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LYSERGIC ACID DIETHYLAMIDE AND SEROTONIN: A COMPARISON OF EFFECTS ON SEROTONERGIC NEURONS AND NEURONS RECEIVING A SEROTONERGIC INPUT[1]

HENRY J. HAIGLER AND GEORGE K. AGHAJANIAN

Departments of Psychiatry and Pharmacology, Yale University School of Medicine and the Connecticut Mental Health Center, New Haven, Connecticut

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ABSTRACT

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Lysergic acid diethylamide (LSD) administered systemically to rats has been shown to reversibly inhibit serotonin (5-HT)-containing neurons of the raphe nuclei (serotonergic neurons). This inhibition could be due to either a direct effect or an indirect action *via* a postsynaptic neuronal feedback loop. To compare the responsivity of serotonergic neurons and postsynaptic neurons to LSD, raphe neurons and neurons in four areas (ventral lateral geniculate, amygdala, optic tectum and subiculum) with an identified 5-HT input from the midbrain raphe nuclei were tested for their response to microiontophoretically ejected and systemically administered LSD. Compared to the raphe, cells in these postsynaptic areas were relatively insensitive to microiontophoretic LSD. Raphe cells could be totally inhibited by LSD at ejection currents too low to have any effect on the postsynaptic neurons. In contrast, 5-HT was very nearly equipotent in depressing the firing of the presynaptic (raphe) cells and the postsynaptic cells. To determine if LSD has any indirect inhibitory effect upon raphe neurons *via* a neuronal feedback, LSD was administered to animals with a mesencaphalic-diencephalic transection. In these animals LSD still produced inhibition of raphe cells at doses comparable to those in control animals. It is concluded: 1) raphe neurons are more sensitive to inhibitory effects of LSD than are postsynaptic neurons (*i.e.*, neurons receiving an identified 5-HT input); and 2) the inhibitory effect of low doses of LSD on the presynaptic (raphe) cells is caused by a direct inhibitory action rather than an indirect action *via* a neuronal feedback.

There is considerable evidence from biochemical studies that *d*-lysergic acid diethylamide (LSD) alters the metabolism of serotonin (5-hydroxytryptamine; 5-HT) in brain (Freedman, 1961; Rosecrans *et al.*, 1967; Andén *et al.*, 1968; Lin *et al.*, 1969; Freedman *et al.*, 1970; Schubert *et al.*, 1970). Dahlström and Fuxe (1965) and Fuxe (1965), using a fluorescence histochemical technique, demonstrated that brain 5-HT is contained primarily within neuronal elements. The perikarya of these neurons are located in the

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Send reprint requests to: Dr. George K. Aghajanian, Connecticut Mental Health Center, Department of Psychiatry, 34 Park St., New Haven, Conn. 06508.

raphe nuclei of the brain stem (Dahlström and Fuxe, 1965) and their projections extend into the forebrain and other areas (Fuxe, 1965; Andén *et al.*, 1966). In the dorsal raphe nucleus of the rat, in contrast to the median and pontine raphe nuclei, the 5-HT-containing (serotonergic) neurons are closely packed and appear to comprise most, if not all, of the neurons in this area. LSD, administered *via* the systemic route, was found to reversibly inhibit serotonergic neurons of the raphe nuclei (Aghajanian *et al.*, 1968, 1970). Since the systemic route of administration was employed, these studies do not discriminate between a possible direct or indirect action of LSD upon raphe cells. It has been suggested that LSD could produce an indirect inhibitory effect by mimicking the action of 5-HT on the postsynaptic cells (*i.e.*, neurons that receive a 5-HT input). This in turn was hypothesized to result in a compensatory negative neuronal feedback to the raphe (Andén *et al.*, 1968; Aghajanian and Freedman, 1968). On the other hand, it has been shown that raphe neurons are inhibited by LSD applied locally by microiontophoresis (Aghajanian *et al.*, 1972; Haigler and Aghajanian, 1973; Bramwell and Gonye, 1973) indicating that LSD can have a direct action upon raphe neurons. In addition, 5-HT applied microiontophoretically has also been found to inhibit raphe cells (Aghajanian *et al.*, 1972). The effect of 5-HT and LSD on raphe cells is additive in that simultaneous ejection of submaximal amounts of 5-HT and LSD produces inhibition which is similar to the inhibition produced by the higher currents of either LSD or 5-HT alone.

Although it appears from the above data that LSD may have a direct effect on raphe neurons, the possibility remains that the drug may be having an even more significant effect on neurons postsynaptic to the raphe. To test this question it would be important to compare the relative potency of LSD and 5-HT at both serotonergic neurons and neurons receiving 5-HT input. Microiontophoretically applied 5-HT has been found to cause an increase or decrease in neuronal firing in nearly every brain region tested. The interpretation of these data is difficult in the absence of detailed knowledge of identifiable 5-HT input to the cells under study (Bloom *et al.*, 1972). Terminals of serotonergic (raphe) neurons have been difficult to detect (Fuxe, 1965; Fuxe and Jonsson, 1967) but a recent modification of the fluorescence histochemical procedure has made it possible to clearly visualize such terminals impinging upon postsynaptic cells (Kuhar *et al.*, 1972; Aghajanian *et al.*, 1973). Some of the postsynaptic areas which receive a uniform 5-HT input are the ventral lateral geniculate, the amygdala (cortical and basal lateral nuclei), the optic tectum and subiculum (Aghajanian *et al.*, 1973). The input in these areas is uniform in the sense that all of the cells appear surrounded by 5-HT terminals. However, the 5-HT input to each cell is not necessarily identical. The effects of both 5-HT and LSD on raphe neurons and on neurons in the above postsynaptic areas were determined in the following experiments.

Materials and Methods

A total of 107 male albino rats (Charles River Laboratories, Wilmington, Mass.), weighing between 230 and 280 g were used. For purposes of recording, animals were anesthetized with halothane and mounted in a stereotaxic instrument. A hole was drilled in the skull and the brain stem transected at the pretrigeminal level using a stereotaxically positioned mechanical wire knife (retractable) as described by Sclafani and Grossman (1969). The transection of the brain stem passes just posterior to the inferior coliculus, through the anterior vermis of the cerebellum, the head of the fourth ventricle, and just anterior to the trigeminal nerve. Such a transection spares the midbrain raphe nuclei but yields a quiescent, unanesthetized animal. The pretrigeminal transection results in what can be considered a "low cerveau isole" type of preparation (Zernicki, 1968). After an additional hole was drilled over the area to be studied, halothane anesthesia was discontinued. Animals prepared in this way were quiescent throughout the rest of the experiment without the need for additional drugs. This preparation was used in all of the microiontophoretic studies and most of the intravenous studies to avoid the possibility that anesthetic agents might potentiate the depressant action and mask the possible excitatory action of 5-HT (Roberts and Straughan, 1967; Boakes *et al.*, 1970). In experiments designed to test the neuronal feedback hypothesis, a transection was placed between the diencephalon and mesencephalon (disconnecting the midbrain raphe from its forebrain projection areas); these animals were maintained on chloral hydrate anesthesia.

The tips of the five barrel micropipettes were broken back to 4 to 5 μm under microscopic control and solutions were directly injected into the various barrels. The prior presence of fiberglass allowed the tips to become filled quickly by capil-

lary action (Tasaki *et al.*, 1968). The use of this fiberglass method ensures that the concentration of drugs in the tip of the electrode is constant and avoids the difficulty of a possible dilution of the drug solution by distilled water. Electrodes filled in this way had impedances (measured at 1000 Hz) in the drug barrels which were consistently in a narrow range of 18 to 22 megohms. With such a narrow range, the possibility of a major variation in transport number (see below) due to shunting of the ejection current through lower impedance pathways was minimized. This narrow range was necessary for comparing the ejection currents of the drugs in different areas. To further standardize the comparison between drug-induced responses of cells in different areas, the duration of microiontophoresis was always 30 seconds for each ejection period at each intensity of ejection current.

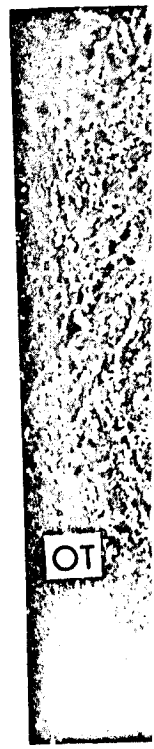
The central (recording) barrel was filled with a 2 M NaCl solution saturated with fast green. One side barrel, the "balance" channel, was filled with 5 M NaCl. The three other barrels were filled with 0.05 M serotonin creatinine sulfate, pH 4.0 (Sigma Chemical Company, St. Louis, Mo.), 0.001 M LSD bitartrate in 0.1 M NaCl, and 0.2 M acetylcholine chloride, pH 6.0 (Calbiochem, Los Angeles, Calif.). The difficulties of major leakage effects and excessive holding currents (Bloom *et al.*, 1964) were avoided by using LSD in a dilute solution. NaCl was added to facilitate the passage of ejecting current (Curtis and Crawford, 1969). Circuitry for the iontophoretic ejection of drugs and current balance was similar to that described by Salmoiraghi and Weight (1967) except that a solid state system was used. The current balance was kept to within ±5 nA. Impedances, measured at 1000 Hz, were 2 to 7 megohms in the recording barrels, 8 to 14 megohms in the balance barrels and 18 to 22 megohms in the drug barrels. Electrical signals were led through a high impedance amplifier, displayed on an oscilloscope and led into an electrical counter whose threshold was set so that it was triggered by the individual discharges (*i.e.*, spikes) of a single neuron. Recordings were of integrated unit rate and consisted of consecutive samples of either 10- or 1-second intervals of the analog output of the electronic counter.

Although the identity of all cells was ultimately determined by histological examination after each experiment, there were certain practical guidelines which were useful during the initial placement of electrodes. Cells in the raphe were recognized as positive-negative spikes with a characteristic regular rhythm and slow rate (0.5–2 spikes/sec) appearing in the midline just ventral to an area of electrical silence (cerebral aqueduct) in frontal planes corresponding to P100 through A650 of König and Klippel (1970). Of the various raphe nuclei, the dorsal raphe nucleus was selected for study because it gives rise to the major 5-HT input to the forebrain (Andén *et al.*, 1966) and because it contains the largest and most homogeneous clusters of 5-HT-containing neurons (Dahlström and Fuxe, 1965). Amygdala cells, primarily in the cortical and basal lateral nuclei (in frontal planes corresponding to A2420-3430 of König and Klippel, 1970) typically appeared as negative spikes and were excited by a low ejection current (1–10 nA) of acetylcholine (ACh). These were found within 1 mm of the ventral surface of the brain (the latter was demarcated by an increase in electrical noise) at an appropriate laterality. Ventral lateral geniculate cells (A2420–3430, König and Klippel, 1970) were recognized by their response to a light moving across the visual field. These were usually found 0.3 to 0.5 mm ventral to the first "swish" response to a moving light (optic tract fiber activity). Neurons in the dorsal lateral geniculate were usually encountered first; these cells, unlike those in the ventral lateral geniculate were characteristically excited by light flashes but not readily by movement of a light across the visual field. Some cells in the optic tectum (superior colliculus; A1270–2420) were similar to the cells in the ventral lateral geniculate in their response to a light stimulus, but other cells in the optic tectum were unresponsive to the light stimuli tested. Cells in the subiculum (A1760–2420) from which we recorded were located in the dorsal subiculum just posterior to the division of the splenum of the corpus callosum.

Two types of "comparison" areas were used. One type consisted of areas which receive a nonuniform 5-HT input (*i.e.*, ventral hippocampus and thalamus). Because of this patchy distribution, cells in these areas may or may not receive 5-HT terminals. The other type of comparison area consisted of areas which receive only a sparse 5-HT input (*i.e.*, dorsal lateral geniculate and reticular formation).

Recording sites were marked by passing a 20 μA cathodal current through the recording barrel for 10 minutes. This resulted in the deposition of fast green in a discrete spot (Thomas and Wilson, 1965). Animals were then perfused with 10% formalin (buffered). Serial sections (50 μm) were then cut, mounted and counterstained with neutral red. A correlation could thus be made between the recording site and the previously established location of 5-HT-containing neurons in the dorsal raphe nucleus (*cf.* fig. 1; Haigler and Aghajanian, 1973), or neurons receiving a dense input of 5-HT terminals (fig. 1) as previously described by Aghajanian *et al.* (1973).

The transport number, an index of the efficiency



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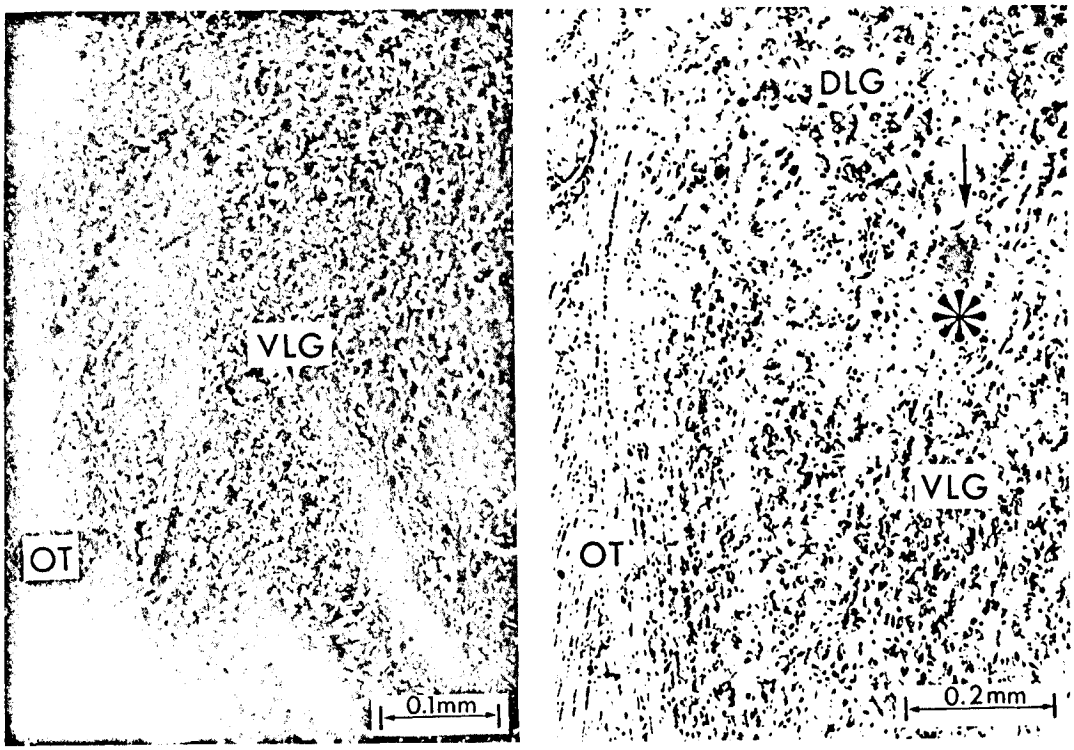

FIG. 1. Correlation of histological localization of recording sites with fluorescence histochemical localization of 5-HT terminals (as illustrated for the ventral lateral geniculate; VLG). The micrograph on the left shows numerous bright, fine fluorescent terminals within the neuropil surrounding the non-fluorescent neurons of the ventral lateral geniculate. Such terminals have previously been shown to be derived from the midbrain raphe nuclei and to be selectively depleted by *p*-chlorophenylalanine, an inhibitor of 5-HT synthesis (Kuhar *et al.*, 1972; Aghajanian *et al.*, 1973). The micrograph on the right illustrates a standard histological preparation which was made after a single unit recording experiment. An electrode tract (arrow) can be seen leading to a fast green spot (above asterisk). In this low power view, portions of the dorsal lateral geniculate (DLG), ventral lateral geniculate and optic tract (OT) are visualized. The electrode tract has traversed the dorsal lateral geniculate and the fast green spot is situated in the dorsal aspect of the ventral lateral geniculate.

of iontophoretic drug release, was determined for 5-HT and LSD: this allows a comparison of our experiments with the experiments of others. The following formula, derived from Curtis (1964) was used to calculate the transport number (n) for both 5-HT and LSD:

$$n = \frac{MFZ}{IT}$$

where M is the moles ejected, F is Faraday's constant (9.65×10^4), Z is the equivalents per mole, I is the current and T is the time of ejection. To determine the moles ejected, tritium-labeled 5-HT and LSD (New England Nuclear Corporation, Boston, Mass.) was used at the same concentrations as in the physiological experiments. Specific activities of 5-HT and LSD were 24 and 1.9 c/mmol, respectively. These solutions were injected into standard five barrel micropipettes; impedances were in the same range as the electrodes used for recording. The tip of the micropipette was immersed into 0.1 ml of 0.9% saline held in a small plastic container. Plastic was used to prevent possible adsorption of LSD onto a glass surface. When the ejection was finished, the entire small container containing the drop of saline was placed in a scintillation counting vial containing 15 ml of Aquasol (New England Nuclear). After vigorous agitation, the vial was placed in a scintillation counter. Counting began after at least 20 minutes were allowed for stabilization from photoactivation. The efficiency was determined by both internal and external standardization. Similar efficiencies were obtained with the two procedures. The number of disintegrations for each sample were converted to the number of moles ejected. The mean transport number of 5-HT using the above technique was

0.219 (number of observations = 18; S.E.M. ±0.009). Bradley and Candy (1970) found a comparable transport number for 5-HT with their "small" pipettes ($t = n = 0.18$). The mean transport number for LSD was 0.0023 (number of observations = 30; S.E.M. ±0.00019). The transport number for LSD was slightly more than 1% of that for 5-HT. Thus, under these conditions, 100 nA of ejection current of LSD would be equivalent to 1 nA of ejection current of 5-HT in terms of the number of drug molecules ejected. Since equal currents of both agents inhibit the raphe (Aghajanian *et al.*, 1972), LSD emerges as being more potent than 5-HT, molecule for molecule, on the rhaphe neurons. Bradley and Candy (1970) have reported 0.02 as a transport number for LSD under their experimental conditions; this is approximately 10 times our figure. This difference can be explained by the fact that the LSD constituted only 1% of the positive ions in our solution since LSD was used in a dilute solution (0.001 M) and NaCl (0.1 M) was added to facilitate passage of current. Thus, for an equal ejection current, considerably less LSD would be ejected under our conditions than under those described by Bradley and Candy (1970).

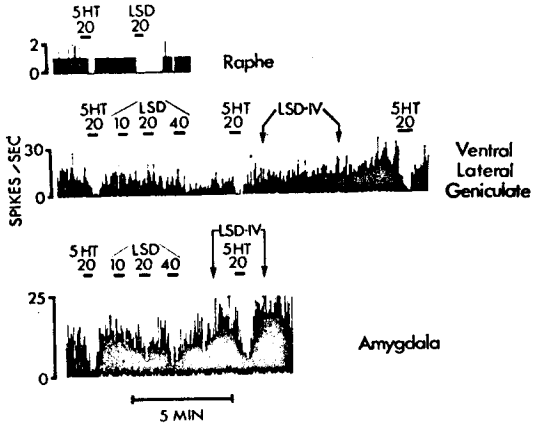

FIG. 2. Comparison of the response of a neuron in the dorsal raphe nucleus with that of neurons in the ventral lateral geniculate and the basolateral nucleus of the amygdala to the microiontophoretic administration of 5-HT and LSD. In the top trace a raphe cell is completely inhibited by the microiontophoresis of both 5-HT and LSD for 30 seconds at an ejection current of 20 nA. In the second trace a cell in the ventral lateral geniculate is inhibited by 5-HT given for 30 seconds at an ejection current of 20 nA. However, LSD has little effect at 10 or 20 nA but produces a partial inhibition at 40 nA. LSD intravenously (20 μg/kg at each arrow) produces an acceleration of firing in the cell. After the intravenous LSD, 5-HT at 20 nA is still capable of producing the same absolute amount of inhibition. In the bottom trace the postsynaptic cell in the amygdala is inhibited by 5-HT (20 nA). The cell is unaffected by LSD at 10 nA and slowed by 20 and 40 nA of ejection current. After 20 μg/kg of LSD intravenously (indicated by the first arrow), the cell shows an acceleration of firing. However, 5-HT (20 nA) is still capable of producing the same absolute amount of a decrease in firing. In this and all subsequent figures of recordings, the horizontal bar indicates the duration of the ejection current; the number above the bar indicates the ejection current in nA. Arrows indicate the time of intravenous injection of LSD. The ordinate represents the integrated rate of firing in spikes per second.

Results

Cells in four areas (the amygdala, the ventral lateral geniculate, the subiculum and the optic tectum), which receive projections from the raphe were tested for their responses to microiontophoretic LSD and 5-HT as well as i.v. administered LSD. Although a number of areas have been previously shown to receive a uniform 5-HT input from the raphe (Aghajanian *et al.*, 1973), these four postsynaptic areas were selected because cells in these areas fired at moderately stable base-line rates. These postsynaptic cells were found to be relatively insensitive to microiontophoretic LSD, being inhibited only 50% by currents (40 nA) four times those necessary to produce total inhibition in the raphe cells (figs. 2–4). In contrast, ejection currents of 5-HT necessary to produce inhibition (at least a 90% decrease in firing) in the raphe (9.3 ± 1.2 nA; number of observations = 22) were very nearly equal to the ejection currents (12.0 ± 0.7 nA; number of observations = 72) necessary to produce inhibition in the postsynaptic cells (figs. 2–3). The currents necessary for inhibition were determined by a procedure of successive approximations to avoid the possibility that the currents used were supramaximal.

In the ventral lateral geniculate we found that both background activity and the response to a light stimulus were blocked or attenuated by 5-HT; LSD was also capable of depressing the background activity of the ventral lateral geniculate cells. However, at low ejection currents LSD did not suppress the synaptic responses evoked by light. These data are in agreement with the findings of Tebēcis and DiMaria (1972). At higher currents the latter authors found that LSD did block light-evoked responses. However, nonspecific depressant effects might have been operative under these conditions since in the higher current range the firing evoked by glutamate and ACh was also depressed (Tebēcis and

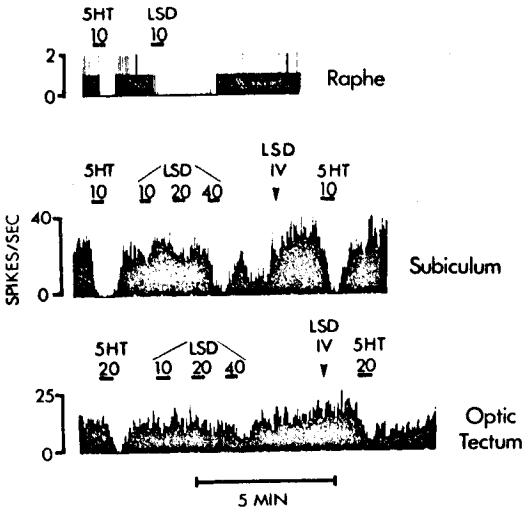

FIG. 3. Comparison of the responses of cells in the dorsal raphe nucleus, the optic tectum and dorsal subiculum to the microiontophoretic administration of 5-HT and LSD. In the top trace a raphe cell is completely inhibited by 5-HT and LSD (30 seconds of a 10-nA ejection current). In the second trace a postsynaptic cell in the subiculum is readily inhibited by 5-HT (30 seconds of a 10-nA ejection current). LSD at 10 or 20 nA of ejection current has only a slight depressant effect. LSD at 40 nA produces a further inhibition. LSD (20 μg/kg) intravenously (indicated by the arrow) produces an acceleration of firing of the cell; 10 nA of 5-HT is still capable of inhibiting the firing of the cell. In the bottom trace a postsynaptic cell in the optic tectum (superior colliculus) is inhibited by 5-HT (20 nA). The cell is slowed to about 50% of control in response to LSD (30 seconds of a 40-nA ejection current). In contrast, 20 μg/kg of intravenous LSD (arrow) produces a 25% increase in the base-line firing. 5-HT is still capable of producing the same absolute amount of decrement in the firing of the cell after intravenous LSD.

DiMaria, 1972). Although the transport number was not determined in the latter experiment it was presumably higher than the 0.002 determined in our study (see "Materials and Methods") since there was less of a dilution of the LSD by NaCl.

It is possible that the 50% inhibition produced by high ejection currents of LSD (40 nA) in the four postsynaptic areas could represent either a weak 5-HT agonistic effect or a nonspecific depressant effect. At least 10 times the current of LSD necessary to produce a 50% inhibition of raphe cells is necessary to approach a 50% inhibition in the areas receiving a uniform 5-HT input (fig. 4). Since a 50% inhibition of firing was not achieved in all areas, a more precise comparison of these dose-response curves was not attempted. At 40 nA of ejection current, LSD occasionally produced decreases in action potential size, indicative of a local anesthetic effect (Curtis, 1968; Hoffer *et al.*, 1971), despite the low transport number under the conditions used (see "Materials and Methods"). For this reason we did not routinely exceed this level of ejection current. Furthermore, even at 40 nA, LSD seemed to have a long-term depressant effect on some postsynaptic and other cells (5–10 minutes) which suggests a nonspecific local toxic action. Neurons in the reticular formation, an area which receives only a sparse 5-HT input, showed only a 20% decrease in base-line firing rate at 40 nA of LSD (fig. 4).

To examine from a different standpoint the relatively lack of sensitivity of the postsynaptic cells to LSD, the drug was administered i.v. at a dose of 20 μg/kg. This dose exceeded the highest

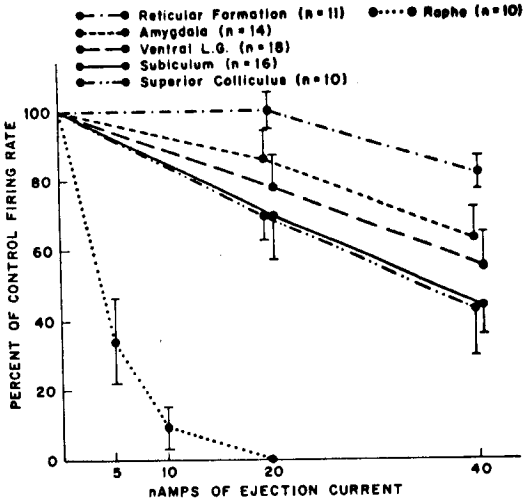

FIG. 4. Effect of LSD on dorsal raphe neurons and postsynaptic neurons when applied microiontophoretically. This is a graphical presentation of the difference in sensitivity to LSD between dorsal raphe cells (presynaptic cells) and cells that receive a 5-HT input (postsynaptic cells) in the amygdala, ventral lateral geniculate, subiculum and optic tectum (superior colliculus). The response of cells in the reticular formation (an area receiving a sparse 5-HT input) to directly applied LSD is also shown. The vertical bars show the S.E.M. The percentage of control firing rate was determined by taking the mean of the firing rate for 10 seconds in a control period and also at the 25 to 35-second interval after the onset of the ejection current. The mean firing rate in the ejection current period was divided by the firing rate during the control period and converted to a percentage.

TABLE 1

Response of dorsal raphe (presynaptic) and postsynaptic neurons to intravenous LSD

Area	No. of Cells	Intravenous Dose of LSD	Percent Change from Control Firing Rate[a]
		μg/kg	
Presynaptic area			
Dorsal raphe nucleus	7	11.6 ± 1.3[b]	−100
Postsynaptic areas			
Superior colliculus	12	20	+49 ± 11[c]
Ventral lateral geniculate	8	20	+40 ± 15[d]
Subiculum	12	20	+23 ± 9
Amygdala	10	20	+15 ± 5[c]

[a] The mean control and postdrug firing rates were determined (*cf.* legend to fig. 6). The postdrug firing rate was determined at the 30- to 40-second interval after the intravenous administration of LSD since inhibition of the raphe would have occurred within 30 seconds. These data were evaluated statistically using Student's *t* test for paired data on the mean of the firing rates before they were converted to the percentage shown.

[b] This is the mean dose ± S.E.M. which produced a total inhibition of firing of the raphe neuron for at least 30 seconds.

[c] $P < .01$.

[d] $P < .05$.

dose (16 μg/kg) necessary to produce total inhibition of firing in the group of raphe cells tested under the same conditions (table 1). The intravenous LSD did not produce an inhibition of firing in the postsynaptic cells; instead, there was an acceleration of firing (figs. 2–3; table 1). To test whether the intravenous LSD might produce this acceleration by antagonizing the inhibitory effect of endogenous 5-HT, 5-HT was administered microiontophetically after the acceleration had occurred (figs. 2–3). It was found that microiontophoretic 5-HT was still effective in inhibiting the postsynaptic cells. By inference, this indicates that the acceleration of firing in the postsynaptic cells is not being produced through a blockade of endogenous 5-HT acting on the postsynaptic cell.

A suggested mechanism through which LSD could indirectly inhibit raphe neurons is that LSD mimics 5-HT at postsynaptic cells, setting into motion a compensatory neuronal feedback inhibition of the presynaptic (raphe) cells (Andén *et al.*, 1968 Aghajanian and Freedman, 1968). This hypothesis is not supported by the data in the present experiments since a prominent direct inhibitory effect is produced by LSD on the raphe neurons in amounts that have little direct effect on the postsynaptic cells. Nevertheless, to further test the neuronal feedback hypothesis, a complete transection was made at the junction between the diencephalon and mesencephalon. This would prevent any neuronal feedback information from postsynaptic cells in the forebrain from reaching the raphe. If the inhibitory effect of LSD on the raphe is dependent on a feedback inhibition emanating from the postsynaptic cells in the forebrain, then such a lesion should prevent or attenuate the inhibition of firing of a raphe neuron produced by intravenous LSD. As is illustrated in figure 5, there is no difference in the inhibition produced by intravenous LSD between a control animal and an animal with a midbrain diencephalon transection. In a series of such comparisons there was no significant difference (t test; $P > .2$) between the mean dose to produce an inhibition of at least 1 minute in the six transected animals (mean dose = 18.3 ± 4.0 μg/kg) and the six control animals (mean dose = 15 ± 2.23 μg/kg). Figure 6 illustrates a typical complete transection. These data indicate that the inhibition of firing of raphe cells produced by intravenous LSD is not dependent on an inhibitory feedback from the postsynaptic cells in the forebrain. This experiment, however, does not fully test the feedback hypothesis because feedback inhibition of the raphe in response to LSD could be mediated by postsynaptic cells in the midbrain.

Some of the cells in areas receiving a non-

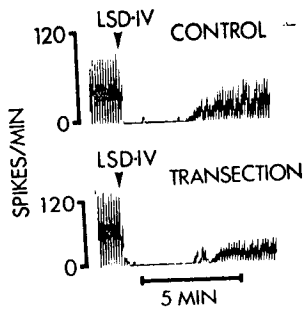

FIG. 5. Comparison of the sensitivity of raphe neurons to intravenous LSD in a control animal and an animal with a mesencephalic-diencephalic transection. The arrow in both cases marks the point at which 20 μg/kg of intravenous LSD were administered. Both cells show a prompt, complete inhibition; the bottom trace is the record of a raphe cell in an animal with a complete transection between the mesencephalon and diencephalon. This transection would prevent any neuronal feedback from the forebrain reaching the dorsal raphe nucleus. Both traces shown here are integrated records, with the ordinate indicating the rate in spikes per minute of neurons in the dorsal raphe nucleus.

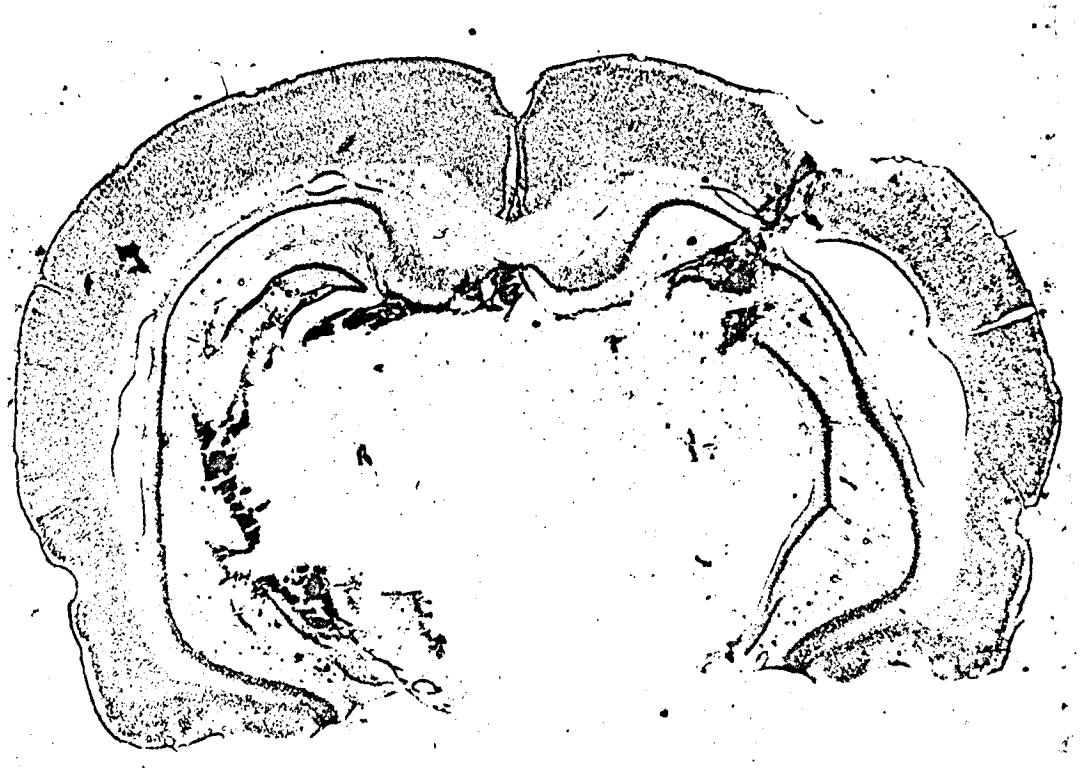

FIG. 6. Histological section showing a mesencephalic-diencephalic transection. This section, which is cut in the same plane as the transection, demonstrates that the mesencephalic-diencephalic separation is complete but that the surrounding cortex and hippocampus are relatively undamaged. All transections were evaluated in this way to ensure that no possible feedback pathways from the forebrain to the dorsal raphe nucleus existed.

uniform input of 5-HT terminals are inhibited by 5-HT; such cells (*i.e.*, in ventral hippocampus and thalamus) have the same relative insensitivity to LSD as do cells in the defined postsynaptic areas (table 2). Cells in the thalamus were either unaffected, inhibited or excited by 5-HT (Table 2). LSD blocked or attenuated the effects of 5-HT in seven of the eight cases in which excitatory responses to 5-HT were observed. The variation in response to 5-HT is possibly explained by the fact that the thalamus does not receive a uniform input of 5-HT terminals. Although cells in the dorsal lateral geniculate receive a sparse 5-HT input, a large proportion of these were readily inhibited by 5-HT; again the response to LSD was minimal (table 2). Typically, cells in the reticular formation, which also receives a sparse 5-HT input, were unaffected by 5-HT. However, in four reticular units there was an acceleration of firing by 5-HT which could be blocked by LSD. These cells, as well as the

TABLE 2

Response of cells in comparison areas to the microiontophoresis of LSD and 5-HT at an ejection current of 20 nA[a]

Area	No. of Cells	Inhibition[b]		Slowing		No Effect		Excitation[c]	
		5-HT	LSD	5-HT	LSD	5-HT	LSD	5-HT	LSD
Thalamus	27	12	0	6	11	1	11	8	0
Dorsal lateral geniculate	24	18	0	6	16	0	8	0	0
Reticular formation	18	0	0	6	7	8	11	4	0
Ventral hippocampus	4	3	0	1	2	0	2	0	0

[a] Twenty nanoamperes is the upper limit of the range of current intensities for both 5-HT and LSD which produced complete inhibition in dorsal raphe neurons (*cf.* figs. 2-4).

[b] Inhibition is defined as at least a 90% decrease in mean firing rate (*cf.* legend to fig. 4).

[c] Excitation is defined as at least a 20% increase in mean firing rate.

thalamic cells accelerated by 5-HT, appeared to be similar in both their response to 5-HT and LSD to those reported by Boakes *et al.* (1970) and Roberts and Straughan (1967).

Discussion

Our principle finding is that the serotonergic neurons of the dorsal raphe nucleus are inhibited at a much lower microiontophoretic ejection current of LSD than are neurons in the ventral lateral geniculate, amygdala, optic tectum and subiculum which receive an identified serotonergic input. At the highest ejection current used (40 nA), LSD does have a depressant effect on these postsynaptic neurons. In sharp contrast to this rather remarkable difference in sensitivity to LSD, 5-HT is equipotent in inhibiting raphe neurons and postsynaptic cells in the four areas studied. In general, the depressant effects of 5-HT and LSD on the postsynaptic cells parallel the data of Curtis and Davis (1962) and Tebēcis and DiMaria (1972) on the lateral geniculate. When LSD is administered in high intravenous doses (70–300 μg/kg), there is evidence that it can depress the firing of cells in the lateral geniculate (Horn and McKay, 1973) and the ventral hippocampus (Aghajanian and Haigler, 1973). This is in agreement with the evidence that LSD at high doses (0.5–2.0 mg/kg) has a 5-HT-like effect on presumed postsynaptic cells in the spinal cord (Andén *et al.*, 1968). Taken together these data indicate that LSD in sufficiently high concentrations may act as a 5-HT agonist on at least some postsynaptic neurons. However, this effect of LSD may be nonspecific since some depression is also obtained at the same ejection current on cells in areas such as the reticular formation, which do not receive a substantial 5-HT input. In any event, at low ejection currents, LSD has a more striking effect on the presynaptic (raphe) neurons.

The experiments in which LSD was administered intravenously further demonstrate a difference in the sensitivity of presynaptic (raphe) and postsynaptic cells to this drug. At low intravenous doses LSD inhibits the raphe cells but not the postsynaptic cells. Instead there tends to be an acceleration in the firing rate of postsynaptic neurons. After low intravenous doses of LSD such an acceleration has also been observed in the lateral geniculate of cats (Mouriz-Garcia *et al.*, 1969; Horn and McKay, 1973). We find that this acceleration is not due to a blockade of the inhibitory effect of endogenous 5-HT since microiontophoretically applied 5-HT is inhibitory even after the intravenous LSD. These data suggest the possibility that at low doses LSD acts primarily by inhibiting the presynaptic (raphe) neurons and produces an acceleration of firing in the postsynaptic cells by releasing them from a tonic inhibitory raphe input. Evidence that the raphe has a tonic inhibitory influence on the postsynaptic cells is 2-fold. First, electrical stimulation of the raphe mimics the effects of the microiontophoretic application of 5-HT to cells in the suprachiasmatic nucleus by causing an inhibition of firing (Bloom *et al.*, 1972). Suprachiasmatic cells receive a documented 5-HT input (Fuxe, 1965; Kuhar *et al.*, 1972; Aghajanian *et al.*, 1973). Secondly, since raphe cells exhibit spontaneous activity under most conditions (Aghajanian *et al.*, 1973; McGinty *et al.*, 1973), they are likely to exert a tonic inhibitory influence on the postsynaptic cells under these same conditions. Bloom *et al.* (1972) have also found that LSD has no effect on the suprachiasmatic cells when applied iontophoretically or given in large parenteral doses. Moreover, LSD did not block the inhibitory effects of 5-HT or the inhibitory effects of raphe stimulation on these cells (Bloom *et al.*, 1972). To test the possibility of a tonic raphe inhibitory influence more thoroughly, further studies are needed in which the effects of raphe stimulation are compared to the effect of 5-HT applied microiontophoretically in other identified postsynaptic areas.

The data reported here demonstrate that both raphe and postsynaptic cells are inhibited by microiontophoretic 5-HT. This indicates that in relation to the serotonergic synapses there is a 5-HT receptor both on the pre- and postsynaptic neurons. Two possible physiological functions can be suggested for a presynaptic indoleamine receptor. The presynaptic receptor could reflect the presence of 5-HT collaterals within or between the various raphe nuclei. Another possibility is that the entire presynaptic membrane, including that of the raphe terminals, is sensitive to 5-HT and structurally related indoleamines such as LSD. This sensitivity could serve as part of a local feedback mechanism at the terminal as has been suggested for central dopaminergic neurons (Carlsson *et al.*, 1972). The block of the

release of ^{3}H-5-HT from isolated brain slices by LSD (Chase *et al.*, 1967) supports the possibility of the existence of such a mechanism. Since the postsynaptic cells are relatively insensitive to LSD it appears that the indoleamine receptor on the postsynaptic cell has different steric requirements from the indoleamine receptor on the presynaptic cell. This argument makes the assumption that because the action of both compounds is inhibitory on the presynaptic cell, they act on the same receptor site; this remains to be tested experimentally.

Data from experiments carried out on *cats* indicate that, in the cortex (Roberts and Straughan, 1967) and the reticular formation (Boakes *et al.*, 1970), 5-HT has an excitatory action on some units, an effect which is blocked by LSD. Fuxe (1965), as these authors indicate, has reported that there are diffuse 5-HT nerve terminals in the cortex and reticular formation of the *rat* CNS. In areas of the rat CNS selected for their dense 5-HT input, we have not found excitatory responses to 5-HT. Instead, we find an inhibitory effect of 5-HT which is weakly mimicked, not blocked, by LSD. However, in areas of the rat CNS where the terminals are low in number (*e.g.*, reticular formation) or nonuniform in distribution (*e.g.*, thalamus), we have found excitatory responses to 5-HT which are blocked by LSD. It has been reported that at pH values lower than 4 there is an increase in the number of excitatory responses in cortical cells which may be due to an ejection of hydrogen ions (Jordan *et al.*, 1972). In view of these results it is necessary to exercise caution in interpreting excitatory response to 5-HT. Furthermore, the excitatory responses to 5-HT are found in areas where it is difficult to ascertain whether or not the cell receives a 5-HT projection. In our studies with cells receiving an identified 5-HT input, 5-HT invariably produced an inhibition of firing.

Both inhibitory and excitatory responses to 5-HT have been reported for raphe cells in the rat (Couch, 1970). The intermingling of cells which are inhibited or excited by 5-HT (Couch, 1970) corresponds quite well with the intermingling of fluorescent and nonfluorescent cells in the pontine or median raphe nuclei (Dahlström and Fuxe, 1965). The increased incidence of cells dorsally in the median raphe which are inhibited by 5-HT (Couch, 1970) correlates with the increasing density of 5-HT-fluorescent cells in the dorsal part of this nucleus (Dahlström and Fuxe, 1965). We have found only inhibitory effects of 5-HT in cells histologically verified to be in the densely packed 5-HT-containing cells of the dorsal raphe nucleus. These data suggest that the excitatory responses to 5-HT in certain portions of the raphe (Couch, 1970) may be accounted for by the nonfluorescent cells interspersed among the 5-HT-containing fluorescent cells. Another possible explanation of these data is that there are two different 5-HT receptors, one which produces inhibition and one which produces excitation.

In conclusion, these data lead to the unexpected conclusion that the serotonergic neurons of the dorsal raphe nucleus are more sensitive to LSD, administered both directly and systemically, than are neurons in the forebrain and other areas with an identified 5-HT input (postsynaptic cells). Both from the microiontophoretic and transection experiments it appears that the effect of LSD on the firing of raphe cells is a direct inhibitory action and is not dependent on a compensatory neuronal feedback to the raphe from the postsynaptic cells in the forebrain. Although LSD applied microiontophoretically at high ejection currents can produce a decrease in firing in the postsynaptic cells, LSD administered in low intravenous doses produces an increase in firing, suggesting that these postsynaptic cells are being released from a tonic raphe inhibition. Such low dose effects are of interest because LSD produces psychotomimetic effects in man in a comparably low dose range. The inhibitory action of LSD on the raphe cells mimics the inhibitory effect of 5-HT in the raphe. 5-HT differs from LSD in that it is equally potent on the postsynaptic and raphe neurons. The difference in sensitivity to LSD between the raphe and postsynaptic cells suggests that steric requirements of the presynaptic indoleamine receptor (or receptors) differ from the postsynaptic indoleamine receptor.

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