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PHARMACOLOGICAL STUDIES UPON NEURONES OF THE LATERAL GENICULATE NUCLEUS OF THE CAT

BY

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Indoles related to 5-hydroxytryptamine, lysergic acid derivatives, phenethylamine derivatives and some other compounds have been applied electrophoretically to the neurones of the lateral geniculate nucleus of the cat anaesthetized with pentobarbitone sodium. Many of these compounds, particularly 4-, 5- and 7-hydroxytryptamine and ergometrine, depress the orthodromic excitation of the neurones by volleys in optic nerve fibres, but do not affect antidromic excitation by volleys in the optic radiation or chemical excitation by L-glutamic acid. It is concluded that the active depressants either block the access of the excitatory transmitter to subsynaptic receptors or prevent the release of the transmitter from optic nerve terminals. The structure-activity relationships of the depressant substances are discussed.

Of recent years considerable interest has been maintained in a series of chemical compounds which have been classified as psychotomimetics since their administration produces clinical syndromes bearing some similarity to naturally occurring psychoses. These substances can be divided into two major groups. One contains an indole nucleus and includes substances such as (+)-lysergic acid diethylamide, certain of the ergot alkaloids, bufotenine, psilocybin and harmine. Members of the other group are related to the catechol amines and include adrenochrome and mescaline. Both groups of substances are of importance since it is possible that their effects are associated with the presence of structurally related compounds as chemical synaptic transmitters within the nervous system. Thus the indolylalkylamine 5-hydroxytryptamine and the catechol amines adrenaline, noradrenaline and 3-hydroxytryptamine have been considered as transmitters although definite evidence is lacking (Erspamer, 1954, 1961 ; Brodie & Shore, 1957 ; Page, 1958 ; Costa, 1960 ; Carlsson, 1959 ; Rothballer, 1959).

There is an extensive literature associated with the psychotomimetics (Kety, 1957 ; Pennes, 1958 ; Bradley, Deniker & Radouco-Thomas, 1959 ; Hofmann, 1959), but there are few reports devoted to descriptions of their mode of action upon particular portions of the central nervous system (see Evarts, 1957). One finding of importance, however, is that the postsynaptic responses of neurones in the lateral geniculate nucleus of the cat, produced by optic nerve impulses, were reduced by an intracarotid injection of either lysergic acid diethylamide or bufotenine (Evarts, Landau, Freygang & Marshall, 1955). The action of lysergic acid diethylamide upon these cells was confirmed by Bishop, Field, Hennessy & Smith (1958) using both intracarotid and intravenous injections. These investigators suggested that the failure

of this compound to affect the spike potentials of presynaptic fibres, the increase it produced in synaptic delay and the finding that repetitive stimulation of the optic nerve overcomes the block produced by lysergic acid diethylamide (Bishop, Burke & Hayhow, 1959) indicated that the compound was interfering with the attachment of the natural excitatory transmitter with its appropriate subsynaptic receptors. The known antagonism of lysergic acid diethylamide towards the action of 5-hydroxytryptamine upon smooth muscle (Gaddum, Hameed, Hathway & Stephens, 1955; Gaddum, 1958) suggests that the excitatory transmitter itself could be related to 5-hydroxytryptamine. The possibility that it might indeed be 5-hydroxytryptamine was rendered unlikely by the failure of Bogdanski, Weissbach & Udenfriend (1957) to detect 5-hydroxytryptamine in this area of the nervous system.

Evarts (1958) extended these investigations and determined the effects of a series of tryptamine derivatives upon synaptic transmission in the lateral geniculate nucleus. Tryptamine and dimethyltryptamine had actions similar to those of lysergic acid diethylamide, but 5-hydroxytryptamine was ineffective. This was not unexpected since the blood-brain barrier is relatively impermeable to this agent (Udenfriend, Weissbach & Bogdanski, 1957). The observation that 2-bromo-(+)-lysergic acid diethylamide, a powerful antagonist of the action of 5-hydroxytryptamine on smooth muscle (Cerletti & Rothlin, 1955), failed to affect lateral geniculate neurones suggested to Evarts that an antagonism towards 5-hydroxytryptamine was insufficient to explain the effects of lysergic acid diethylamide upon these neurones. Bishop, Burke, Davis & Hayhow (1960) also report the relative ineffectiveness of 5-hydroxytryptamine upon these cells and the lower potencies of psilocybin, 2-bromo-(+)-lysergic acid diethylamide and bufotenine compared with that of lysergic acid diethylamide.

When drugs are administered intravenously or intra-arterially the presence of diffusional barriers, such as the blood-brain barrier, may create serious difficulties when attempts are being made to study the pharmacology of centrally located neurones. The relative potencies of a series of compounds determined in this fashion may not be directly related to drug action at neuronal receptors, but may depend upon the relative abilities of the substances to penetrate the barrier. One method of drug administration which circumvents the blood-brain barrier depends upon the electrophoretic application of substances locally into the extracellular environment of neurones. This technique can be conveniently combined with extracellular recording of spike responses of single neurones (see Curtis & Eccles, 1958; Curtis & Watkins, 1960), and multiple drug-applying pipettes can be used so that the actions of several agents can be compared.

The present experiments, in which a series of indoles, catechol amines and some other compounds were applied electrophoretically to neurones of the lateral geniculate nucleus of the cat, were designed as an attempt to determine the nature of the excitatory transmitter of these cells. A preliminary report has been published (Curtis & Davis, 1961).

METHODS

The experiments were carried out upon groups of neurones and single neurones located in the dorsal part of the lateral geniculate nucleus of cats anaesthetized with pentobarbitone

sodium. After resection of the right eyeball, exposure of the optic nerve (Bishop, Jeremy & Lance, 1953) and removal of the bony vault over the left parieto-occipital cortex, the animal's head was placed in a rigid frame. Ear-plugs were used and the head was aligned so that Horsley-Clarke co-ordinates could be used for locating the nucleus. The right optic nerve was suspended clear of orbital tissue and was stimulated electrically. A small "lens-end" lamp was mounted so that controlled light flashes could be used to stimulate the left eye. In some experiments the geniculate neurones were activated antidromically by embedding a bank of 6 to 13 steel stimulating electrodes into the gyrus marginalis of the ipsilateral parietal cortex. These were insulated, except at their tips, which were placed 4 to 5 mm below the surface of the cortex amongst the terminating optic radiation axons (see Bishop & Davis, 1960).

The animal was suspended by pelvic and mid-thoracic clamps so that its body was held horizontally above the base of the animal frame. In this fashion respiratory movements of the brain were reduced to a minimum and artificial respiration was not necessary. The animal's temperature was maintained in the region of 36.5 to 38.5° C by means of heating pads.

The pia-arachnoid of the cortex through which microelectrodes had to pass was widely removed and the exposed brain tissue was continuously irrigated with a mammalian Ringer solution (Liley, 1956) at 37° C, the excess being removed by suction. Extracellular potentials were recorded by means of the central recording barrel of five-barrel electrodes, the total diameter of the electrode tip being 6 to 10 μ (Curtis & Eccles, 1958). The microelectrodes were carried by a micromanipulator which was rigidly attached to the head frame and permitted movements to be made in three directions. The central barrel of the five-barrel electrodes usually contained 5 M sodium chloride solution, but in many experiments a solution was used which permitted histological identification of the area from which records had been taken. This solution was similar to that described by Galifret & Szabo (1961), but potassium salts were avoided. The solution initially contained 2.5 M sodium citrate and 1.25 M cupric chloride, neutralized to *p*H 7 with sodium hydroxide. The specific resistance of this solution (13 ohm cm at 20° C) was much higher than that of 5 M sodium chloride (approximately 5 ohm cm), and the resistances of electrodes filled with the sodium citrate-copper complex were approximately 2.5 times higher than if they had been filled with 5 M sodium chloride. Consequently, equal portions of the complex mixture and 5 M sodium chloride were used, the specific resistance of the solution being approximately 8 ohm cm. Following the use of the electrode for recording purposes an anionic current of $2\text{–}4\times10^{-6}$ A was passed for 3 to 4 min. At the termination of the experiment the animal was perfused first with 0.15 M sodium chloride solution and then with 10% formol saline. After further fixation, frozen sections of the geniculate region were cut and stained with acid thionin (Laskey, 1949) or methylene blue followed by phloxine. Almost invariably a "lesion" was observed as a poorly stained circular area 300 to 600 μ in diameter. Many such lesions could be made in the one preparation, and by using these, together with the tracks made by the comparatively large electrodes, maps were made giving the location of neurones from which records had been taken.

The extracellular spike responses were of negative-positive voltage sequence and 0.2 to 1 mV in magnitude. When spikes were larger than this, and particularly when the spike was initially positive, the cell responses were altered by current flowing from the drug barrels (see Curtis & Koizumi, 1961). The responses were recorded photographically from an oscilloscope after suitable amplification and a negative-capacitance probe was used in order to obviate the comparatively large capacitance to earth of the recording electrode. In some experiments the frequency of firing of single neurones, evoked orthodromically or by drug application, was measured by a rate meter. After amplification the spikes were used to trigger a pulse generator, the constant-voltage output pulses of which were integrated electrically over a selected period. This potential, proportional to the number of pulses, was displayed by an ink-writer and a period of 0.2 sec was provided between counts in order to cancel the previous total. An integration interval of 1 sec was used when measuring the frequency of firing evoked by an excitant substance, but when spikes were elicited synaptically they were counted for 2 to 4 sec after the stimulus. The spike potentials, together with the output pulses of the pulse generator, were observed on a double-beam oscilloscope, and the triggering

level of the generator could be altered to discriminate between spikes of different size. This was important as it was often difficult to record the spikes of a single neurone in complete isolation, presumably because of the density of neurones in the nucleus.

Because of this difficulty of recording spike responses of single neurones many experiments were carried out using the negative focal potentials generated by many cells, activated either synaptically or antidromically. Similar potentials have been utilized when studying the effects of amino-acids upon spinal neurones (Curtis, Phillis & Watkins, 1959, 1960). In the spinal cord, field potentials were reduced in magnitude by both depressants and excitants of individual cells. Consequently, although the use of such potentials will at least indicate whether or not a substance has an effect upon neuronal membrane, be it excitant or depressant, additional studies have to be made upon single cells in order to determine the mode of action of the agent.

The four peripheral barrels of the five-barrel electrodes were filled with aqueous solutions of the compounds being tested. Since these barrels were first filled with distilled water which was then replaced with drug solution from the top, the comparative instability of many solutions made necessary the storage of the electrodes in an atmosphere of oxygen-free nitrogen and in the absence of light. The storage time was limited to 48 to 60 hr. In some cases dry electrodes were filled from the top by means of pressure or centrifugation. In this way very unstable compounds were tested within 30 min of preparing a solution. The nature of the substances studied did not necessitate the use of solutions of very low *p*H (see Curtis & Watkins, 1960). For most substances saturated aqueous solutions of suitable salts were made and the active material passed electrophoretically as a cation. In some cases, however, particularly with lysergic acid diethylamide and 2-bromo-(+)-lysergic acid diethylamide, solutions were acidified to *p*H 3 to 3.5 in order to obtain maximal ionization within the electrode. L-Glutamic acid, certain acidic indole derivatives and psilocybin were applied as anions from solutions made to *p*H 8 with sodium hydroxide. Both 5- and 6-hydroxytryptamine were available only as complexes with creatinine sulphate. The assumption that a cationic electrophoretic current would pass equal quantities of the tryptamine derivative and creatinine was tested by diluting 4-hydroxytryptamine with equimolecular amounts of creatinine. Creatinine itself was inert, and the apparent potency of the mixture, as a depressant of geniculate neurones, was half that of 4-hydroxytryptamine. Consequently a factor of two was allowed for when rating the potencies of 5- and 6-hydroxytryptamine. All currents are expressed as nA (10^{-9} A), cationic and anionic currents applying cations and anions respectively from the electrode tip.

The compounds used in this investigation were either commercially available or obtained from other investigators.

RESULTS

5-Hydroxytryptamine

5-Hydroxytryptamine was readily shown by the electrophoretic technique to be a depressant of the synaptic firing of lateral geniculate neurones. Although not the most active depressant, it was used as a basis for the comparison of the potencies of structurally related compounds, since it was readily available and the experimental evidence indicated that the other compounds had a similar mode of action. Accordingly, the results of using this indole derivative will be discussed in detail before presenting an analysis of structure-activity relationships. Throughout the experiments the 5-hydroxytryptamine creatinine sulphate complex was used. Control experiments were performed in several animals using creatinine alone. When applied as a cation from a solution of *p*H 3 using comparatively large electrophoretic currents (100 to 200 nA), this compound had no action upon geniculate neurones (see Table 4, compound 21). Thus it can be assumed that the observed effect of

passing cations from the solution of the complex (*p*H 3.3) was due to 5-hydroxytryptamine. In practically every instance when it was applied, the depressant action of 5-hydroxytryptamine was obvious when currents of the order of 10 to 40 nA were used.

The focal potentials generated synaptically in the lateral geniculate nucleus are complex waveforms, the complexity in part depending upon the site of recording (see Bishop & Davis, 1960). The two postsynaptic potentials (r_1 and r_2) correspond in latency and threshold to the two presynaptic potentials (t_1 and t_2), which in turn are associated respectively with low- and high-threshold afferent fibres of the optic nerve. 5-Hydroxytryptamine depressed both types of orthodromically evoked postsynaptic focal potentials, but was without action upon focal potentials generated antidromically. This is illustrated in Fig. 1, the potentials A to E being evoked by

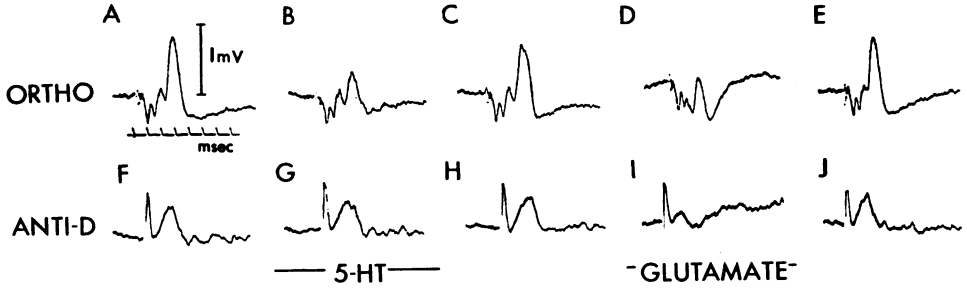


Fig. 1. Focal potentials recorded from the lateral geniculate nucleus by means of the centre barrel of a five-barrel electrode: ORTHO: A to E, evoked by maximal stimulation of the contralateral optic nerve: ANTI-D, F to J, evoked by stimulation of the ipsilateral optic radiation. A and F are control responses, negativity recorded as an upward deflection, as in all figures. B, G—recorded 32 and 29 sec respectively after starting an electrophoretic current of 20 nA which passed 5-hydroxytryptamine (5-HT) from one barrel of the electrode for 40 sec. C, H—48 and 55 sec after the cessation of this current. D, I—recorded 21 and 24 sec after an electrophoretic current of 150 nA began to pass L-glutamate ion from another barrel of the electrode. E, J—52 and 60 sec after the termination of the L-glutamate application. Time marker—msec; voltage calibration—1 mV.

maximal stimulation of the contralateral optic nerve. The antidromic potentials F to J were the result of stimulating optic radiation fibres. After the control responses A and F, a cationic current of 20 nA passed 5-hydroxytryptamine from one barrel of the electrode for 40 sec. The responses B and G were recorded 32 and 29 sec after the beginning of this current flow, and C and H 48 and 55 sec after its cessation. Only the orthodromic potential (B) was altered in magnitude; the antidromic potential (G) was not changed. Similar effects were observed when larger electrophoretic currents were used. In contrast to this action, the passage of glutamate ions, from another barrel of the electrode, depressed both forms of focal potential. Thus, shortly after the series A to C, F to H, a similar experiment was performed in which both the orthodromic (D) and antidromic (I) potentials were almost abolished within 20 sec of applying glutamate ion. Recovery occurred (E, J) within 50 sec of the termination of the application. The depression of both orthodromic and antidromic potentials by glutamic acid is in accordance with the effects observed

when this amino-acid is applied electrophoretically near neurones in the spinal cord (Curtis, Phillis & Watkins, 1960). An explanation has been given which depends upon the production of a conductance change in postsynaptic neuronal membrane by this substance. The failure of 5-hydroxytryptamine to affect antidromic potentials therefore suggests that this compound does not alter the membrane conductance of geniculate cells. Supporting evidence for this conclusion was obtained when single neurones were studied (see below).

When the exploring microelectrode passes through the geniculate nucleus, the recording of a large t_1 response in association with an r_1 focal potential, in either the A or B layer, has been taken to indicate that the electrode is recording near the terminals of the low-threshold optic tract fibres. Such a potential is illustrated in Fig. 2 A, where the t_1 and r_1 components are labelled. Application of 5-hydroxytryptamine using a current of 35 nA for 30 sec reduced the size of the r_1 postsynaptic potential to approximately 50% (B) and recovery (C) occurred within 45 sec. The t_1 spike was unaltered. A similar finding has been observed when lysergic acid diethylamide was administered by intracarotid injection (Bishop, Burke & Hayhow,

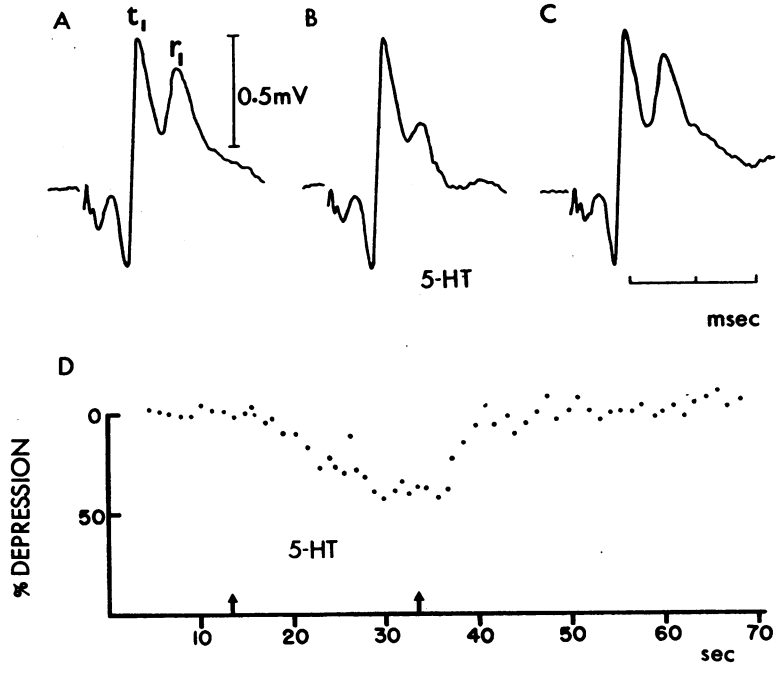


Fig. 2. A to C, focal potentials recorded from the lateral geniculate nucleus by means of the central barrel of a five-barrel electrode and evoked by stimulation of low-threshold optic nerve fibres. The t_1 and r_1 responses are marked. A—control. B—25 sec after a current of 35 nA began to pass 5-hydroxytryptamine (5-HT) from one barrel of the electrode. C—45 sec after this current was terminated. Time marker—msec: voltage calibration—1 mV. D, Ordinates—percentage depression of an r_2 postsynaptic focal potential recorded from the lateral geniculate nucleus and evoked by stimulation of the optic nerve approximately once per sec. 5-Hydroxytryptamine (5-HT) was applied electrophoretically using a current of 20 nA which was turned "on" at the first arrow and "off" at the second. Abscissa—time in sec.

1959), and the results were interpreted as indicating the lack of action of the indole on transmission in the presynaptic nerve fibres. This is possibly a correct interpretation of the results, but in the present experiments the t_1 response might be associated more with axons passing through the localized area of recording and of drug application than with those terminating in it. A further possibility is that the applied substance reduces the release of transmitter from the presynaptic terminals, an effect which may occur even when there is no appreciable decrease in the presynaptic spike potential.

When applied to negative field potentials generated by optic nerve impulses, a characteristic of the action of 5-hydroxytryptamine was the rapidity with which field potentials recovered after the application. The results from one experiment are plotted in Fig. 2 D. The field potential was evoked by the high-threshold optic nerve fibres and the control response was reduced by approximately 40% within 16 sec of applying 5-hydroxytryptamine with an electrophoretic current of 20 nA. This current flowed for 20 sec and the field potential recovered within 16 sec. The rate of recovery was prolonged when larger amounts of the indole were applied, but usually, following a depression of 40 to 60%, was of the order of 15 to 60 sec. Since 5-hydroxytryptamine was used as a standard when comparing the potency of related compounds (see below), it was necessary to establish how frequently the drug could be applied in order to obtain relatively constant depressions and rates of recovery. The usual time of application was 20 sec, the field potentials being evoked once per sec. Provided a period of at least 3 min elapsed between successive applications of 5-hydroxytryptamine, the depressions obtained, and the rates of recovery, were reasonably constant. With more frequent applications both the magnitude of depression and the time of recovery progressively increased.

When the effect of 5-hydroxytryptamine upon single neurones was studied the observations made upon field potentials were confirmed. It was not possible to excite all cells antidromically, since the stimulating electrodes were placed in a circumscribed portion of the optic radiation. However, since the main purpose of antidromic excitation was to test the excitability of the neurones a more convenient test was to apply L-glutamate ion. Without exception glutamate ion excited every neurone upon which it was applied, thus extending the observations that have been made upon spinal interneurones (Curtis, Phillis & Watkins, 1960) and neurones within the brain stem (Curtis & Koizumi, 1961). It was assumed that if a chemical compound, applied from one barrel of the electrode, diminished the frequency of cell discharge which was evoked by the application of glutamate ion from another barrel, then the depressant agent was probably altering the excitability of the neuronal membrane. Such an effect was observed when depressant and excitant amino-acids were applied simultaneously to spinal neurones (cf., Curtis & Watkins, 1960), and in this instance several factors probably contributed to the antagonism. In the case of indole derivatives it is unlikely that these could occupy the receptors with which glutamic acid interacts (see Curtis & Watkins, 1960); and consequently if indoles did depress the neuronal response to glutamate this would presumably be the result of an interaction between the indole and some other component of the postsynaptic membrane. In several instances spike potentials

of single neurones were produced by three methods, orthodromically by impulses in optic nerve fibres, antidromically by impulses in optic radiation fibres and chemically by application of L-glutamate ion. Such conditions offered an excellent opportunity for determining the mode of action of 5-hydroxytryptamine, and these observations were confirmed upon a further 20 neurones which were excited only synaptically and chemically.

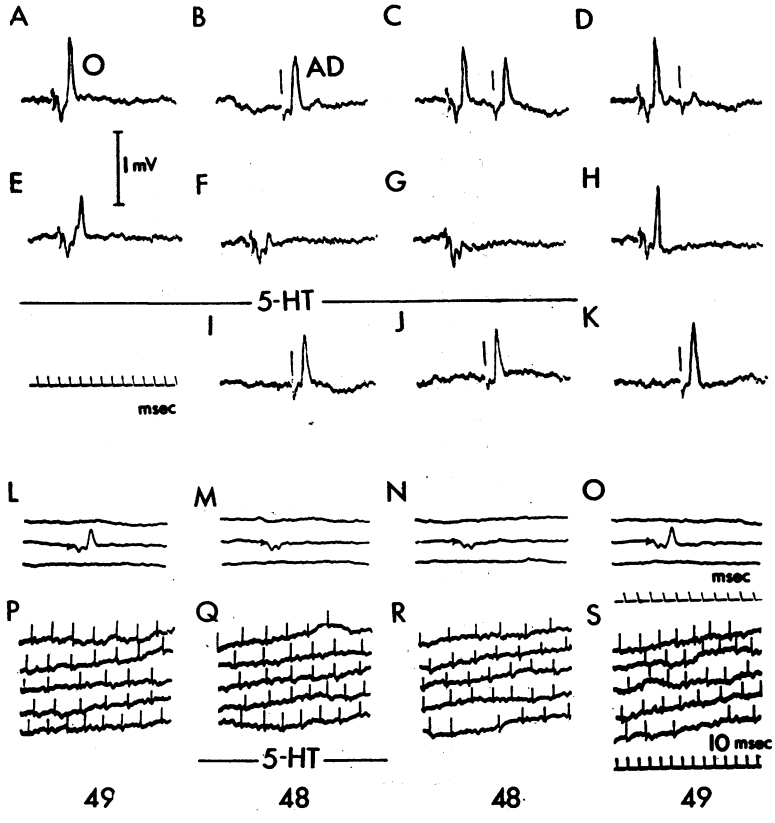


Fig. 3. Postsynaptic spike responses recorded from a single lateral geniculate neurone which was fired orthodromically by a volley in the contralateral optic nerve (A, E to H, L to O); antidromically by stimulation of the ipsilateral optic radiation (B, I to K); and chemically by application of L-glutamic acid electrophoretically (P to S). A, B—control responses. C, D—the orthodromic spike preceded the optic radiation stimulus by 2.9 and 2.4 msec respectively. E, F, I, G and J—recorded 6, 7, 11, 16 and 17 sec respectively, after a current of 20 nA began to pass 5-hydroxytryptamine (5-HT) into the extracellular environment of the neurone. H, K—9 and 14 sec after the termination of this current. L, P—control responses. M, Q—6 and 30 sec after the beginning of the application of 5-hydroxytryptamine (5-HT) by a current of 20 nA for 40 sec. N, R, O and S—7, 15, 110 and 120 sec after the cessation of this current. P, Q, R and S were recorded on film moving parallel to the Y axis of the oscilloscope (approximately 7 frames per sec), and are representative of the maximum frequencies evoked by the amino-acid, which was applied for periods of 10 sec using a current of 100 nA. The figures beneath the records give the number of spikes per sec in each run. Time markers—msec for A to K beneath E, msec for L to O beneath O, 10 msec for P to S beneath S. Voltage calibration—1 mV for A to K. The records L to S were recorded at approximately half this amplification.

The spike responses of Fig. 3 are from a neurone located towards the anterior end of the lateral geniculate nucleus. This cell responded monosynaptically to impulses in the low-threshold optic nerve fibres (A, E to H, L to O), antidromically to stimulation of the optic radiation (B, I to K) and was excited by glutamate ion applied electrophoretically (P to S). Due to slight over-correction for the capacitance of the recording system the spike shape is irregular in the series A to K. Slight variations of spike size were associated with small respiratory movements, but typically the orthodromic spike was slightly larger and of shorter duration than the antidromic (compare A and B). Both spikes were " all or none " in nature and were superimposed upon smaller negative waves which in the case of the antidromic potential was probably a field potential generated by other cells. The antidromic spike had a latency of 0.9 msec, measured from the stimulus. When this subcortical stimulus was preceded by an orthodromic spike at an interval of 2.4 msec (D) antidromic invasion was absent, whilst at an interval of 2.9 msec (C) a spike was produced. These observations establish that the spike evoked by subcortical stimulation was indeed antidromic and was not produced synaptically by impulses in nerve fibres in the optic radiation (Bishop, Burke & Davis, 1962). The responses E, F, G, I and J were recorded 6, 7, 16, 11 and 17 sec respectively after the beginning of a current of 20 nA which applied 5-hydroxytryptamine for 23 sec. H and K were recorded 9 and 14 sec respectively after this current was terminated. The antidromic spike potential was unaffected by the indole (I, J and K) whilst the orthodromic spike was blocked. Initially (E) there was an increase in spike latency revealing a small negative potential which possibly consists mainly of the excitatory postsynaptic potential of the single neurone (F). Eventually this also was depressed (G) by 5-hydroxytryptamine. Recovery was rapid, the orthodromic spike potential being present 9 sec after the application ceased (H). Several min after this series was recorded the effect of 5-hydroxytryptamine upon the excitation of this cell by L-glutamate ion was determined (L to S). Orthodromic responses were elicited every sec (L to O) and L-glutamate ion was applied for periods of 10 sec (100 nA) every 20 to 30 sec. The maximum frequency of firing for each test was measured and specimens of the records are illustrated (P to S) together with the number of spikes per sec. After the controls L and P, 5-hydroxytryptamine was applied for 40 sec using an electrophoretic current of 20 nA. The orthodromic spike failed within 6 sec (M), but 30 sec after the 5-hydroxytryptamine application started there was no significant alteration in the excitation of the neurone by glutamate ion. During the recovery phase the frequency of firing by the amino-acid was unaltered 15 sec (R) and 120 sec (S) after the 5-hydroxytryptamine current ceased, whilst at 7 sec (N) the orthodromic responses were still blocked. These responses remained blocked for approximately 70 sec, the recovered spike at 110 sec being shown in O.

The failure of 5-hydroxytryptamine to affect the firing of geniculate neurones by L-glutamic acid is illustrated also in Fig. 4. This neurone could not be fired antidromically, but was fired synaptically by impulses in low-threshold optic nerve fibres, the spike being superimposed upon a negative field potential (A). Glutamate ion was applied for test periods of 10 sec using an anionic current of 110 nA, and specimen records, together with the maximum frequency of firing evoked towards the end of the applications, are illustrated (E, F and G). Again, as in Fig. 3,

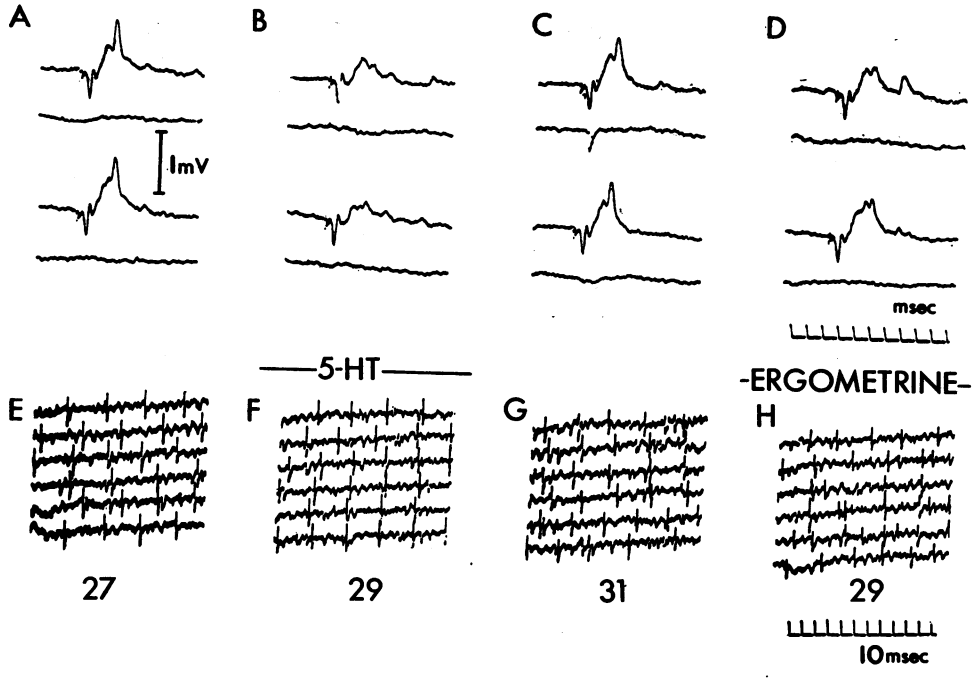


Fig. 4. Responses recorded near a single neurone in the lateral geniculate nucleus and evoked orthodromically (A to D) and chemically by the electrophoretic application of L-glutamic acid (E to H) using an anionic current of 110 nA. As in Fig. 3, records E to H are representative of the maximum firing rate evoked by the amino-acid and the actual maximum frequencies (spikes per sec) evoked during the 10-sec application are given below the records. The orthodromic spikes are superimposed upon a slower negative potential. A, E—control responses. B, F—recorded 34 and 28 sec after a current of 20 nA commenced to pass 5-hydroxytryptamine (5-HT) near the neurone. C, G—34 and 48 sec after the cessation of the application of 5-hydroxytryptamine. D, H—38 and 43 sec after a current of 10 nA commenced to apply ergometrine from another barrel of the five-barrel electrode. The records E to H have been retouched in order to show more clearly the spikes of the cell under investigation. Timer—msec for A to D; 10 msec for E to H. Voltage calibration—1 mV.

5-hydroxytryptamine blocked orthodromic excitation of the neurone but failed to influence the firing produced by glutamate ion.

In many experiments records were obtained from single neurones in the A_1 layer of the lateral geniculate nucleus that were activated by stimulation of the ipsilateral eye with light. The response of such a cell is shown in Fig. 5, the marker at the left of the records indicating the duration of the light flash. The application of 5-hydroxytryptamine also depressed the orthodromic activation of this cell (B), recovery occurring after cessation of the application (C). 5-Hydroxytryptamine failed to reduce the frequency of firing evoked by L-glutamic acid when the excitant amino-acid was applied to several of these cells.

Other compounds used in the investigation

Since it can be concluded from the previous section that the excitatory transmitter released at the synapses between optic nerve fibres and lateral geniculate neurones

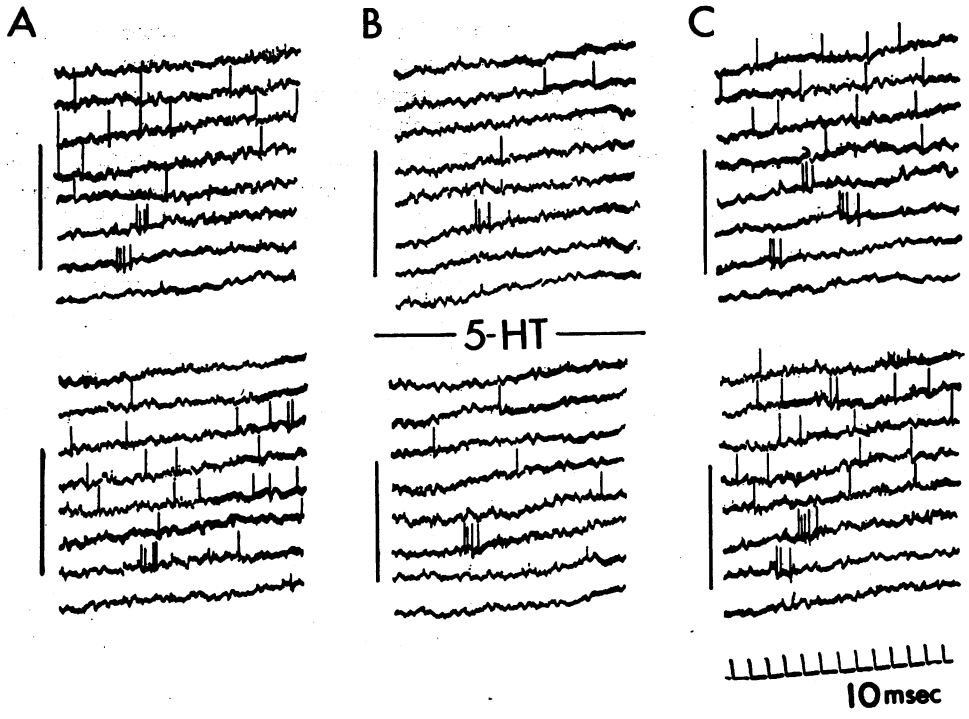


Fig. 5. Spike responses of a single lateral geniculate neurone recorded by means of the central barrel of a five-barrel electrode and evoked by a light flash applied to the ipsilateral eye. The duration of the flash (approximately 550 msec) is shown by the vertical bar at the left of the records. The responses were recorded on film moving parallel to the Y axis of the oscilloscope, the sweeps being 140 msec in duration and the delay between sweeps 1 msec. In each block of records the responses on the lower traces precede those above, so that the blocks should be read from below upwards. A—control. B—recorded 16 (upper) and 18 sec (lower) after a current of 20 nA commenced to pass 5-hydroxytryptamine (5-HT) from one barrel of the electrode. C—32 (upper) and 36 sec (lower) after this current was terminated. Time marker—10 msec.

may be related to 5-hydroxytryptamine (see Discussion), the investigation was extended to include as many structurally similar compounds as possible. Many of these were chosen for their ability to prevent the action of 5-hydroxytryptamine upon smooth muscle, some for their psychotomimetic properties and others for steric reasons. A series of sympathomimetic amines was tested since it is possible that these may interact with receptors for tryptamine derivatives (Blaschko, 1957; Vane, 1960; Woolley, 1960). Furthermore, the observations of Marrazzi (1957) suggest that indoles as well as catechol amines may depress the responses of cortical neurones.

Tables 1 to 4 list the compounds tested. Table 1 includes simple indole derivatives related to tryptamine; Table 2, lysergic acid derivatives; Table 3, compounds related to phenethylamine; and Table 4, miscellaneous compounds selected for various reasons. When comparing the potency of a particular substance with 5-hydroxytryptamine, electrophoretic currents were used that produced identical depressions of a postsynaptic focal potential. Usually 5-hydroxytryptamine was

applied using a current of 10 to 20 nA for 20 to 40 sec, the depression produced being of the order of 30 to 60%. After a period of 3 to 4 min the other substance was then passed for the same time interval using a current to produce a similar depression. The current which was used was chosen arbitrarily at first, until some idea was gained of that necessary to produce a depression identical with that of 5-hydroxytryptamine. It was then assumed that potencies were inversely proportional to the electrophoretic currents, and a value of 12 was chosen for 5-hydroxytryptamine, allowance being made for the fact that only a half of a particular electrophoretic current would carry 5-hydroxytryptamine from a solution of the creatinine sulphate complex. This method of determining potency assumes that the rates of ejection of, and therefore the local concentrations attained by, different substances are proportional to current flow (see Curtis, Perrin & Watkins, 1960). Within the electrodes most compounds were almost totally ionized, but on reaching the extracellular fluid the degree of ionization would depend upon the dissociation constant of the agent and the prevailing *p*H. For most of the compounds of the tables a large proportion of the applied substance would remain fully ionized in the extracellular medium, but for some, particularly 2-bromo-(+)-lysergic acid diethylamide and lysergic acid diethylamide, the proportion ionized would be low.

All compounds were tested using r_1 and r_2 postsynaptic responses in at least three different sites in the nucleus and in at least two different preparations. Since the recovery of the potential after the application was often very slow (see below) the assessment of potencies by matching a depression produced by 5-hydroxytryptamine was very time-consuming. As this was not the prime consideration of the investigation, in many instances the depression produced by 5-hydroxytryptamine was not exactly matched by other compounds. In these cases it was assumed that if the depressions were within $\pm 10\%$ of the standard depression produced by 5-hydroxytryptamine, the current which would produce an equal depression could be calculated from an assumed linear relationship between current and depression. For this reason, as well as for that discussed above concerning the relationship between current flow and the rate of ejection of different compounds, the potencies given in the tables are only approximate.

Many compounds, and particularly those found to be powerful depressants, were applied to single neurones. This was done because, as has been pointed out in Methods, both depressants and excitants of single cells may depress the potentials generated by groups of cells, by reducing the number of neurones contributing spike and excitatory postsynaptic potentials (see Curtis, Phillis & Watkins, 1960). Care was taken to observe any excitation of individual cells, and in addition, with many substances, the effect upon the spikes evoked by L-glutamate ion was determined. No excitant of lateral geniculate neurones was observed amongst the compounds listed, and, as no alteration in the excitation of the cells by the amino-acid was observed with all of the substances with which this particular test was performed, it is assumed that the structurally similar depressants had a common mode of action.

The use of " backing " currents to prevent the diffusion of compounds from the tips of electrodes (Curtis & Eccles, 1958) precludes a close analysis of the onset of drug effects when the substances are applied electrophoretically (Castillo & Katz,

1957). Most of the substances which were tested produced maximum depression of negative field potentials within the first 10 to 20 sec of application. With some, however, particularly tryptamine, psilocin, bufotenine, 4-methoxytryptamine, 5-methoxytryptamine, lysergic acid diethylamide, ergometrine and methylergometrine, the maximum effect was delayed for 20 to 40 sec. In these cases the delay presumably arises because of a low rate constant for the production of the drug-receptor complex.

Fig. 4 illustrates the effect of ergometrine (Table 2—2) upon a single geniculate neurone which was fired synaptically by impulses in the low-threshold optic nerve fibres (C) and chemically by L-glutamate ion (G). 38 sec after a current of 10 nA began to pass ergometrine from the electrode the synaptic firing was suppressed (D), and this was similar to the effect of 5-hydroxytryptamine upon this cell when it was applied previously (B). Testing with L-glutamic acid shortly after this showed no significant alteration in the firing rate (H). The responses of another cell are illustrated in Fig. 6 Again this was fired synaptically (A) and chemically (C). The

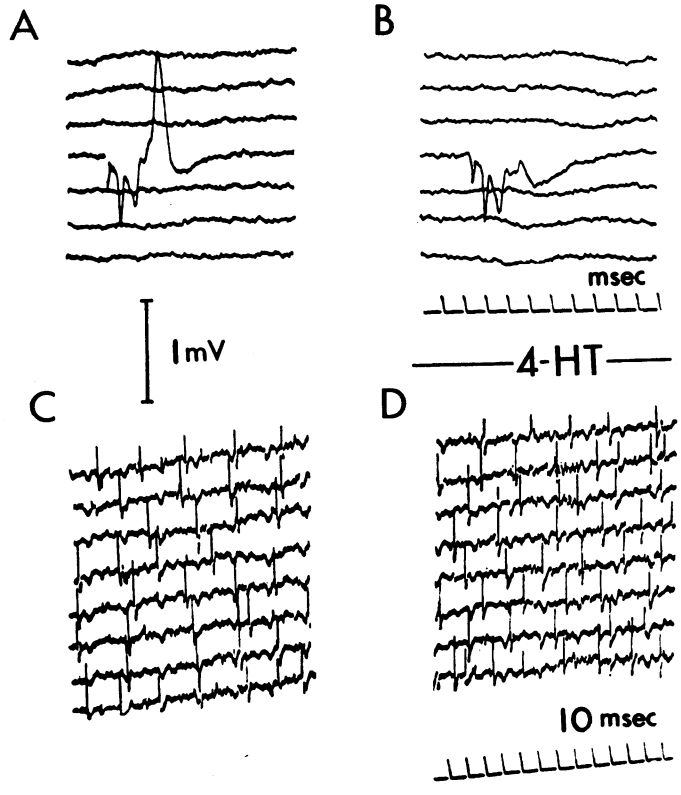
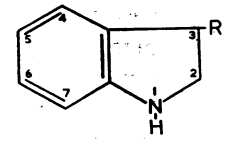


Fig. 6. Responses recorded in the lateral geniculate nucleus near a single neurone and evoked by optic nerve stimulation (A, B) and by application of L-glutamic acid as an anion from one barrel of the five-barrel electrode using a current of 100 nA. (C, D). These spikes have been retouched. A, C—control; B, D—34 and 44 sec after the beginning of a current of 5 nA which was used to apply 4-hydroxytryptamine (4-HT) from another barrel of the electrode. Time marker—msec for A, B; 10 msec for C, D. Voltage calibration—1 mV.

TABLE 1



Structural feature of interest	Name of compound	Common name	No.
(*a*) Variation of 3 position side-chain	Indol-3-ylacetic acid		1
	γ-Indol-3-ylbutyric acid		2
	Tryptamine		3
	Indol-3-ylacetamidine		4
(*b*) Position of phenolic hydroxyl group	4-Hydroxytryptamine	4-HT	5
	5-Hydroxytryptamine	5-HT, serotonin	6
	6-Hydroxytryptamine	6-HT	7
	7-Hydroxytryptamine	7-HT	8
(*c*) Methylation of phenolic hydroxyl group	4-Methoxytryptamine		9
	5-Methoxytryptamine		10
	N-Acetyl-5-methoxytryptamine	Melatonin	11
(*d*) ω-*N*-Alkylation	*N'N'*-Dimethyltryptamine		12
	N'N'-Diethyltryptamine		13
	4-Hydroxy-*N'N'*-dimethyltryptamine	Psilocin	14
	5-Hydroxy-*N'N'*-dimethyltryptamine	Bufotenine	15
	6-Hydroxy-*N'N'*-dimethyltryptamine		16
	7-Hydroxy-*N'N'*-dimethyltryptamine		17
(*e*) Side-chain methylation	α-Methyltryptamine		18
	4-Hydroxy-α-methyltryptamine		19
	4,α-Dimethyltryptamine		20
(*f*) Variations of 5-HT side-chain	5-Hydroxyindol-3-ylacetic acid	5HIAA	21
	5-Hydroxytryptophan	5HTP	22
	5-Hydroxyindol-3-ylacetamidine		23
(*g*) Miscellaneous indole derivatives	5-Amino-3-ethyl-2-methylindole	5-HT-amino analogue	24
	5-Dimethylamino-3-ethyl-2-methylindole	Medmain	25
	5-Dimethylamino-3-ethyl-1,2-dimethylindole	Methyl medmain	26
	3-(2-Aminoethyl)-1-benzyl-5-methoxy-2-methylindole	BAS	27
	3-(2-Dimethylaminoethyl)-1-benzyl-5-methoxy-2-methylindole		28
	N'N-'-Dimethyl-4-phosphoryloxytryptamine	Psilocybin	29

INDOLE DERIVATIVES

1. The *p*H of the aqueous solution after adjustment with the acid, alkali or salt shown in parenthesis.
2. Potency indicates ratios of activity as depressants of the orthodromic excitation of lateral geniculate neurones relative to 5-hydroxytryptamine = 12.
3. Duration of depressant activity relative to that of 5-hydroxytryptamine = 1.
4. Actual values not measured but were greater than 4.

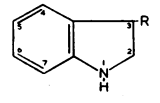
Ring substituent	Side-chain, R	*p*H[1]	Predominant ion species	Potency[2]	Duration[3]
	$-CH_2.CO_2H$	8 (NaOH)	Anion	0	
	$-CH_2.CH_2.CH_2.CO_2H$	8 (NaOH)	Anion	0	
	$-CH_2.CH_2.NH_2$	4 (HCl)	Cation	2–3	4–10
	$-CH_2.C(NH).NH_2$	5 (*p*-Toluene sulphonic acid)	Cation	1	1–3
4-OH	$-CH_2.CH_2.NH_2$	4 (Oxalic acid)	Cation	18–24	24–36
5-OH	$-CH_2.CH_2.NH_2$	3·3 (Creatinine sulphate)	Cation	12	1
6-OH	$-CH_2.CH_2.NH_2$	3·5 (Creatinine sulphate)	Cation	4–5	4
7-OH	$-CH_2.CH_2.NH_2$	5 (Oxalic acid)	Cation	12–18	6–10
$4-OCH_3$	$-CH_2.CH_2.NH_2$	3 (HCl)	Cation	6	5–10
$5-OCH_3$	$-CH_2.CH_2.NH_2$	3 (HCl)	Cation	6	5–10
$5-OCH_3$	$-CH_2.CH_2.NH(CO.CH_3)$	3 (HCl)	Cation	<1	Prolonged[4]
	$-CH_2.CH_2.N(CH_3)_2$	3 (Oxalic acid)	Cation	1–2	8–12
	$-CH_2.CH_2.N(C_2H_5)_2$	5 (HCl)	Cation	1	10–15
4-OH	$-CH_2.CH_2.N(CH_3)_2$	4 (HCl)	Cation	2–3	Prolonged[4]
5-OH	$-CH_2.CH_2.N(CH_3)_2$	3 (HCl)	Cation	6	20–40
6-OH	$-CH_2.CH_2.N(CH_3)_2$	6 (HCl)	Cation	1	4–8
7-OH	$-CH_2.CH_2.N(CH_3)_2$	6 (HCl)	Cation	2	3–6
	$-CH_2.CH(CH_3).NH_2$	6 (Methane sulphonic acid)	Cation	1	20
4-OH	$-CH_2.CH(CH_3).NH_2$	4 (Maleic acid)	Cation	1–2	10–15
$4-CH_3$	$-CH_2.CH(CH_3).NH_2$	4 (Maleic acid)	Cation	<1	1
5-OH	$-CH_2.CO_2H$	7 (Cyclohexyl ammonium salt)	Anion	0	
5-OH	$-CH_2.CH(NH)_2.CO_2H$	3·5 (HCl)	Cation	<1	8
5-OH	$-CH_2.C(NH).NH_2$	5·5 (*p*-Toluene-sulphonic acid)	Cation	2	1–2
$2-CH_3$; $5-NH_2$	$-CH_2.CH_3$	3·3 (HCl)	Cation	1	3
$2-CH_3$; $5-N(CH_3)_2$	$-CH_2.CH_3$	3 (HCl)	Cation	1–2	3–4
1, $2-CH_3$; $5-N(CH_3)_2$	$-CH_2.CH_3$	3·5 (HCl)	Cation	<1	3
1-Benzyl; $2-CH_3$; $5-OCH_3$	$-CH_2.CH_2.NH_2$	4 (HCl)	Cation	1–2	3–6
1 Benzyl; $2-CH_3$; $5-OCH_3$	$-C(CH_3)_2.CH_2.NH_2$	6 (HCl)	Cation	<1	2
$4-O.PO(OH)_2$	$-CH_2.CH_2.N(CH_3)_2$	8 (NaOH)	Anion	2–3	20–30

application of 4-hydroxytryptamine (Table 1—5) completely suppressed the orthodromic firing (B), but the rate of firing evoked by the amino-acid was virtually unaltered (D). In Figs. 4 and 6 the recovery after ergometrine and 4-hydroxytryptamine respectively are not illustrated, as both of these substances have a prolonged depressant effect.

The tables also show the recovery times of the depressant action of the various compounds, relative to that of 5-hydroxytryptamine. These figures are based on a comparison of recovery times which were observed after 5-hydroxytryptamine and the compound were applied to produce equal depressions. Again these figures are approximate, and as with the potencies several groups of substances become apparent. In general, compounds less potent than 5-hydroxytryptamine, which had the briefest action, were also longer-acting.

As far as structure-activity relationships are concerned not many closely related compounds were available. However, some features are noteworthy and allow of some speculation concerning the nature of the receptor site for the compounds. The relationships can be summarized in reference to sections of the individual tables.

Table 1



I

(*a*) Most indoles (I) that were depressants had a 2-aminoethyl side-chain in position 3. In some cases the amino-group was absent (Table 1—21, 24, 25 and 26), but these compounds were of low potency. Indoles having neither a side-chain amino-group nor a ring substituent were inactive (Table 1—1, 2). An acetamidine side-chain was less effective than a 2-aminoethyl side-chain (compare Table 1—3 and 4).

(*b*) The activity of tryptamine (Table 1—3) was increased by the presence of a phenolic hydroxyl group, particularly in position 4 but also in position 7 (Table 1—5 and 8 respectively). 5-Hydroxytryptamine had the briefest duration of action of all the compounds tested.

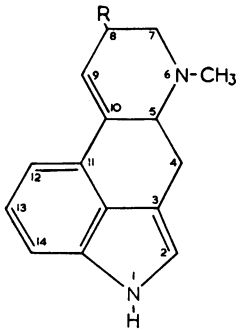
(*c*) Methylation of the 4- and 5-hydroxyl groups (Table 1—9 and 10 respectively) reduced the depressant activity of 4- and 5-hydroxytryptamine. These compounds were similar in potency and were less prolonged in action than was 4-hydroxytryptamine. *N*-Acetyl-5-methoxytryptamine (melatonin, Table 1—11) was of low potency, presumably because of the acylation of the amino-nitrogen atom.

(*d*) Alkylation of the amino-group resulted in a reduction of the activities of tryptamine and hydroxytryptamines (Table 1—12 to 17). In the case of tryptamine, diethyl was more effective than was dimethyl substitution in reducing activity. 4-Hydroxy-*N'N'*-dimethyltryptamine (psilocin—Table 1—14) was less potent than was 5 hydroxy-*N'N'*-dimethyltryptamine, which is in contrast to the potencies of the parent compounds, 4- and 5-hydroxytryptamine (Table 1—5 and 6 respectively).

(*e*) The potencies of tryptamine and of 4-hydroxytryptamine were reduced by methylation of the α-carbon atom in the side-chain (Table 1—18 and 19). The importance of hydroxyl substituents in the ring is also suggested by the difference in

TABLE 2

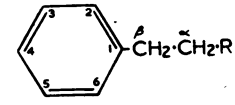
LYSERGIC ACID DERIVATIVES



1. The *p*H of the aqueous solution after adjustment with the acid, alkali or salt shown in parenthesis.
2. Potency indicates ratios of activity as depressants of the orthodromic excitation of lateral geniculate neurones, relative to 5-hydroxytryptamine=12.
3. Duration of depressant activity relative to that of 5-hydroxytryptamine=1.
4. Actual values not measured but were greater than 4.

Structural feature of interest	Name of compound	Common name	No.	Ring substituent	Side-chain R	*p*H[1]	Predominant ion species	Potency[2]	Duration[3]
(*a*) Various amido-groups	(+)-Lysergic acid diethylamide	LSD-25	1		.CO.N(C_2H_5)$_2$	3 (HCl)	Cation	2–3	20+
	(+)-Lysergic acid 2-hydroxy-1-methylethylamide	Ergometrine	2		.CO.NH.CH(CH_3).CH_2OH	4·5 (Maleic acid)	Cation	9–12	20–30
	(+)-Lysergic acid 1-hydroxymethylpropylamide	Methylergometrine	3		.CO.NH.CH(C_2H_5).CH_2OH	5·5 (HCl)	Cation	6–9	Prolonged[4]
	—	Ergotamine	4		Peptide	4 (Tartaric acid)	Cation	0	
(*b*) Ring substituents	1-Methyl-(+)-lysergic acid 1-hydroxymethylpropylamide	Methysergide (UML-491)	5	1-CH_3	.CO.NH.CH(C_2H_5).CH_2OH	6 (Maleic acid)	Cation	1–2	Prolonged[4]
	2-Bromo-(+)-lysergic acid diethylamide	BOL-148	6	2-Br	.CO.N(C_2H_5)$_2$	3 (Tartaric acid)	Cation	1	Prolonged[4]
	12-Hydroxy-(+)-lysergic acid 2-hydroxy-1-methylethylamide	12-Hydroxy-ergometrine	7	12-OH	.CO.NH.CH(CH_3).CH_2OH	6 (Maleic acid)	Cation	6	Prolonged[4]

TABLE 3



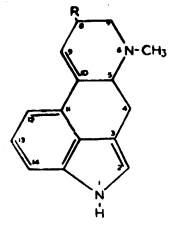
Structural feature of interest	Name of compound	Common name	No.
(*a*) Variation in amino-alkyl side-chain	Phenethylamine		1
	(+)-α-Methylphenethylamine	Dexamphetamine	2
(*b*) Ring substitution	4-Hydroxyphenethylamine	Tyramine	3
	3,4-Dihydroxyphenethylamine	Dopamine	4
	3,4,5-Trimethoxyphenethylamine	Mescaline	5
(*c*) Side-chain and ring substituted compounds with hydroxyl group in position β	(−)-2-Methylamino-1-phenylpropan-1-ol	Ephedrine	6
	(−)-1-(*m*-Hydroxyphenyl)-2-methylaminoethanol	Phenylephrine	7
	(−)-2-Amino-1-(3,4-dihydroxyphenyl)ethanol	Noradrenaline	8
	(−)-1-(3,4-Dihydroxyphenyl)-2-methylaminoethanol	Adrenaline	9
	1-(3,4-Dihydroxyphenyl)-2-isopropylaminoethanol	Isoprenaline	10

depressant potencies between 4-hydroxy-α-methyltryptamine and 4,α-dimethyltryptamine (Table 1—19 and 20 respectively).

(*f*) Alterations to the side-chain of 5-hydroxytryptamine reduce potency. The presence of a basic group was necessary for activity, 5-hydroxyindol-3-ylacetic acid being inert (Table 1—21). As with tryptamine, a phenolic hydroxyl group in position 5 increased the activity of indol-3-ylacetamidine (compare Table 1—4 and 23).

(*g*) Most of the miscellaneous indoles are of interest because they block the action of 5-hydroxytryptamine upon smooth muscle of various types (Woolley, 1958 ; Gaddum, 1958 ; Cerletti, 1959). *N'N'*-Dimethyl-4-phosphoryloxytryptamine (Table 1—29) was an active depressant, of similar potency to 4-hydroxy-*N'N'*-dimethyltryptamine (Table 1—14). The other compounds (24 to 28) were relatively inactive.

Table 2



II

Lysergic acid derivatives were of interest because they can be considered as indoles substituted at positions 3 and 4, these corresponding to positions 3 and 11 respectively of the lysergic acid nucleus (II).

PHENYLETHYLAMINE DERIVATIVES

1. The *p*H of the aqueous solution after adjustment with the acid, alkali or salt shown in parenthesis.
2. Potency indicates ratios of activity as depressants of the orthodromic excitation of lateral geniculate neurones, relative to 5-hydoxytryptamine =12.
3. Duration of depressant activity relative to that of 5-hydroxytryptamine=1.
4. Actual values not measured but were greater than 4.

Ring substituents	Side-chain substituents	R	*p*H[1]	Predominant ion species	Potency[2]	Duration[3]
		NH_2	5 (HCl)	Cation	<1	2–4
	α-CH_3	NH_2	6 (H_2SO_4)	Cation	<1	2–4
4-OH		NH_2	3 (HCl)	Cation	1	1–4
3-OH; 4-OH		NH_2	4 (HCl)	Cation	2	1–2
3-OCH_3; 4-OCH_3; 5-OCH_3		NH_2	6 (H_2SO_4)		1–2	1–4
	α-CH_3; β-OH	$NH.CH_3$	5 (HCl)	Cation	<1	1–4
3-OH	β-OH	$NH.CH_3$	6 (HCl)	Cation	<1	2
3-OH; 4-OH	β-OH	NH_2	3·3 (Tartaric acid)	Cation	1	1–2
3-OH; 4-OH	β-OH	$NH.CH_3$	4 (Tartaric acid)	Cation	<1	1–2
3-OH; 4-OH	β-OH	$NH.CH(CH_3)_2$	6 (H_2SO_4)	Cation	<1	1

(*a*) Lysergic acid diethylamide was less potent than 5-hydroxytryptamine and its duration of action was prolonged. Ergotamine was inactive. An unexpected finding was the high potencies of both ergometrine and methylergometrine (Table 2—2 and 3 respectively). In view of this, the effect of intravenously injected ergometrine was determined upon geniculate potentials in one animal. In this preparation, an intravenous dose of 300 μg/K of lysergic acid diethylamide reduced a r_1 postsynaptic focal potential by 70% so that only a synaptic potential was recorded (Bishop, Field, Hennessy & Smith, 1958 ; Bishop & Davis, 1960). Three hours later, when almost complete recovery had occurred, administration of 440 μg/K of ergometrine maleate (which would produce an overall body concentration of approximately 10^{-6} M, equivalent to that produced by lysergic acid diethylamide) reduced the r_1 potential by approximately 60%. There was thus little difference between these two lysergic acid derivatives when administered intravenously.

(*b*) The potency of (+)-lysergic acid derivatives was reduced by ring substituents. 2-Bromo-(+)-lysergic acid diethylamide was not very active (Table 2—6), and the introduction of a methyl group in position 1 reduced the activity of methylergometrine (compare Table 2—3 and 5). Further, a hydroxyl group in position 12 reduced the depressant action of ergometrine (compare Table 2—2 and 7).

Table 3

Phenethylamine derivatives were generally of low potency when compared with the depression produced by 5-hydroxytryptamine. Since potencies are very approximate, little is gained by discussing the differences between the compounds in detail.

Dopamine (3-hydroxytyramine) and mescaline (Table 3—4 and 5 respectively) were the most powerful depressants in this series, whilst adrenaline and noradrenaline (Table 3—9 and 8 respectively) were less effective. The most active compounds have a terminal primary amino-group and either hydroxyl groups (Table 3—4 and 8) or methoxyl groups (Table 3—5) in positions 3 and 4.

Table 4 includes miscellaneous compounds tested for various reasons. The inhibitors of mono-amine oxidase (section b) and the non-indolic blocking agents of 5-hydroxytryptamine and of adrenaline (section d) will be discussed below. The other compounds were selected for their indole or indole-like structure (Table 4—1, 2, 3, 4, 13, 14, 15) or merely because of interest in their possible central action. None were very potent depressants of the synaptic excitation of lateral geniculate neurones; those with very weak actions included harmaline, reserpine, strychnine, morphine, pethidine, methadone, benzimadazole, β-naphthylguanidine and chlorpromazine.

Enzyme inhibitors and drug-antagonism

The rapidity with which field potentials recovered after an application of 5-hydroxytryptamine, compared with the slower recovery observed after 4-hydroxytryptamine and lysergic acid diethylamide, suggested the possibility that 5-hydroxytryptamine might be moved, in part, from the site of application by enzymes. There is evidence that a mono-amine oxidase might be responsible for removing 5-hydroxytryptamine from mammalian tissues (Blaschko, 1958), and consequently a series of inhibitors of this enzyme were tested for their ability to delay the recovery of field potentials after depression by 5-hydroxytryptamine. Further, these enzyme inhibitors were applied to single neurones that were fired synaptically in order to determine their effect upon the number of spikes so elicited. In this way it was hoped to detect the enzymes associated with the removal of excitatory transmitter substances (cf., Curtis, Phillis & Watkins, 1961). The substances used in this section of the investigation (Table 4—section b) were isoniazid, iproniazid phosphate, sodium *p*-chloromercuribenzoate and ephedrine. Using these compounds it was hoped to cover a series of enzymes which might be involved not only in the removal of 5-hydroxytryptamine but also in the inactivation of catechol amines (see Curtis, Phillis & Watkins, 1960, 1961). None of these agents prolonged the synaptic firing of geniculate neurones and none had any effect upon the recovery of field potentials after an application of 5-hydroxytryptamine. As shown in Table 3, ephedrine was a weak depressant of the neurones and in some experiments iproniazid (Table 4—5) was also shown to have a very low depressant potency.

It was also of interest to determine whether substances known to antagonize the action of 5-hydroxytryptamine upon smooth-muscle preparations would prevent its depressant action upon geniculate neurones. Many of these compounds (cf., Gaddum, 1958; Woolley, 1958) were themselves depressants and consequently antagonism was difficult to detect. However, the prior electrophoretic administration of 2-bromo-(+)-lysergic acid diethylamide, methysergide (UML-491), harmaline, reserpine and chlorpromazine did not alter significantly the depression produced by subsequent doses of 5-hydroxytryptamine. In addition, dibenamine (Table 4—11), a powerful antagonist of 5-hydroxytryptamine upon uterine smooth

TABLE 4

MISCELLANEOUS COMPOUNDS

1. The *p*H of the aqueous solution after adjustment with the acid, alkali, or salt shown in parenthesis.
2. Potency indicates ratios of activity as depressants of the orthodromic excitation of lateral geniculate neurones, relative to 5-hydroxytryptamine=12.
3. Duration of depressant activity relative to that of 5-hydroxytryptamine=1.
4. Actual values not measured but were greater than 4.

Feature of interest	Name of compound	Common name	No.	*p*H[1]	Predominant ion species	Potency[2]	Duration[3]
(*a*) Indole nucleus	3,4-Dihydro-7-methoxy-1-methyl-9-pyrid (3,4-b) indole	Harmaline	1	4 (HCl)	Cation	1	9–15
	—	Yohimbine	2	5 (HCl)	Cation	0	—
	3 4,5-Trimethoxybenzoyl methyl reserpinate	Reserpine	3	3·5 (Acetic acid)	Cation	1–2	Prolonged
	—	Strychnine	4	7 (HCl)	Cation	1	1–3
(*b*) Inhibitors of mono-amine oxidase	1-Isonicotinoyl-2-isopropylhydrazine	Iproniazid	5	3 (Phosphoric acid)	Cation	<1	1
	Isonicotinic acid hydrazide	Isoniazid	6	H_2O	Cation	0	—
	p-Chloromercuribenzoate		7	9 (NaOH)	Anion	0	—
(*c*) Morphine and related compounds	—	Morphine	8	4·7 (H_2SO_4)	Cation	1	2–3
	Ethyl 1-methyl-4-phenyl-piperidine-4-carboxylate	Pethidine	9	6 (HCl)	Cation	1	6–7
	(±)-6-Dimethylamino-4,4-diphenyl-heptan-3-one	Methadone	10	6 (HCl)	Cation	1	Prolonged[4]
(*d*) Sympathomimetic amine antagonists	Dibenzyl(2-chloroethyl)amine	Dibenamine	11	(HCl)	Cation	0	—
	2-(*N*-3-Hydroxyphenyl-4-toluidinomethyl)-imidazoline	Phentolamine	12	(HCl)	Cation	0	—
(*e*) Miscellaneous	5-Azaindole		13	3 (HCl)	Cation	0	—
	Benzimidazole		14	4·6 (HCl)	Cation	<1	1–4
	1,3,7-Trimethylxanthine	Caffeine	15	3 (HCl)	Cation	0	—
	β-Naphthylguanidine		16	5 (HCl)	Cation	<1	4–6
	2-Chloro-10-(3-dimethylaminopropyl)-phenothiazine	Chlorpromazine	17	5 (HCl)	Cation	<1	Prolonged[4]
	10-(2-Dimethylaminopropyl)phenothiazine	Promethazine	18	5 (HCl)	Cation	0	—
	1-Methyl-2-(3-pyridyl)pyrrolidine	Nicotine	19	5·5 (HCl)	Cation	0	—
	4-(2-Aminoethyl)imidazole	Histamine	20	5 (HCl)	Cation	0	—
	1-Methylhydantoin-2-imide	Creatinine	21	3 (H_2SO_4)	Cation	0	—

muscle (Gaddum, Hameed, Hathway & Stephens, 1955), and phentolamine (Table 4—12), an adrenergic blocker (Trapold, Warren & Woodbury, 1950), failed to affect either synaptic firing of geniculate neurones or the depression by 5-hydroxytryptamine.

DISCUSSION

The local electrophoretic application of certain indoles and catechol amines to neurones of the lateral geniculate nucleus prevents these cells from responding to volleys in optic nerve fibres. Intracellular records were not obtained from the neurones and the direct excitability of the postsynaptic membrane could not be tested. However, since the compounds did not affect the excitability of the cells when this was tested either antidromically or by the application of L-glutamic acid, three possible modes of action can be excluded. In the first place, it is unlikely that the depression is due to an alteration of intracellular processes which follows the penetration of the neuronal membrane by the compounds (see Vane, 1959; Greenberg, 1960). Secondly, the failure of these substances to depress chemical and antidromic excitability suggests that they do not change the conductance of the postsynaptic membrane. Thus the action of these agents is not that expected of inhibitory transmitters and differs from that of the depressant amino-acids (cf., Curtis, Phillis & Watkins, 1959). Finally, the observations indicate that the electrically excitable component of the postsynaptic membrane is not stabilized by an interference with spike-generating mechanisms, such as occurs with procaine (Curtis & Phillis, 1960). Since impulse conduction in presynaptic fibres was probably unaffected, two remaining sites exist with which the chemical substances may interact.

An important possible site of action is the subsynaptic receptor which is specialized for combination with the excitatory transmitter released from optic nerve terminals. The drug-receptor complex could be compared with that produced when curare-like compounds prevent the access of acetylcholine to cholinoceptive receptors without changing the conductance of the postsynaptic membrane (Castillo & Katz, 1957). The other possibility which cannot at present be excluded is a presynaptic site of action of these compounds, and recently considerable importance has been attached to this type of pharmacological interaction (see Riker, 1960; Koelle, 1961). The rapidity with which 5-hydroxytryptamine affects transmission at lateral geniculate synapses suggests that the compound does not interfere with the synthesis and storage of the excitatory transmitter. However, 5-hydroxytryptamine could disturb membrane processes associated with transmitter release, possibly by interfering with presynaptic membrane sites through which transmitter is discharged. A diminution in transmitter release would also occur if the added compounds depolarized the presynaptic terminals (Hagiwara & Tasaki, 1958), but this would probably be reflected in an alteration in spike potentials recorded from the fibres.

The question of the site of action of 5-hydroxytryptamine and related compounds cannot be resolved finally until the transmitter substance itself can be applied to the synapses. A similar problem arises from the investigation of Hill & Usherwood (1961), who found that certain tryptamine analogues depress neuromuscular transmission in the jumping leg of the locust, and suggest an action of these compounds

similar to that proposed above. The previous criteria which were considered to establish a postsynaptic site of action of lysergic acid diethylamide at geniculate synapses (Bishop, Burke & Hayhow, 1959) are not satisfactory and could equally well be used to argue a presynaptic site. Thus the increase in the synaptic delay and the reduction in the block which is produced by repetitive stimulation of the optic nerve could also be accounted for by a depression in the amount of transmitter released per impulse. The depressant indoles and catechol amines may indeed interact with both pre- and post-synaptic sites, but the possibility exists, particularly if the action is postsynaptic, that the receptor with which these compounds combine is that specialized for the transmitter responsible for excitation of the neurones. If this is the case, the transmitter may bear some structural similarity to these active depressants. This possibility guided the selection of many of the substances tested, and, although an excitant was not discovered, the structure-activity relationships which were revealed merit some discussion. It is unlikely that the concentrations of substances such as 4-hydroxytryptamine and 5-hydroxytryptamine were beyond what might be considered a physiological range. Lysergic acid diethylamide was found to be a comparatively weak depressant, yet an intravenous dose of 300 to 400 μg/kg is sufficient to block transmission at lateral geniculate synapses (Bishop, Burke & Hayhow, 1959). Assuming a uniform distribution throughout the animal the local concentration would be of the order of 10^{-6} M. Since 4-hydroxytryptamine and 5-hydroxytryptamine were effective depressants when applied with lower electrophoretic currents than were necessary with lysergic acid diethylamide, it is improbable that local concentrations of these compounds, necessary to depress neuronal responses, exceeded this level.

The molecular similarity between the active depressant compounds and the transmitter may not necessarily be very close. It must be remembered that many extremely potent curare-like compounds with actions at cholinoceptive synapses bear little superficial resemblance to acetylcholine. Nevertheless the structural requirements for combination with receptors at lateral geniculate synapses are of importance, since, apart from cholinergic synapses, this is the only central synapse for which a specific blocking agent is known. 5-Hydroxytryptamine (Curtis, Phillis & Watkins, 1961), tryptamine, α-methyltryptamine and ergometrine (Curtis & Davis, unpublished observations) are inactive when applied to spinal neurones. Since no difference was observed in the effects of indoles when they were applied to neurones which were fired monosynaptically by impulses in low- and high-threshold optic nerve fibres, it can be assumed that both groups of fibres release the same transmitter.

As far as the relatively simple indoles are concerned (Table 1), maximum depressant activity was associated with an unsubstituted 2-aminoethyl side-chain and a phenolic hydroxyl group in position 4 (Table 1—5). Activity was reduced by substituents along the chain or on the terminal nitrogen atom. Removal of the phenolic hydroxyl group or its replacement by methoxyl or methyl groups also reduced activity. Consequently it is probable that the receptor with which these compounds interact has two active sites, one for the hydroxyl group, the other for the terminal amino-group. The diminished potency of 6-hydroxytryptamine, com-

pared with that of the 5- and 7- and particularly with the 4-hydroxy compounds, suggests that the active receptor sites are slightly closer together than the minimum distance which separates the ω-amino-group and the 6-hydroxyl group. If the receptor–hydroxyindolylalkylamine interaction involves two relatively fixed receptor sites, it is unlikely that the indole nitrogen atom participates directly in the complex formation, since the position of this atom, relative to receptor atoms, would vary in the complexes formed by the different hydroxytryptamines. The geniculate receptor differs in this respect from that postulated for the interaction of certain tryptamine derivatives with the heart of *Venus mercenaria* (Greenberg, 1960). The potencies and long durations of activity of 4- and 7-hydroxytryptamine are presumably associated with the ready access of these substances to the receptor and the formation of stable complexes. On the other hand, the combination of a relatively high depressant activity with a brief duration, which is shown by 5-hydroxytryptamine, suggests that either the complex formed in this case is relatively unstable or that 5-hydroxytryptamine is removed rapidly from its site of action. Other related compounds are less effective and generally prolonged in action, presumably because membrane structures interfere with their access to and removal from the receptors.

The compounds of Table 3 were relatively ineffective depressants, the most active having a terminal basic group together with hydroxyl or methoxyl groups attached to the ring. This may indicate that, although the indole nitrogen atom is not directly involved in the drug-receptor interaction, the larger resonating system of the indole nucleus, compared with the phenyl structure, may be important. One significant finding is that adrenaline, noradrenaline and dopamine are not transmitters acting upon lateral geniculate neurones.

The active depressants derived from lysergic acid (Table 2) are of importance since they penetrate the blood-brain barrier and are potent when administered systemically. When given by intravenous injection to cats the effects of large doses (1 to 2 mg/kg) of both ergometrine (Brown & Dale, 1935) and lysergic acid diethylamide (Bishop, Field, Hennessy & Smith, 1958) are similar, and in smaller doses both substances depress transmission through the lateral geniculate nucleus. The high potencies of ergometrine and methylergometrine, when applied electrophoretically (Table 2—2 and 3), suggest the possibility that these compounds interact with membrane receptors by means of the terminal hydroxyl group of the side-chain and the nitrogen atom at position 6. In certain conformations of the amido-group, the hydroxyl group and the nitrogen atom at position 6 may be separated by distances similar to those between the primary amino-group and the phenolic hydroxyl group of the active hydroxytryptamines. It is of interest that the phenolic hydroxyl group of 12-hydroxyergometrine (Table 3—7) does not have the same potency-increasing effect as does the 5-hydroxyl group in the simple indole series. This may be attributed to the fixed distance between the nitrogen atom at position 6 and the 12-hydroxyl group in the lysergic acid series being different from that required for phenolic-hydroxyl-group participation in the drug-receptor complex.

The lysergic acid derivatives were applied electrophoretically from solutions in which they would be predominantly in the ionic state. However, at the *p*H of the

extracellular fluid, the degree of ionization of substances such as lysergic acid diethylamide and 2-bromo-(+)-lysergic acid diethylamide (*p*Ka 5.9 and 5.4 respectively) would be less than that of ergometrine (*p*Ka approximately 6.7). Consequently, if the interaction of these compounds with membrane receptors was predominantly electrostatic in character, the low potency of lysergic acid diethylamide and 2-bromo-(+)-lysergic acid diethylamide may be due to the low extracellular concentrations of the charged active species. On the other hand, the low potencies of 2-bromo-(+)-lysergic acid diethylamide and methysergide (Table 2—6 and 5) may be associated with an interference by the groups attached in position 1 and 2 of the ring to the interaction of the molecules with the membrane receptor sites.

The determination of the nature of the excitatory transmitter at lateral geniculate synapses was not assisted by the application of enzyme inhibitors. The failure of iproniazid, isoniazid and *p*-chloromercuribenzoate to prolong or increase the effectiveness of both the transmitter and 5-hydroxytryptamine probably excludes enzymic inactivation by mono-amine oxidase as an important factor in the removal of either substance from the receptor sites. It is of interest that Erspamer, Glässer & Mantegazzini (1960) have compared the pharmacological effects of 4- and 5-hydroxytryptophan. The central effects of these compounds are similar, and are presumably the consequence of enzymic decarboxylation to the respective amines (Udenfriend, 1958 ; Erspamer, Glässer, Nobili & Pasini, 1960 ; Erspamer, Glässer, Pasini & Stoppani, 1961). However, the central effects of 4-hydroxytryptophan exceeded those of 5-hydroxytryptophan in duration, and it has been suggested that this may be associated with the less efficient enzymic oxidation of 4-hydroxytryptamine by amine oxidase, compared with that of 5-hydroxytryptamine (Erspamer, Ferrini & Glässer, 1960). However, in the present experiments, the very prolonged action of both 4-hydroxytryptamine and of tryptamine, which is metabolized rapidly in the presence of amine oxidase (Erspamer, Ferrini & Glässer, 1960), and the failure of amine oxidase inhibitors to affect the recovery rate of neurones after an application of 5-hydroxytryptamine, suggest that enzymic oxidation may be relatively unimportant in removing these compounds after electrophoretic application. It is possible that other enzyme processes are involved, and, as indicated above, the stability of the drug-receptor complex may be an important factor in determining the duration of action, the agent being removed from the receptor region by diffusion.

The analogues of 5-hydroxytryptamine and other compounds which prevent its action at the receptors of some peripheral tissues (see Gaddum, 1958 ; Gyermek, 1961) have actions upon geniculate neurones which are identical with that of 5-hydroxytryptamine, and it can be assumed that all of the substances interact with the same receptor. The 5-hydroxytryptamine-like activity of some of these compounds has been emphasized (Shaw & Woolley, 1956), and, since the structure-activity relationships of tryptamine derivatives vary from tissue to tissue (see Erspamer, 1952 ; Barlow & Khan, 1959a, 1959b, 1959c ; Vane, 1959 ; Greenberg, 1960 ; Hill & Usherwood, 1961), it is important that the site of the actual receptor involved should be specified when discussing these relationships (see Woolley, 1959 ; Gyermek, 1961). The failure of 2-bromo-(+)-lysergic acid diethylamide to reproduce

the psychic manifestations which follow the administration of lysergic acid diethylamide (Cerletti & Rothlin, 1955), in spite of the observation that both compounds effectively block the action of 5-hydroxytryptamine upon smooth muscle, was considered to be convincing evidence that the symptoms were not directly associated with a blocking of a central action of 5-hydroxytryptamine (see Gaddum & Vogt, 1956 ; Rothlin, 1957 ; Vogt, 1958). Other actions of 5-hydroxytryptamine analogues were envisaged (see Woolley, 1958), but these were still concerned with a possible role of 5-hydroxytryptamine as a neurohumoral agent. The present observations permit yet another postulate, namely, that lysergic acid diethylamide, and other tryptamine-like compounds with actions upon the central nervous system, are interfering with the synaptic activity of a transmitter identical with or similar to that responsible for the excitation of lateral geniculate neurones. It is also conceivable that at some central synapses 5-hydroxytryptamine is a transmitter, and consequently the effects produced by intravenous injections of tryptamine derivatives (Szara, 1957 ; Tedeschi, Tedeschi & Fellows, 1959 ; Vane, Collier, Corne, Marley & Bradley, 1961) or by the systemic or intraventricular application of 5-hydroxytryptamine analogues and antagonists (Gaddum & Vogt, 1956 ; Purpura, 1956a, 1956b ; Rovetta, 1956 ; Ginzel, 1957 ; Bradley, 1958 ; Malcolm, 1958 ; Smythies, Koella & Levy, 1960) will be complex phenomena depending upon which synapses are affected and whether the compounds mimic or block the action of the transmitters. The possibility must also be considered that 5-hydroxytryptamine-like compounds may be operating normally in the central nervous system, not as excitatory or inhibitory transmitters, but as postsynaptic blocking agents of transmitter action. The difficulties inherent in this concept of neuronal regulation have been discussed recently (Curtis, 1961). The finding that lysergic acid diethylamide suppresses the firing of hippocampal neurones (Brücke, Gogolak & Stumpf, 1961) may indicate that the transmitter responsible for the excitation of these cells is similar to that released at geniculate synapses. However, unlike the lateral geniculate nucleus, the hippocampus is rich in 5-hydroxytryptamine (Bogdanski, Weissbach & Udenfriend, 1957 ; Paasonen, Maclean & Giarman, 1959), and it is conceivable that the lysergic acid diethylamide effect is one of antagonism towards a synaptic action of 5-hydroxytryptamine.

The psychotomimetic action of lysergic acid diethylamide and of related compounds, in the human subject, is produced by doses which are much lower than those necessary to depress lateral geniculate responses in the cat. However, the depression of potentials generated by many cells, or even the prevention of firing of one cell, are relatively crude methods of assessing alterations in neuronal function. With smaller doses of these compounds subtle alterations in the responses of certain neurones, produced by the same mechanisms by which larger doses block these responses, may be sufficient to cause psychic manifestations.

Finally, these results can be compared with those reported by other investigators. The influence of the blood-brain barrier upon the pharmacology of compounds injected intravenously or intra-arterially is shown by examining the findings of Evarts *et al.* (1955), Evarts (1958) and Bishop, Burke, Davis & Hayhow (1960). When injected intra-arterially, lysergic acid diethylamide was about ten times as

potent as bufotenine as a depressant of synaptic transmission through the lateral geniculate nucleus. In contrast, when applied locally, bufotenine was approximately twice as potent as lysergic acid diethylamide. Again, psilocybin injected intra-arterially was a poor depressant of lateral geniculate neurones (Bishop, Burke, Davis & Hayhow, 1960) when compared with lysergic acid diethylamide, yet when applied locally the two compounds were of similar potency. In the earlier investigations 5-hydroxytryptamine was either inactive (0.1 mg intra-arterially ; Evarts, 1958) or very variable and relatively weak (Bishop, Burke, Davis & Hayhow, 1960). The high potency of this compound when applied locally indicates that the blood-brain barrier effectively limits its access to the neurones when it is administered systemically.

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