inhibitory effects of noradrenaline

and 5-HT on neurones in the spinal cord (Engberg & Ryall, 1966) and thalamus (J. W. Phillis and A. K. Tebēcis, unpublished observation). The animals used in the present investigation were therefore anaesthetized with nitrous oxide and halothane (Fluothane, ICI) or nitrous oxide and methoxyflurane (Penthrane, Abbott). These combinations of anaesthetics have been used in a concurrent series of experiments with thalamic neurones on which monoamines also have marked effects, and comparable results have been obtained in both areas. The action of cholinomimetic compounds on lateral geniculate neurones will be described in a subsequent paper (Phillis, Tebēcis & York, 1967).

METHODS

Anaesthesia was induced in the cats used in these experiments by an intravenous injection of thiopentone sodium (Intraval Sodium, May & Baker). Supplementary doses of thiopentone sodium were administered during the preparation of the animal up to the stage of mounting it in the stereotaxic frame, at which point it was connected to a gas anaesthetic machine (Commonwealth Industrial Gases, Midget Mark I) and anaesthesia was subsequently maintained with mixtures of nitrous oxide, oxygen and halothane (Fluothane, I.C.I.) or nitrous oxide, oxygen and methoxyflurane (Penthrane, Abbott). The levels of halothane or methoxyflurane were adjusted to maintain the animal in a state of light anaesthesia (stage 3, level I).

The right optic nerve was exposed in some animals by resection of the right eyeball, and the left parieto-occipital cortex was exposed. After aligning the animal in a frame, a stereotaxically oriented glass micropipette was inserted into the cortex lateral to the lateral geniculate nucleus, cut and left in position to serve as a marker for the alignment of multiple barrel electrodes and to assist in selection of the area of brain used in subsequent histology. A bipolar stimulating electrode was placed on the exposed optic nerve and a Grass PS 2 Photostimulator mounted in front of the left eye.

A series of 5–6 bipolar concentric stimulating electrodes were inserted into the gyrus marginalis in the left visual cortex so that their tips were located approximately 4 mm below the cortical surface. These were used to stimulate the terminal portions of the optic radiation fibres in order to activate antidromically neurones in the ipsilateral LGN. The exposed cortical surface was covered with a 2–3 mm thick layer of 4 % agar in a physiological saline of the following composition: Na, 152·8 m-equiv/l.; K, 2·65 m-equiv/l.; Ca, 1·05 m-equiv/l.; Cl, 157·6 m-equiv/l.; glucose, 763 mg/l. (based on the estimations of Bito & Davson, 1966). A small area of the cerebral cortex overlying the lateral geniculate nucleus was left exposed for the insertion of micropipettes.

Evoked field potentials and spike potentials were recorded through a sodium chloride (2 M) filled barrel of the multiple barrelled-pipettes used in these experiments. The recording barrel was connected to a negative capacitance probe (Bioelectric Instruments) and potentials were displayed on a Tektronix 565 oscilloscope after suitable amplification, and in some instances recorded on film. The output of the preamplifier was also connected in parallel to another oscilloscope, two spike intensifiers (Kellett, Phillis & Veale, 1965) and an audio amplifier. The second oscilloscope was used to monitor the output pulses of one of the spike intensifiers which was connected to an electronic counter (Hewlett Packard 5214 L). This spike intensifier operated as a variable voltage gate and pulse generator, enabling the rejection of all spike discharges smaller than those of the unit primarily under observation, and providing the counter with a uniform series of pulses corresponding to cell discharges. Gate

times on this counter range from 10 μsec to 100 sec, but it was most frequently used with gate times of 0·1–1 sec. The output of the counter was displayed on an ink recorder (Texas Instruments Recti/Riter) using the analog output coupling stage of a Hewlett Packard 562 A digital recorder.

The construction and filling of five-barrelled electrodes has already been described in detail (Krnjević & Phillis, 1963*b*) and the nine-barrelled electrodes used in these experiments were made and filled in a similar manner. Aqueous solutions of the various monoamines were made up at a pH of 3·5–5 to improve their stability, and the filled electrodes were stored in the dark at 4° C. One barrel of each nine- and of some of the five-barrelled electrodes was filled with a solution of 0·1 N hydrochloric acid for making lesions in the LGN (McCance & Phillis, 1965). L-glutamate was always present in another barrel. Drugs were applied as cations, with the exception of L-glutamate. A retaining potential was applied to all drug-containing barrels to prevent the diffusion of actively ionized compounds. The potential was usually adjusted so that the 'braking current' was of the order of 5 nA. In particular, when using the nine-barrelled electrodes, it was sometimes observed that neuronal spikes rapidly disappeared after the initial contact with a neurone but reappeared if the 'braking currents' were increased to 20 nA. Such large 'braking currents' were not used routinely as they were associated with longer latencies before the appearance of drug-induced effects, presumably a result of the removal of the drug from the immediate vicinity of the electrode tip. This in turn made it difficult to compare the relative potencies of the compounds tested.

In the present investigation the following substances were dissolved in aqueous solutions at the given concentrations: 5-hydroxytryptamine creatinine sulphate (May & Baker) 0·1 M; 5-hydroxytryptamine bimaleinate (Koch-Light Laboratories) 0·2 M; 3,4-dihydroxyphenylethylamine (dopamine) (Calbiochem and Koch-Light Laboratories) 1·0 M; L-noradrenaline bitartrate (British Drug Houses) 1·5 M; D-lysergic acid diethylamide hydrogen tartrate (lysergide) (Sandoz, whose generosity in donating drug samples is appreciated) 0·1 M; Sodium L-glutamate (British Drug Houses) 1·5 M; acetylcholine chloride (British Drug Houses) 1 M.

At the termination of most experiments, the animals were perfused with a 0·9 % saline solution followed by 10 % formol saline. After further fixation in formol saline, 50 μ serial sections of the LGN were cut on a freezing microtome and stained with Luxol Fast Blue and Neutral Red (Lockard & Reers, 1962). Photomicrographs of whole sections were taken with a Nikon 6 C Shadowgraph.

RESULTS

5-HT. Many of the cells tested with 5-HT were located by applying L-glutamate continuously as the electrode tip passed through the dorsal lateral geniculate nucleus. Subsequent identification of the location of such cells in the nucleus was made possible by making lesions at the recording site. Passage of hydrogen ion by a current of 4–10 μA for 5–10 sec results in the formation of a small (100–150 μ diameter) lesion and rows of these lesions are readily seen in sections through the nucleus. The technique has been extensively employed in related experiments on the cerebellum and thalamus (McCance & Phillis, 1964; McCance, Phillis & Westerman, 1966) and has the advantages of reliability and rapidity. Local tissue lesions are also useful as a means of assessing the local or remote origin of evoked potentials within the brain. Locally evoked negative fields will reverse to positive potentials after the destruction of activated cells whereas distantly

evoked potentials remain unchanged (McCance, Phillis & Westerman, 1965; Malliani & Zanchetti, 1965).

Examples of two lesions are shown in Fig. 1*A*. This is a photomicrograph of a transverse section through the hippocampus and lateral geniculate nucleus at an antero-posterior level of +3·5. Two electrode tracks are

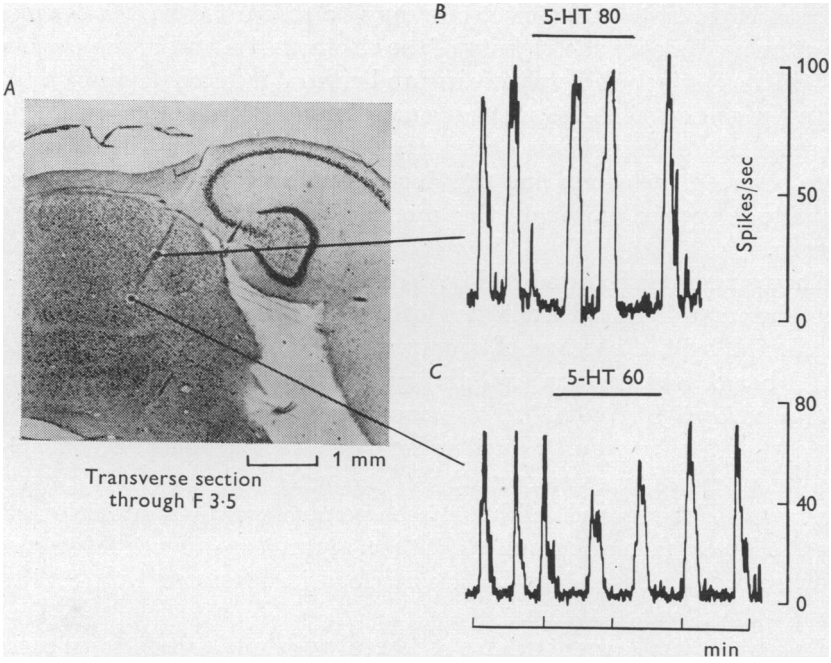

Fig. 1. Comparison of effects of 5-HT on L-glutamate firing of two lateral geniculate neurones. (*A*) 50 μ frozen section cut at *A–P* stereotaxic co-ordinate +3·5. Position of cells indicated by acid lesions. (*B*) and (*C*). Firing frequencies of the two cells evoked by repeated applications of L-glutamate (80 nA). 5-HT applied during periods indicated by horizontal solid line. In this and subsequent records the firing frequency of cells was plotted by means of an electronic counter and ink recorder. Drug applications indicated by horizontal solid lines above and/or below trace.

visible on the section, the more lateral track being punctuated by two lesions. The responses from the two cells located at the lesion sites are shown in Fig. 1*B, C*. The usual technique adopted for testing the depressant monoamines on L-glutamate excitation was to apply the latter at approximately 30 sec intervals for a period of 10–15 sec. When the current through the L-glutamate barrel had been adjusted to give a consistently repeatable response, a cationic current was passed through the monoamine containing barrel, applications of L-glutamate being continued at 30 sec intervals. Any inhibitory action of the monoamine will be apparent as a reduction in the

rate of firing of the cell induced by L-glutamate. A series of monoamine applications was made on each neurone tested, the cationic current through the barrel containing the monoamine being increased in magnitude after each application until either an appreciable reduction in the L-glutamate excitation was apparent or the neurone was assessed as being insensitive to the monoamine in question. The passage of large outward (cationic) currents through a micro-electrode may be associated with a depressant effect on neurones in the vicinity of the electrode tip, and it was frequently difficult to distinguish between drug and current induced depressions when large (in excess of 80-100 nA) cationic currents were employed and only minimal depressant effects observed. Cells that came into this category were assessed by comparing the depressant effects of currents of equal magnitude passed through the monoamine containing and recording barrels.

The neurones illustrated in Fig. 1 differed in their sensitivity to 5-HT. The glutamate-induced excitation of the unit illustrated in Fig. 1*B* was unaffected by the passage of a current of 80 nA for 90 sec through the 5-HT barrel whereas the excitability of the second unit was markedly depressed by 5-HT (60 nA). The same current was used to apply L-glutamate to both cells (80 nA). Further increases in the magnitude of the 5-HT applying current were associated with a greater depression of glutamate excitation of the second cell but there was no discernible inhibition of the first. It is clear from the results that the L-glutamate-induced firing of some neurones in the lateral geniculate nucleus is depressed by 5-HT more readily than that of others.

Difficulties were experienced in repeatedly passing large cationic currents through 5-HT containing electrodes used in these experiments and this has hindered attempts to assess the relative proportions of 5-HT sensitive and insensitive cells. It was frequently impossible to pass currents greater than 40 nA through 5-HT barrels and the possibility that neurones which were unaffected by these amounts of the drug would have responded to larger doses cannot be excluded. Furthermore, the 5-HT was associated with creatinine or bimaleinate which would have carried a considerable proportion of the iontophoretic current. The mean value of the transport numbers for 5-HT in four pipettes of a similar type to those used in these experiments was 0·14 and therefore considerably lower than that for adrenaline (0·21) and acetylcholine (0·42) (Krnjević, Mitchell & Szerb, 1963; Krnjević, Laverty & Sharman, 1963). Consequently there is some justification for the assumption that the potency of 5-HT can be doubled when comparing it with compounds that are not associated with a cationic complex (Curtis & Davis, 1962).

Such considerations must be borne in mind when evaluating the signifi-

cance of any comparative results, and although 5-HT depression of L-glutamate induced excitation was evident with only one third of the neurones tested, it is possible that the proportion would have been considerably greater had it been feasible to increase the magnitude of the 5-HT applying currents. Similar considerations inevitably apply to other sections of the results obtained with 5-HT.

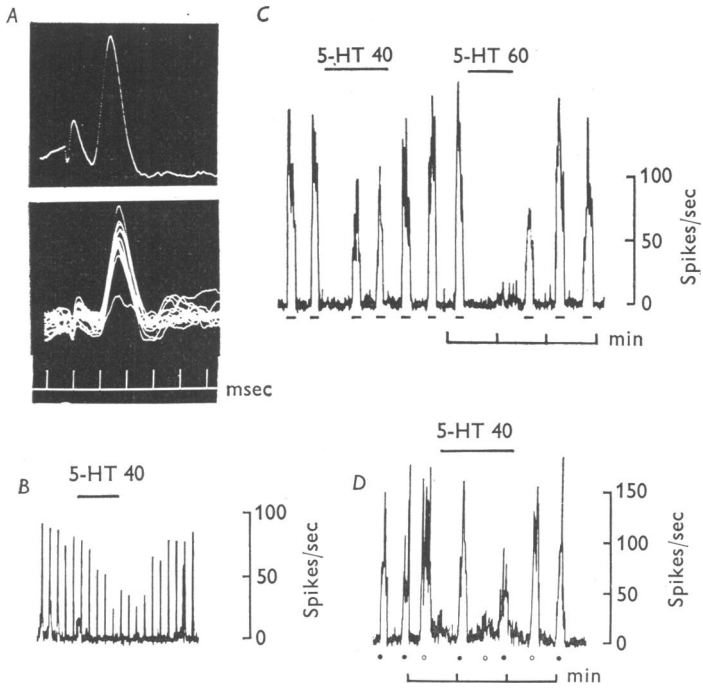

Fig. 2. (*A*)–(*D*) are the responses of one neurone. (*A*) Antidromic spikes evoked by single and repetitive (100/sec) stimulation of ipsilateral visual cortex. (*B*) Bursts of firing evoked by light flashes in left eye at 10 sec intervals and recorded with electronic counter. 5-HT (40 nA) applied during period indicated. (*C*) Depression of L-glutamate (40 nA) firing by 5-HT (40, 60 nA). (*D*) Comparison of depression of acetylcholine (40 nA, open circles) and L-glutamate (40 nA, filled circles) firing by 5-HT (40 nA).

Many of the neurones that were initially located by the application of L-glutamate also responded to optic nerve or visual stimulation and some could be antidromically activated by stimulation of the terminal portion of the optic radiation in the visual cortex. The invasion of an antidromic spike establishes that a cell is a part of the geniculo-cortical projection system and the following criteria have been adopted to distinguish such responses from those mediated orthodromically by either corticofugal fibres (Widén & Marsan, 1960) or recurrent collaterals (O'Leary, 1940);

(*a*) a constant latency of < 1·2 msec measured from the cortical stimulus, (*b*) the ability to follow repetitive stimulation at frequencies of 80–100/sec and (*c*) responses to two shocks at short intervals (about 1 msec).

The spikes in Fig. 2*A* are from a unit which responded to cortical stimulation with an all-or-none spike superimposed on a small negative field. The spike appeared with a constant latency of 1 msec and would follow repetitive cortical stimulation at 100/sec. It can therefore be assumed that this was a geniculo-corticate neurone. It also responded to brief flashes of light delivered at 10 sec intervals to the left (ipsilateral) eye with a high frequency burst of spikes. Records of a series of responses to flashes of light are presented in Fig. 2*B*. During the period of application of 5-HT (40 nA), the number of spikes discharged in response to each light flash was considerably reduced. The response returned to normal within 60 sec of the cessation of 5-HT application. L-glutamate induced firing of this cell was also depressed by 5-HT (Fig. 2*C*). Concurrent with the abolition of the L-glutamate response by 5-HT (60 nA), there was a failure of invasion of the antidromic spike (see also Fig. 3). This neurone was also excited by acetylcholine as were many of the neurones tested (Phillis *et al.* 1967). In a previous investigation, using pentobarbitone sodium anaesthetized cats, acetylcholine was found to have a very weak excitant action on lateral geniculate neurones (Curtis & Davis, 1962) because it failed to activate neurones unless a background discharge was provided, either by the application of L-glutamic acid or by synaptic action. In the present investigation, acetylcholine and related compounds had profound excitatory effects on lateral geniculate neurones. Sufficient amounts of the drugs to cause an effect were sometimes released by diffusion from the electrode alone. In this respect, the effects of acetylcholine on some geniculate neurones are as potent as those observed on Renshaw cells in the spinal cord (Curtis & Eccles, 1958; Curtis, Phillis & Watkins, 1961*b*), thalamic neurones (McCance *et al.* 1966) and granule layer cells in the cerebellar cortex (McCance & Phillis, 1964).

A comparison of the effects of 5-HT on firing induced by L-glutamate (filled circles, 40 nA) and acetylcholine (open circles, 40 nA) is shown in Fig. 2*D*. After recording the control responses, 5-HT (40 nA) was applied for 90 sec, during which period the responses to L-glutamate were considerably reduced and those to acetylcholine almost abolished. A depressant effect of 5-HT on acetylcholine excitation has previously been described (Curtis & Davis, 1962), but the results were obtained from cells which were simultaneously being activated synaptically by optic nerve stimulation or light flashes, and the reduction in acetylcholine excitation was attributed to a block of this facilitatory background activation. The unit illustrated in Fig. 2 responded to acetylcholine with a high frequency

discharge in the absence of any controlled background facilitation and therefore the block of acetylcholine activation cannot be explained in this manner.

The antidromic responses of another unit, shown in Fig. 3*A* (1–4), fulfilled the criteria listed above and it was accordingly classified as a geniculo-cortical neurone. After applying 5-HT (100 nA) for 30 sec, invasion of the

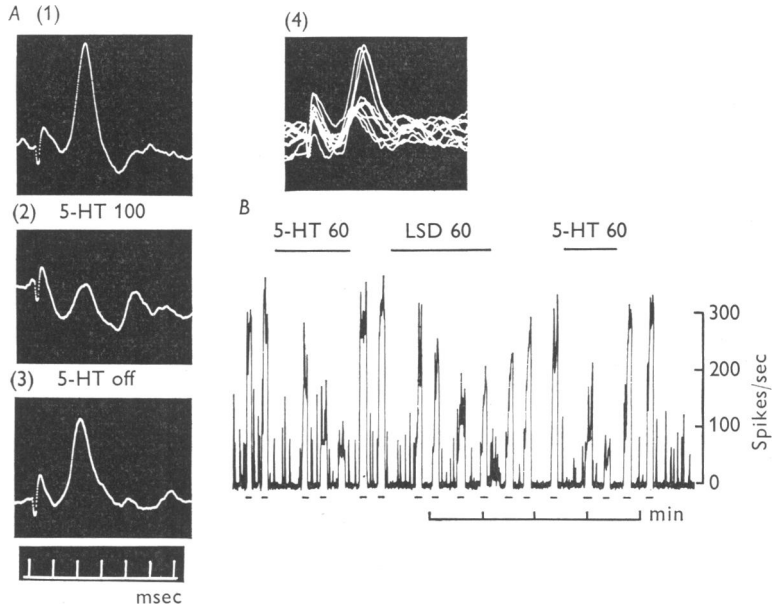

Fig. 3. *A*. Antidromic spikes of geniculo-cortical neurones. *A* (1), Control response. *A* (2), response after 5-HT (100 nA) applied for 30 sec. *A* (3), recovery of invasion 25 sec after termination of 5-HT. *A* (4), superimposed responses with cortical stimulus straddling threshold. (*B*) L-glutamate (20 nA) and spontaneous firing of same cell depressed by 5-HT (60 nA) and lysergide (LSD, 60 nA). Lysergide did not antagonize 5-HT.

antidromic spike failed, leaving a small negative field potential (Fig. 3*A* (2). The response recovered rapidly after cessation of the 5-HT application (Fig. 3*A* (3)). Figure 3*A* (4) was recorded by superimposing several sweeps during a period when the intensity of cortical stimulation was just at threshold for this fibre and clearly illustrates the all-or-none nature of the response. 5-HT also depressed L-glutamate excitation of this neurone (Fig. 3*B*) as did lysergide (LSD).

Invasion of an antidromic spike was not blocked by 5-HT on all the lateral geniculate neurones tested, even though L-glutamate or acetylcholine excitation was depressed. Such a finding is not incompatible with a theory which suggests that 5-HT acts by stabilizing the post-synaptic

membrane, as the safety margin for the antidromic propagation of a spike to lateral geniculate neurones may be higher than that for spinal motoneurones. In a similar investigation the action of the depressant monocarboxylic amino acid, γ-aminobutyric acid, on Betz cells in the feline cerebral cortex, it was also found that antidromic spikes were considerably less susceptible to blocking than those evoked by synaptic or chemical stimulation, and the same explanation is likely to be valid (Krnjević & Phillis, 1963*b*). γ-Aminobutyric acid readily prevents the invasion of an antidromic spike into motoneurone somas (Curtis, Phillis & Watkins, 1959), where the safety factor for spike propagation is smaller, perhaps as a result of the greater cell size.

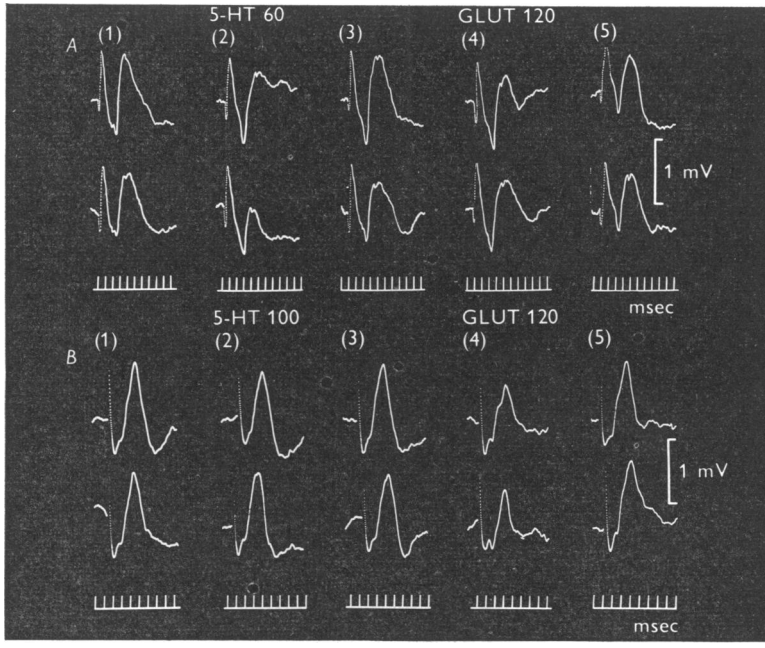

Fig. 4. Focal potentials evoked by maximal stimulation of the contralateral optic nerve. Each pair of vertically superimposed responses was recorded at 2 sec intervals *A* (1, 3, 5), are control responses; *A* (2), was recorded after 5-HT (60 nA) had been applied for 35 sec and *A* (4), after L-glutamate (120 nA) application for 25 sec. *B* (1, 3, 5), are another series of control responses recorded at another position in the nucleus. 5-HT (100 nA) for 45 sec had little depressant action; L-glutamate (120 nA) depressed the potential. Time marker-msec, voltage calibrations—1 mV.

There was considerable variation in the effects of 5-HT on focal potentials evoked in the lateral geniculate nucleus by optic nerve or visual stimulation. Two series of records evoked by maximal stimulation of the contralateral optic nerve are presented in Fig. 4, each pair of vertically super-

imposed potentials being members of a consecutive sequence. The focal potential illustrated in Fig. 4*A* was depressed by the application of 5-HT (60 nA) for a period of 35 sec and the recovery lasted 40 sec after the applying current was terminated. However 5-HT (100 nA) applied for 45 sec had little effect on another synaptically evoked field in the same animal (Fig. 4*B*). Possible explanations for the failure of 5-HT to depress the second field are either that the responsible cells were insensitive to its effects or that the electrode was recording an electrotonically propagated potential, generated at a sufficient distance from the electrode tip to be unaffected by drug applications. The second alternative is unlikely to have been the reason for the failure of 5-HT to depress the second focal potential as L-glutamate (120 nA) caused a comparable amount of depression of both evoked potentials. The depression of orthodromically evoked potentials by an excitant amino acid is in accordance with the effects that have been observed when L-glutamate is applied iontophoretically near neurones in the spinal cord (Curtis, Phillis & Watkins, 1960).

Lysergide. Intracarotid injection of lysergide and related substances depress the evoked response in the lateral geniculate nucleus to optic nerve stimulation (Evarts, Landau, Freygang & Marshall, 1955; Bishop, Field, Hennessy & Smith, 1958). It has been suggested that this is the result of these compounds interfering with the attachment of the natural excitatory transmitter to its subsynaptic receptors (Bishop, Burke & Hayhow, 1959). The results obtained by Curtis & Davis (1962) substantiate this hypothesis as iontophoretically applied lysergide depressed synaptic firing without affecting L-glutamate induced excitation. In other areas of the central nervous system, such as the cerebral and pyriform cortices (Krnjević & Phillis, 1963*a*; Legge *et al.* 1966), lysergide has a depressant effect on amino acid excitation as well as depressing synaptic firing. Lysergide is known to antagonize the peripheral and some central effects of 5-HT (Gaddum, 1958; Gaddum & Hameed, 1954; Woolley, 1958; Bond & Guth, 1964) and its effects on synaptic transmission in the lateral geniculate nucleus have been adduced as evidence that 5-HT is an excitatory transmitter in the lateral geniculate. However, in the present experiments, when lysergide was tested on neurones in the lateral geniculate nucleus, it depressed both synaptic- and amino acid-induced firing and could not be shown to interfere with the depressant action of 5-HT.

The photographic record (Fig. 5*A*) and the initial portion of the ink recorder trace (Fig. 5*B*) were recorded simultaneously from the same cell. The unit was firing spontaneously and responded to the application of L-glutamate (20 nA) with a rapid discharge of spikes. The rate of spontaneous firing decreased progressively during the period of application of lysergide by a current of 40 nA and eventually the unit ceased to discharge

spontaneously. It is also evident from the ink recorder trace that there was a reduction in the magnitude of the L-glutamate effect. After cessation of the lysergide application, the L-glutamate response recovered within 70–80 sec although the cell did not resume spontaneous firing at the original rate. A further application of lysergide (80 nA) caused even more marked reduction in the response to glutamate ion.

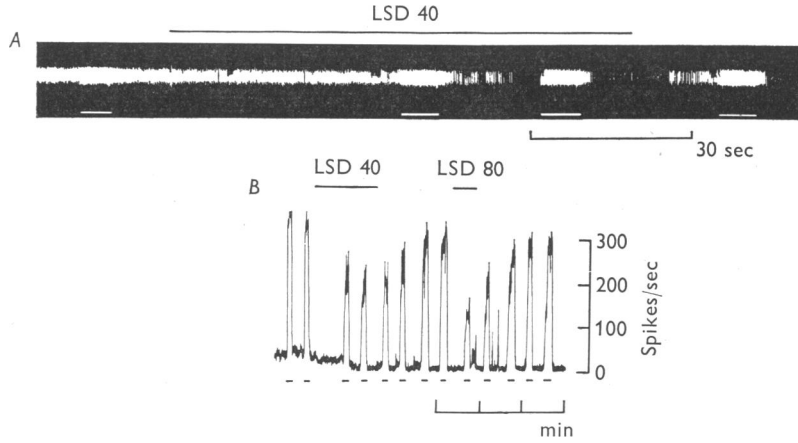

Fig. 5. (*A*) and (*B*). Photographic and ink records from same neurone. The initial portion of trace in (*B*) was recorded simultaneously with (*A*). Lysergide (LSD, 40 nA) depressed spontaneous and L-glutamate (20 nA) firing without altering spike size. LSD (80 nA) had a more pronounced effect. L-glutamate applications indicated by horizontal white lines.

Because of the duration of these depressant effects of lysergide, it was difficult to ascertain whether it also antagonized the action of 5-HT. A 5-HT depressed cell which was also tested with lysergide is shown in Fig. 3*B*. After recording a control response to 5-HT (60 nA), lysergide (60 nA) was applied for 2 min. This caused a smaller depression than did the 5-HT, and it continued for a longer period after the application had ceased. When 5-HT was tested 80 sec after the termination of the lysergide application, it caused a comparable depression to that observed in the control response. Lysergide and 5-HT were tested on several cells in this manner and it was never possible to demonstrate any antagonism between them.

Dopamine and noradrenaline. These compounds had a potent depressant action on many of the neurones tested in the lateral geniculate nucleus, blocking synaptic and antidromic activation as well as amino acid and acetylcholine excitation. A comparison of the effects of these two substances on L-glutamate excitation of two cells are shown in Fig. 6. Dopamine

(20 nA) strongly depressed the glutamate-induced firing of both cells and noradrenaline had a comparable effect when applied with a current of 40 nA. The second cell was also depressed by lysergide (25 nA) whereas the first unit was not affected when lysergide was applied with a current of 35 nA.

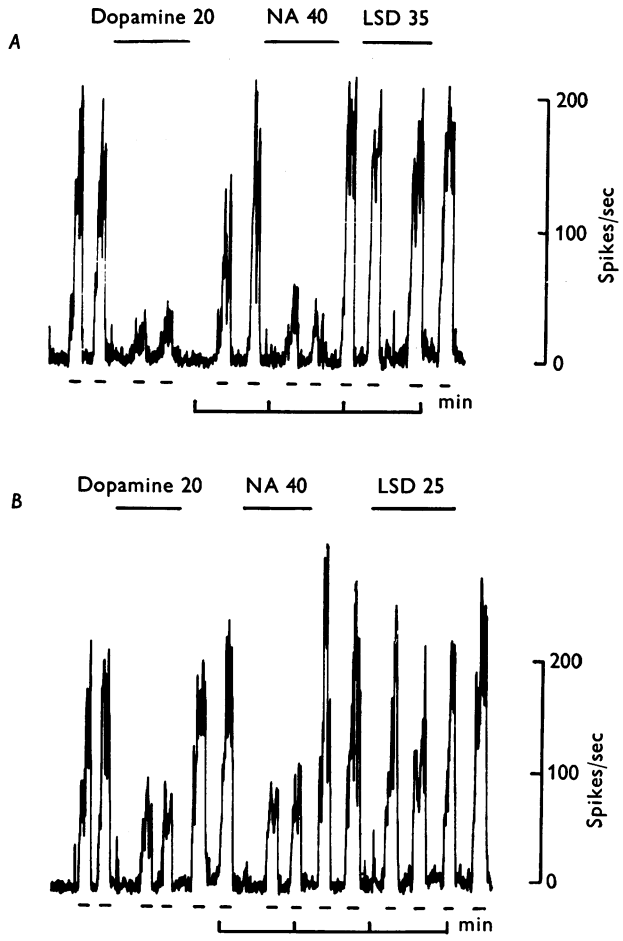

Fig. 6. Depression of L-glutamate firing by dopamine and noradrenaline. (*A*), L-glutamate (40 nA) firing depressed by dopamine (20 nA) and noradrenaline (NA, 40 nA) but not by lysergide (LSD, 35 nA). (*B*), another neurone, L-glutamate firing (50 nA) depressed by dopamine, noradrenaline and lysergide (LSD, 25 nA).

Differences between the sensitivity of lateral geniculate neurones to catecholamines and 5-HT or lysergide were frequently apparent. For a given electrode the relative potencies of dopamine and noradrenaline

remained constant for all the neurones tested. Although there was a considerable variability between the results obtained with different electrodes, dopamine was more potent as a depressant than noradrenaline. The transport numbers for a series of noradrenaline containing electrodes ranged from 0·02 to 0·37 (Krnjević *et al.* 1963). This factor is probably the major cause in the variability of our results with this compound. A comparison of the relative potencies of the catecholamines with 5-HT or lysergide revealed a considerable fluctuation from cell to cell, suggesting that catecholamines may be acting on a different receptor from 5-HT and lysergide.

Dopamine and noradrenaline depressed the synaptic firing of lateral geniculate neurones in response to optic nerve or visual stimulation in an analogous manner to 5-HT. Similarly the time course of the depression of synaptic firing was closely related to that of the inhibition of glutamate firing, which suggests that the two processes are related. The application of larger amounts of dopamine or noradrenaline to sensitive cells also depressed the invasion of antidromic spikes.

DISCUSSION

The excitability of many neurones in the dorsal lateral geniculate nucleus was depressed by monoamines and lysergide. This depression was manifested by a reduction or abolition of the effects of optic nerve or visual stimulation, by a failure in some instances of an antidromically propagating spike to invade the cell soma, and by a depression of the excitant effects of L-glutamate or acetylcholine.

The significance of these results is apparent when the responses of lateral geniculate neurones to iontophoretically applied monoamines are compared with those of neurones elsewhere in the central nervous system. It is clear that monoamines can have an essentially similar depressant action on cells in many regions of the feline central nervous system, including the spinal cord (Engberg & Ryall, 1966; Weight & Salmoiraghi, 1966), cerebral cortex (Krnjević & Phillis, 1963*a*), pyriform cortex (Legge *et al.* 1966), hippocampus (Biscoe & Straughan, 1966), caudate nucleus (Bloom, Costa & Salmoiraghi, 1965), thalamus (Andersen & Curtis, 1964; Phillis & Tebēcis, 1967), hypothalamus (Bloom, Oliver & Salmoiraghi, 1963) and brain stem (Bradley & Wolstencroft, 1965) and to this list can now be added the lateral geniculate nucleus. Excitant effects of the monoamines have been observed in several areas of the nervous system, but these were not evident in the present experiments on the LGN. The possibility that more extensive testing, or larger applications of the substances used, would have revealed excitant effects cannot be excluded.

The failure to observe a depression of excitability of all neurones tested suggests that there is some variability in the distribution of membrane receptors for monoamines in the lateral geniculate nucleus. It also excludes the likelihold that these compounds were acting on γ-aminobutyric acid type receptors as suggested by Krnjević & Phillis (1963*a*). γ-Aminobutyric acid was not routinely carried in the micropipettes used in these experiments, but it can be anticipated that receptors for this substance would have been present on all the neurones tested, since the widespread sensitivity of cells in the central nervous system to the actions of the excitant and depressant amino acids makes it likely that receptors for these compounds are inherently present in the soma membrane of nerve cells. An exhaustive study of structure–activity relations of amino acids (Curtis, Phillis & Watkins, 1961*a*) has led to the conclusion that the excitant and depressant amino acids act on the same or very similar sites on the membrane (Watkins, 1965) and as L-glutamate excited all of the neurones tested in this survey it can be assumed that they would also have had receptor sites for γ-aminobutyric acid. Accordingly, if monoamines combine with amino acid receptors, they should have depressed all the cells tested, but this did not occur.

Differences in the relative sensitivities of cells in the lateral geniculate nucleus to the depressant actions of catechol and indole amines may indicate that these two groups of compounds are acting on different types of receptors, as has also been suggested for their actions on neurones in the spinal cord (Engberg & Ryall, 1966).

The consistency with which monoamines produce a reduction in excitability of sensitive neurones, manifested by a decreased effect of synaptic, chemical and antidromic stimulation, indicates that these compounds act post-synaptically. The presence of both catecholamine and 5-HT containing nerve terminals in the lateral geniculate nucleus (Fuxe, 1965) raises the possibility that they may in fact be inhibitory transmitters in this nucleus. Alternatively, they could be acting by stimulating the release of inhibitory transmitter from presynaptic terminals or on non-synaptic receptors on the post-synaptic membrane. Although difficult to rule out entirely, it is unlikely that these compounds are acting presynaptically and, on the evidence currently available, a post-synaptic locus of action must be postulated. Attachment of the monoamines to their receptors on the post-synaptic membrane may increase the permeability of the membrane to chloride ion or possibly both chloride and potassium ions, the resultant change in the conductance of the membrane being directly responsible for the stabilization of the membrane. Further investigation with intracellular recording techniques and a comparison of the reversal potentials of the drug and synaptically induced inhibitory potentials would be required to

provide more direct evidence that these compounds are acting as inhibitory transmitters.

Evidence has recently been obtained suggesting that dopamine acts as the inhibitory transmitter in the caudate nucleus. An increased output of dopamine from the caudate nucleus in response to stimulation of nucleus centromedianus thalami (CM) has previously been reported by McLennan (1964). When caudate neurones are induced to fire by DL-homocysteic acid, they respond to CM stimulation by a depressant response. Furthermore, in this nucleus, administration of the α-adrenergic blocking agent phenoxybenzamine (dibenzyline) abolishes the inhibitory actions of both dopamine and CM stimulation (McLennan & York, 1967; York, 1967).

The failure of several groups of investigators to detect significant amounts of catecholamine and 5-HT in the optic nerves and lateral geniculate nuclei (Amin *et al.* 1954; Vogt, 1954; Bogdanski *et al.* 1957; Cobbin *et al.* 1965) is not inconsistent with the hypothesis that this nucleus receives a monoaminergic innervation. A monoaminergic fibre system projecting from the brain stem to the diencephalon and telencephalon has recently been proposed by Andén *et al.* (1966) and Shute & Lewis (1966). The cell bodies for this system are situated in the lower brain stem and their axons ascend in the medial forebrain bundle. The dorsal lateral geniculate nucleus receives a dense innervation with catecholamine and 5-HT containing nerve fibres but does not contain any monoamine-positive nerve cell bodies (Fuxe, 1965). The ascending fibres are very thin (1–2 μ); which may explain why it is difficult to detect their contents in extracts of the lateral geniculate nucleus.

This monoaminergic projection system, which the present results would suggest is inhibitory in action, may be complementary to the cholinergic projection system postulated by Shute & Lewis (1966). Acetylcholine has an excitatory action on cells in the lateral geniculate nucleus (Curtis & Davis, 1963; Phillis *et al.* 1967) and between them the two systems would be expected to control the level of excitability in this nucleus. Facilitatory and inhibitory effects of stimulation of the mesencephalic reticular formation upon neurones in the LGN have been described by Suzuki & Taira (1961).

The differences between the findings presented in this paper and those described by Curtis & Davis (1962) can most readily be attributed to the different anaesthetics used in the two surveys. Engberg & Ryall (1966) and Weight & Salmoiraghi (1966) have recently shown that catechol and indole amines depress neurones in the cat spinal cord, although this was not apparent in an earlier investigation (Curtis *et al.* 1961*b*), in which cats anaesthetized with pentobarbitone sodium were used. Administration of pentobarbitone sodium to unanaesthetized decorticate cats reduced the

depressant effect of noradrenaline on spinal interneurones and on a Renshaw cell (Engberg & Ryall, 1966). A comparison of the relative percentages of spinal neurones depressed by noradrenaline and 5-HT in unanaesthetized and diethyl ether anaesthetized cats indicates that diethyl ether does not possess this action of the barbiturates (Weight & Salmoiraghi, 1966). Our experiments on thalamic and lateral geniculate neurones suggest that halothane and methoxyflurane are also satisfactory in this respect.

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