Specificity of Serotonergic Inhibition in Limulus Lateral Eye

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ABSTRACT The receptor specificity for synaptically mediated lateral inhibition in Limulus lateral eye retina was studied by structure-activity correlations of the action of the putative indoleaminergic neurotransmitter, serotonin (5-HT), and its isomers and structural analogs, tryptamine (TRYP), 6-hydroxytryptamine (6HT), 5,6-dihydroxytryptamine (5,6-DHT), 5-hydroxydimethyltryptamine (5-HDMT), and 5-hydroxytryptophan (5-HTP). The 5-HT blockers, lysergic acid diethylamide (LSD), bromo-LSD (BOL), and cinanserin, were also tested. The inhibitory action of the indoleaminergic agonists is highly structure-specific. An hydroxyl group in the 5 position of the indole nucleus, sterically unencumbered by hydroxyls in neighboring positions, is essential. In order of decreasing potency, 5-HT, 5-HDMT, and 5-HTP are active agonists; TRYP, 6-HT, and 5,6-DHT are inactive. Configuration and mobility of the side chains of the active agonists also affect the interaction, and these side-chain characteristics correlate with agonist potency. The receptors for inhibitory action and for transmembranal transport in reuptake are different. Both active agonists and inactive analogs appear to be taken up (Adolph and Ehinger, 1975. Cell Tissue Res. 163:1-14). LSD and BOL have bimodal actions: direct inhibition and agonist blockade. These actions may be mediated via low-specificity presynaptic uptake receptor sites rather than the highly specific, postsynaptic, agonist receptor sites.

INTRODUCTION
The inhibitory interactions among the ommatidia of the compound lateral eye of the horseshoe crab, Limulus polyphemus, are classic examples of chemically mediated, synaptic activity. The functional properties of the lateral eye, including the prepotent effects of the inhibitory interactions, have been reported in detail in the work of Hartline and colleagues (Ratliff and Hartline, 1974). Serotonin (5-hydroxytryptamine; 5-HT) is the most likely candidate for the neurotransmitter mediating lateral inhibition in the eye; its synaptic mechanisms and neurochemical and histochemical identification have been previously reported (Adolph and Tuan, 1972; Adolph and Ehinger, 1975; Adolph, 1976).

The lysergic acid derivatives lysergic acid diethylamide (LSD) and BOL (bromo-LSD) have bimodal, agonist-antagonist actions, and bufotenine, the dimethylated analog of serotonin (N,N-dimethyl 5-HT; 5-HDMT), has an
agonistic action, on inhibition in the lateral eye. Histochemical studies of 5-HT presence in situ in the lateral eye by the formaldehyde fluorescence technique of Falck and Hillarp demonstrated avid uptake of, and resultant fluorescence enhancement by, the neurotoxic indoles 5,6-dihydroxytryptamine (5,6-DHT) and 6-hydroxytryptamine (6-HT). Thus the structure of the putative transmitter, the role of transmitter-receptor specificity, and structure-activity correlations seem to be promising avenues for elucidating the synaptic mechanisms underlying lateral inhibition.

In the work reported here, 5-HT and its isomers and structural analogs, tryptamine (TRYP), 6-HT, 5,6-DHT, 5-hydroxytryptophan (5-HTP), and bufotenine (5-HDMT) were tested. In addition, the action of the 5-HT blockers LSD, BOL, and cinanserin on light-evoked and 5-HT-induced inhibition in the lateral eye was examined. The results indicate that highly specific transmitter-receptor interactions underlie lateral inhibition. First, an hydroxyl group in the 5 position of the indole nucleus, sterically unencumbered by other hydroxyls in neighboring positions, is essential. Second, side-chain configuration has a secondary importance on the interaction. Third, the receptors for the inhibition and for active transmembranal transport in reuptake are different. And, fourth, the bimodal effects of the tested lysergates (LSD and BOL) may depend on their interaction with presynaptic receptors.

**METHODS**

The methods used have been described in detail (Adolph, 1976). The preparation consisted of a thin slice, along an anterior-posterior locus, of the lateral eye, which exposed the ommatidia and included the bulk of the optic nerve fiber bundle. Recordings of electrical activity originating in the eccentric cells of ommatidia were made either extracellularly, using nerve fibers drawn into glass suction electrodes, or intracellularly via glass micropipette electrodes that usually impaled the cell body. Drugs were applied either by microiontophoresis from single or multiple microelectrodes ejecting into the region of synaptic neuropil proximal to the test ommatidium, or by gross perfusion under a constant-volume paradigm. All the substances tested were obtained from commercial suppliers, except cinanserin (SQ 10,643), a gift from E. R. Squibb and Sons, Inc., Indianapolis, Ind., and LSD and BOL, obtained from the U. S. Food and Drug Administration/Bureau of Narcotics and Dangerous Drugs.

**RESULTS**

Response of an active eccentric cell to microiontophoretically applied pulses of 5-HT is shown in Fig. 1. The lower trace shows membrane potential recorded by an intracellular microelectrode during a series of iontophoretic ejections of equal quantities of 5-HT (constant-charge basis), recorded on the upper trace. After an initial latent period, the response to the initial pulse is a rapid hyperpolarization of membrane potential (ca. 4–5 mV) followed by a less rapid recovery to the original prepulse level. Before the 5-HT pulse, the cell had a steady level of spontaneous spike firing of about four or five per second. At the peak of the 5-HT effect, spike firing rate is reduced to one every 2–3 s. A substantial iontophoresis current-associated noise during the ejection pulse produces a large artifact but does not obscure the response. A second 5-HT pulse is ejected after an interval of ~90 s. During this interval
the firing rate and membrane potential returned to control levels. The second response sequence begins in a manner essentially equivalent to the initial one, but is interrupted by a third 5-HT pulse after only 30 s. A pattern of 5-HT pulses every 25 s is then repeated several times. The inhibitory response to the repeated 5-HT pulses increases for the first four repetitions, with each pulse producing a greater reduction, and finally suppression, of spike firing. There is a concomitant increase in the membrane potential hyperpolarization to a maximum level of ~8 mV below the control level. The last five 5-HT pulses show a developing desensitization effect: a slow depolarization of membrane potential and resumption of spike firing even during continued 5-HT applications.

Summation and desensitization effects are not peculiar to responses evoked by 5-HT iontophoresis in the eye. Light-evoked lateral inhibition mediated by the natural inhibitory transmitter, inhibition evoked by a perfusion paradigm, and cross-effects between natural transmitter lateral inhibition and applied 5-HT, all exhibit these effects (Adolph, 1976).

The actions of 5-HT and 6-HT on the intracellularly recorded, light-evoked, eccentric cell response are illustrated in Fig. 2. The indoleamines were applied in a perfusion regime as 20 μM solutions in artificial sea water (ASW). Fig. 2 A is the response to a 3-s light flash shortly after a control perfusion with
ASW. It is typical of the usual eccentric cell response when the eye is partially dark-adapted. Resting membrane potential (ca. -50 mV) exhibits some noise reflecting spontaneous potential fluctuations of retinular cells electrically coupled to the second-order cell. Transient depolarization is followed by repolarization and then by a steady level of maintained depolarization of membrane potential during the light flash. Superimposed on the transient and steady components are spike action potentials firing at rates proportional to the transient peak and steady potential levels. After the light is turned off,
suggests that there was no long-lasting occupation of, or competition for, 5-HT receptor sites by the antecedent 6-HT.

A similar result of inaction by 6-HT compared to inhibition by 5-HT is depicted in Fig. 3. The indoleaminergic isomers were focally applied to the synaptic neuropil via twin iontophoretic electrodes rather than grossly perfused over all the eye structures. The initial 1-s pulse of 5-HT (70 nA) produced the usual transient membrane potential hyperpolarization and accompanying spike inhibition (IPSP). An equivalent quantity of 6-HT ejected from the twin pipette had no effect; a subsequent 5-HT pulse reproduced the IPSP. It is noteworthy that the charge quantity that was ejected (70 nC) carried only ca. 70 fmol (70 × 10⁻¹⁵ mol) of indoleamine per pulse (assuming a transport number ca. 0.1, as described in Adolph, 1976). A 5-HT concentration at the micromolar level is required in a perfusion regime to evoke comparable inhibitory effects.

5,6-DHT is an isomeric analog of 5-HT—that is, it has an hydroxyl at the 5 position on the indole and an additional hydroxyl at the 6 position. The pharmacological action of this dihydroxylated indole might be a clue to receptor specificity for the 5-hydroxy configuration. Would the receptor accept only a pristine 5-hydroxyindole structure, or would such a structure encumbered by a neighboring hydroxyl group still fit? From the results of experiments of the type illustrated by Fig. 4, the answer to the second part of the question is no. In the experiment shown, 5-HT and 5,6-DHT were ejected from twin iontophoretic micropipettes with their tips in the synaptic neuropil proximal to the ommatidium impaled by the recording electrode. An initial 1-s pulse of 5,6-DHT had no effect on continuous light-evoked spike firing. Then a 1-s pulse of 5-HT produced a short-latency IPSP and complete spike suppression. These effects, which began well before the iontophoretic pulse concluded, suggest that the tips of the iontophoretic pipettes were quite closely apposed to a synaptic receptor region. The inhibition ended after ~10 s, a relatively short recovery time, due to the small, albeit potent, quantity of 5-HT ejected (25 fmol). Then 5,6-DHT was reapplied, again with no effect. Other experiments, not reported here, in which alternating sequences of 5,6-
DHT and 5-HT were applied by a perfusion regime, also showed that 5,6-DHT is ineffective.

Tryptamine (TRYP) differs from 5-HT by having no hydroxyl group attached to the indole nucleus. Comparison of the pharmacological effects of the two analogs should give some indication of whether the hydroxyl group is necessary. All the hydroxylated indole analogs that we tested except the 5 hydroxyl, i.e., 5-HT itself, are inactive. Fig. 5 depicts the results of the application of TRYP, 5-HT, and NaCl (as control for current effects) to the region of synaptic neuropil via a triple-barrel iontophoretic electrode. The same iontophoresis current intensity (58 nA) was used during the 10-s current pulses. Transport numbers of TRYP and 5-HT are probably of the same order of magnitude (Haigler and Aghajanian, 1977), so that the applied quantity of the two substances is comparable. TRYP had no effect nor did a control current flow from the NaCl barrel. 5-HT produced short-latency, transient membrane hyperpolarization of ca. 9-10 mV, and spike inhibition. 5,6-DHT is ineffective. 1-s time marks.

The effect of side-chain structure on inhibitory response was of interest in light of the extreme specificity of the receptor to the indole component. 5-Hydroxytryptophan (5-HTP), the naturally occurring precursor of 5-HT,
differs from the latter only in having a carboxyl group at C(11) of the side chain. The critical indole components of both molecules are identical. The carboxyl group on the 5-HTP side chain reduces its agonist potency; this effect is illustrated in Fig. 6. Perfusion of an eye with 100 μM 5-HTP resulted

**Figure 5.** Responses to iontophoretic applications of TRYP, 5-HT, and NaCl (control), from triple-barrel electrode. Spike firing in response to constant, dim illumination of ommatidium. (Upper panel) Action of TRYP, NaCl current control, and 5-HT pulses onto synaptic neuropil proximal to ommatidium. All pulses 10 s by 58 nA. TRYP is ineffective both directly and via cross-effects with 5-HT. The actions are not contaminated by current-induced artifacts, shown by NaCl control. (Lower panel) Dose-response series evoked by pulses through 5-HT barrel. All pulses were 10 s. The 58 nA response to 5-HT can be considered as a fourth response in the series. Pulses were close enough in time that there may have been some desensitization effect.

**Figure 6.** Eccentric cell firing rate changes in response to perfusions by 5-HTP and 5-HT. Intracellular recording from eccentric cell during steady, dim illumination of ommatidium. A 100 μM 5-HTP perfusion reduced firing rate to half that of the control, whereas a subsequent 1 μM 5-HT perfusion completely inhibited spike firing for a prolonged period.
in a 50% peak reduction in firing rate. After an ASW flush, the eye was perfused with 1 μM 5-HT. Spike activity was completely abolished; spontaneous recovery did not begin even after 15 min. Subsequent recovery was then initiated by an ASW flush. Thus, side-chain structure does have some significance in the specificity and intensity of the inhibitory response. Replacing a C(11) hydrogen by a carboxyl decreased the inhibitory effect by at least 200 times (concentration difference of 100 times and peak effect difference of 2 times). However, the 5-HT effect was a saturating one and not strictly comparable to the 5-HTP effect. Based on other experimental assessments of 5-HT activity reported in this paper and in earlier ones, there may be a 20-times effect difference between equal concentrations of applied 5-HT and 5-HTP. If that is the case, then in this experiment the overall difference in inhibitory action between 5-HT and 5-HTP would be 2,000 times.

Several aspects of LSD action on lateral eye function are seen in Fig. 7. In that experiment, the concentration of applied LSD was 6 μM and that of 5-

![Figure 7. LSD blockade of light-evoked lateral inhibition. Firing rates of extracellularly recorded spike action potentials. (○) Control spike response of optically isolated ommatidium to light stimulus in the absence of lateral inhibition. (●) Inhibitory decrement/control, i.e., inhibitory constant. Inhibitory decrement is the difference between control spike response and the response during activation of lateral inhibition by concurrent illumination of ommatidia neighboring the test unit. Any direct pharmacological actions affect the neighbors as well as the test unit; thus, dividing the decrement by control response compensates for direct effects. For example, direct reduction of an inhibitory unit's firing rate would result in less inhibition on the test unit even though the inhibitory stimulus light intensity was unchanged. (Adapted from Adolph [1976].)
HT was 7.25 μM. The pH of the LSD solution was adjusted to ca. 7.5 with NaOH. In related experiments using LSD and BOL, the range of concentrations of LSD tested was 0.5-20 μM; BOL concentration was 20 μM. One aspect of LSD action is its bimodality, comprising direct inhibition and 5-HT action blockade, evidenced by the control response following LSD perfusion and the subsequent 5-HT perfusion. Control response measures spike firing rate of a single ommatidium, stimulated by a periodically repeated test light flash applied only to that ommatidium. Optical isolation of the test ommatidium is produced by using a fiber optic light guide directly applied to the cornea of the eye segment. Under such conditions there is very little, if any, light-stimulated lateral inhibition. Shortly after the LSD perfusion, control firing rate drops rapidly and then begins a slow rise back toward its pre-LSD level. In contrast to this direct inhibition of spike firing by the LSD, a subsequent perfusion by 5-HT, even though preceded by an ASW flush, has no pharmacological effect on the control response, i.e., the usual inhibitory action of 5-HT has been blocked.

Another aspect of LSD action shown in this experiment is its blockade of lateral inhibition with an onset time-course concurrent with its 5-HT blockade, but with a prolonged action that terminates only after the ASW flush. Lateral inhibition is shown by the solid line. As previously reported (Adolph, 1976, p. 425 and Fig. 8), the actions of LSD are proportional to concentration. LSD thus concurrently blocks both 5-HT-activated inhibition and natural transmitter-mediated lateral inhibition. BOL (20 μM) was found to have similar effects on lateral eye function, although with less potency than LSD at that concentration. However, BOL undergoes some decomposition in the presence of traces of free halogens, such as chlorine or bromine, and because the perfusion solutions were made up in ASW, the BOL solutions may have been somewhat degraded, so that comparisons of effectiveness and potency with LSD solutions are not rigorous.

The results of experiments reported here and in earlier papers (Adolph and Ehinger, 1975; Adolph, 1976) and previously unreported findings are summarized in Table I. The three 5-hydroxylated indoleamines, 5-HT, 5-HDMT, and 5-HTP, and the lysergic acid derivatives, LSD and BOL, had inhibitory actions. 5-HT was most potent of the three analogic agonists, 5-HDMT was next in potency, and 5-HTP was least active. LSD and BOL both acted in a distinct manner, exhibiting a mixed effect consisting of an initial agonistic inhibition followed by antagonistic blockage of either exogenous 5-HT-induced inhibition or natural-transmitter-evoked inhibition. Three other analogs or isomers of 5-HT—namely TRYP, 6-HT, and 5,6-DHT—were found to be inactive in producing inhibition, nor did they compete with the active agonist 5-HT for putative receptor sites. Cinanserin, a substance that has been found to have serotoninergic antagonism in some invertebrate systems (Rubin et al., 1964), is inactive in Limulus lateral eye.

**DISCUSSION**

Our findings indicate that the inhibitory action of the indoleaminergic agonists is highly structure-specific. An hydroxyl group in the 5 position of the indole
### Table I

**Structure-Activity Relationships Among the Various Agonists, Antagonists, and Inactive Substances**

| Substance                                      | R₅ R₆ R₁₁ R₁₂ | Notes                                      |
|------------------------------------------------|----------------|--------------------------------------------|
| 5-Hydroxytryptamine (5-HT, serotonin)         | OH H₂ H₃      | Strongest inhibition                       |
| 5-Hydroxy-dimethyl-tryptamine (5-HDMT, bufotenine) | OH H₂ (CH₃)₂  | Moderate inhibition                        |
| 5-Hydroxytryptophan (5-HTP)                   | OH H COOH H₃  | Weaker inhibition                          |
| Lysergic acid diethylamide (LSD, lysergide, delysid) | H H            | Part of mixed activity, direct inhibition and 5-HT structure block (see drawing) |
| Bromolysergic acid diethylamide (bromo-LSD; Bol-148) | H H (ditto)   | (ditto) Bromine attached at C(2) position, slightly less mixed activity than LSD. |
| Tryptamine (TRYP)                             | H H H₃        | No inhibition; Does not compete with 5-HT for receptors. |
| 5,6-Dihydroxytryptamine (5,6-DHT)             | H OH H₃       | (ditto)                                    |
| Dimethylamino-propylthio-cinnamamide (CINANSERIN; SQ10643) | OH OH H₃      | (ditto)                                    |

**Diagram**

![Diagram of molecular structures](image)
nucleus, sterically unencumbered by hydroxyls in neighboring positions, is essential. Thus, 5-HT, 5-HDMT, and 5-HTP are active in varying degree, whereas TRYP, 6-HT, and 5,6-DHT are inactive. The active indoleamines appear to occupy receptor sites as well as to induce an inhibitory response, since they elicit desensitization to subsequent indoleaminergic applications in addition to cross-desensitizing the inhibition evoked by the natural inhibitory transmitter (Adolph, 1976). LSD and BOL also have a direct-effect inhibitory action as well as blocking actions of the agonists and natural inhibitory transmitter (Adolph, 1976). The direct inhibitory action of the lysergic acid derivatives is unlikely to occur via the same receptor sites occupied by the agonists during their actions since LSD and BOL have unhydroxylated, tryptamine-like nuclei as their indoleaminergic backbone, and tryptamine was found to be ineffective in binding to the inhibitory receptor sites (no cross-desensitization property) nor did it evoke inhibition. Some speculations on the mechanism of LSD/BOL actions will be given later in this Discussion.

The degree of inhibitory effect of the three active agonists appears to be correlated with side-chain configuration and may indeed be dependent upon it. In Fig. 8, identically oriented drawings of space-filling models of 5-HT, 5-HDMT, and 5-HTP illustrate the structural differences exhibited by their C₈-N₂ side chains. The “interaction space” can be thought of as related to the space-filling volume of the side-chain. The ratio of these volumes for 5-HT:5-HDMT:5-HTP is 1:1.64:1.74. In the pharmacological experiments, the agonists were active in the same order: 5-HT produced strongest inhibition, 5-
HDMT moderate inhibition, and 5-HTP weaker inhibition, for a given agonist concentration. Side-chain configuration may modulate agonist pharmacological potency by causing varying degrees of steric hindrance to the binding of the 5-hydroxylated indole nucleus to the primary receptor site. The smaller the "interaction space" of the side chain, the less probability there is for steric hindrance in the indole-receptor binding interaction. A secondary binding site may be involved in agonist-receptor interactions. Such a secondary site might be on the same receptor molecule that interacts, in a highly specific manner, with the 5-hydroxylated indole locus. The agonist side chain-receptor molecule interaction is strikingly less specific and may exhibit allosteric character involving the primary, indole nucleus. Alternatively, the secondary site might reside in a distinct secondary receptor molecule. There is some isomorphism between the C3-N2 side chain of 5-HT and the corresponding component of the double ring structure in LSD/BOL (Wesemann, 1974), as can be seen in the structural drawings in Table I. However, the orientation of that component is restricted by its being part of the double ring structure. So also is its accessibility for interaction with a putative side-chain receptor. The 5-HT side chain, in contrast, is highly mobile. Kelley and Adamson (1973) calculated that the range of pyrrole to side-chain inter-nitrogen distances is restricted to 6.00-6.04 Å for LSD; the range is 3.80-6.04 Å for 5-HT and 5-HDMT. This distinction based on side chains is another, albeit weak, argument against LSD/BOL interacting with the agonist receptor.

The receptors for agonist- and natural transmitter-evoked inhibition and those for reuptake appear to be different. In the work reported by Adolph and Ehinger (1975), the low specificity of the uptake mechanism was qualitatively demonstrated by the uptake and fluorescence-enhancing properties of 5,6-DHT and 6-HT, as well as 5-HT, in formaldehyde histofluorescence studies. There was marked increase in fluorescent processes, especially "beaded" axons and varicosities associated with presynaptic terminals, in addition to some general enhancement of cell body fluorescence. The pharmacological results of the present study indicate that 5,6-DHT, 6-HT, and TRYP do not interact with the receptor involved in the inhibition since they neither act as inhibitory agonists nor occupy receptor sites to produce cross-desensitization of 5-HT and natural transmitter action.

LSD and BOL exert a bimodal action: an initial inhibition followed by blocking of 5-HT and natural transmitter-evoked inhibition. In view of the strict structural specificity found for the 5-HT receptor, it is unlikely that the lysergic acid derivatives act via these receptors. LSD and BOL have nonhydroxylated, indoleamine constituents analogous to tryptamine; the latter does not interact with the inhibitory receptor. One alternative hypothesis for LSD action is that there is a specific postsynaptic receptor for LSD which is coupled to an inhibitory response in addition to blocking the specific 5-HT receptor. This latter effect might be through some allosteric, structural masking or occupation of part of the 5-HT receptor, or through involvement with some stage in the receptor to ionic permeability change transduction process. It seems that the postsynaptic LSD receptor interactions would be strongly
dependent on the double-ring side chain rather than on the indole component, since the analogous TRYP was completely ineffective in such interactions.

A more attractive hypothesis for LSD action in the present system is based on its interaction with the presynaptic uptake receptor and modulation of its action in storage and inactivation of 5-HT. Fig. 9 shows hypothetical modes and sites of action of various indoleamines. 5-HT released from the presynaptic ending interacts in a highly structure-specific manner with the postsynaptic receptor. The inhibition is primarily due to membrane hyperpolarization resulting from increased potassium ion permeability and net outward potassium ion flow in response to the receptor activation (Adolph, 1976). The agonist action is terminated by reuptake into the presynaptic region and is mediated by the relatively nonspecific receptor coupled to the reuptake mechanism. LSD and BOL can be structurally recognized by the reuptake receptor, most likely based on the indoleamine constituent. Perhaps because of the relatively large, mobility-restricted, double-ring side chain of LSD, the molecule is not readily transported into the cell and persists in occupying the reuptake receptor sites. The result of such a blockage could be threefold (a) initial restriction of 5-HT reuptake and therefore increased amounts capable

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**Figure 9.** Hypothetical inhibitory synapse illustrating mechanisms of putative 5-HT transmitter release and inactivation/storage reuptake, high-specificity postsynaptic receptor, and nonspecific presynaptic receptor for reuptake in blockade by LSD. The active agonists 5-HT, 5-HDMT, and 5-HTP are indicated in order of potency on the inhibitory response, presumably mediated by the postsynaptic receptor. The ionic expression of the transmitter postsynaptic receptor interaction is an increase in potassium permeability and resultant membrane hyperpolarization.
of interacting with postsynaptic receptors to produce an inhibitory response; (b) increased concentration of 5-HT in the synaptic region due to the restricted reuptake process and, after the initial inhibitory response, a prolonged occupation of postsynaptic 5-HT receptors, and desensitization of the transmitter-receptor to inhibitory response transduction process, i.e., functional "block" of the response; and (c) ultimate reduction of the presynaptic pool of 5-HT available for release as transmitter, resulting in less 5-HT release and subsequent inhibitory action at postsynaptic loci (i.e., blockade). Aspect (b) might be considered blockade via modulation of postsynaptic processes, whereas aspect (c) involves blockade resulting from modulation of presynaptic processes. There would most likely be differences in the occurrence of the two blockade mechanisms, with (b) preceding (c) in time. An alternative presynaptic locus for LSD interaction could be at LSD-specific presynaptic receptors, although such a mechanism does not seem to be the most economical one since the response to the LSD-receptor interaction would of necessity be bimodal—that is, an initial increase of 5-HT release followed by a decrease in release.

Haigler and Aghajanian (1977) proposed that LSD exerts its serotoninergic antagonism at loci on rat raphe neurons that are presynaptic to neurons postsynaptically responsive to 5-HT and located in the amygdala and lateral geniculate. Their mechanism is based on direct action of LSD and other indoleamines on the presynaptic receptor to produce one component of the bimodal action; less efficient activation of postsynaptic 5-HT receptors produces the second component.

There are two important distinctions between the mechanism of serotoninergic antagonism by LSD proposed in this paper and that of Haigler and Aghajanian: (a) our work concerns peripheral serotoninergic synapsis in an invertebrate, whereas theirs treats the mammalian (rat) CNS; (b) our work hypothesizes a modulatory role of LSD on ongoing 5-HT synapsis with the bimodal pattern of action a consequence of that modulation; theirs proposes direct LSD interaction with pre- and postsynaptic receptors of varied specificity toward agonists and antagonists.

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