

# Presynaptic Uptake Blockade Hypothesis for LSD Action at the Lateral Inhibitory Synapse in *Limulus*

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**ABSTRACT** We investigated the action of LSD at the putative indoleam-nergic lateral inhibitory synapse in the lateral eye of *Limulus polyphemus*. We recorded extracellular and intracellular voltage responses from eccentric cells while producing inhibition either by light or by antidromic stimulation of the optic nerve in the presence of LSD, serotonin (5-HT), chlorimipramine, or a bathing medium whose high  $Mg^{++}$  and low  $Ca^{++}$  concentrations partially or completely blocked synaptic transmission. We found (a) light-evoked and antidromically stimulated lateral inhibition is enhanced during superfusion of low (1–5  $\mu M$ ) concentrations of LSD and suppressed by higher (5–20  $\mu M$ ) concentrations; (b) these actions of LSD are markedly reduced by bathing the retina in a medium high in  $Mg^{++}$  and low in  $Ca^{++}$ ; (c) very low concentrations of chlorimipramine, a putative uptake blocker of serotonin, appear to mimic actions of LSD both on eccentric cell firing rate and on lateral inhibition; (d) superfused 5-HT depresses lateral inhibition at all superthreshold concentrations (0.1–25  $\mu M$ ). These results suggest that LSD's action may require an intact inhibitory transmitter release and postsynaptic response mechanism, whereas serotonin exerts a direct postsynaptic effect. We propose that LSD blocks presynaptic uptake of transmitter at the lateral inhibitory synapse. The concentration dependence of LSD's action can be accounted for as follows: low concentrations partially restrict transmitter reuptake, thereby prolonging the lifetime of the transmitter in the synaptic cleft and thus increasing the magnitude and duration of postsynaptic inhibition. Higher concentrations cause more presynaptic uptake sites to be blocked; this causes accumulation of transmitter in the synaptic cleft, which causes a functional blockade of the synapse because of postsynaptic desensitization. As an alternative, we propose a hypothesis based on LSD action at presynaptic autoreceptors. Similar hypotheses can account for many aspects of LSD's action in mammalian brain.

## INTRODUCTION

Studies of the compound lateral eye of the horseshoe crab, *Limulus polyphemus*, have yielded many basic principles of visual physiology and

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neural integrative action (Ratliff and Hartline, 1974). Behind each of this eye's facets lies an ommatidium containing about a dozen receptor cells electrically coupled to a spike-generating eccentric cell. Eccentric cells of neighboring ommatidia form inhibitory synapses with each other. Serotonin (5-hydroxytryptamine [5-HT]) is the favored candidate for the lateral inhibitory transmitter. 5-HT depresses the eccentric cell firing rate when it is administered via superfusion (Behrens and Wulff, 1970; Adolph and Tuan, 1972) or iontophoresis (Adolph, 1976; Adolph and Kass, 1979). This 5-HT inhibition is specific for the structure of 5-HT; any modification of the hydroxyl group in the 5 position of the indole nucleus or of nearby structures reduces its effectiveness (Adolph and Kass, 1979). There is some neurochemical and histochemical evidence for the presence of an indoleamine in the *Limulus* retina (Adolph and Tuan, 1972; Adolph and Ehinger, 1975), although Battelle (1980) was unable to find evidence for 5-HT synthesis.

While exploring the possible role of 5-HT as the lateral inhibitory transmitter in *Limulus*, Adolph (1976) used high concentrations of *d*-lysergic acid diethylamide (LSD) to suppress both light-evoked and 5-HT-induced inhibition. The action of LSD at a putative 5-HT synapse appeared to support the hypothesis that LSD blocks the postsynaptic receptor to the 5-HT transmitter, as is presumed to occur in the mammalian brain (Aghajanian, 1972). However, the structure of the LSD molecule is inappropriate for the structural specificity inferred for the postsynaptic receptor in *Limulus* (Adolph and Kass, 1979). The presynaptic uptake mechanism (Adolph and Ehinger, 1975) is less specific than the postsynaptic inhibitory mechanism, and thus may allow the uptake receptor to accommodate the LSD molecule (Adolph and Kass, 1979). On the basis of these last two findings, Adolph and Kass suggested that in *Limulus*, LSD may suppress both light-evoked and 5-HT-induced inhibition by blocking presynaptic uptake of the transmitter. This blockade might cause the accumulation of transmitter in the synaptic cleft, leading to depression of the firing rate of eccentric cells and desensitization of the postsynaptic receptor to further synaptically released inhibitory transmitter (Kass et al., 1979, 1980; Kass, 1981). In this study we provide additional experiments whose results support a hypothesis for LSD action in *Limulus* lateral eye based on a presynaptic mechanism. Caution must be used in extending, to a vertebrate, interpretations of findings obtained in an invertebrate; however, it appears that a similar presynaptic mechanism could account for several of the actions of LSD that have been reported for mammalian brain (Kass, 1981).

## MATERIALS AND METHODS

### *Animals and Preparation*

Adult *Limulus polyphemus* were maintained in an artificial seawater (ASW) aquarium kept at 18°C with a pH of 7–8; they were fed periodically. A lateral eye was

excised along with ~5 cm of optic nerve. The superior edge of the eye just above the optic nerve was sliced with a razor blade. The sliced eye was sealed into the two-compartment perfusion chamber with petroleum jelly, so that the eye formed the front of the front compartment. After the recording chamber was placed in a light-proof and electrically shielded recording cage, the optic nerve was pulled through the 1-mm hole that separated the front from the back chamber. One or two small pieces of cotton were carefully wedged between the hole and the optic nerve to eliminate flow between the two chambers. No mixing between chambers was observed when a dye was placed in either the front or the back chamber and allowed to stand overnight ( $N =$  five experiments).

#### *Optical Arrangement*

A small (76  $\mu\text{m}$ ) and a large (1.5 mm) light spot were transmitted directly to the corneal surface of the eye by a steel-clad individual glass fiber and a light-pipe (American Optical Corp., Bedford, MA), respectively. Barlow (1969) showed that proper positioning of the 76- $\mu\text{m}$  glass fiber could effectively (optically) isolate a single ommatidium and eccentric cell. The sources of light were glow modulators (R1131C; Sylvania/GTE, Warren, PA), which could be controlled manually or by a computer, and whose mean light intensity was controlled by duty-cycle modulation of a 1-kHz train of light pulses (circuit designed by T. G. Uter; produced by Soltronic, Inc., Solana Beach, CA). Neutral density filters (Eastman Kodak Co., Rochester, NY) were used to attenuate the beams.

#### *Electrophysiological Stimulation and Recording*

The eye could also be stimulated by (a) intracellular injection of current, (b) extracellular iontophoresis of drugs, (c) sending antidromic impulses to the retina by shocking the optic nerve, or (d) perfusing modified solutions of ASW into the chamber. The intracellular micropipette electrode was omega-dot glass filled with 3 M  $\text{K}_2\text{SO}_4$  or KCl. The iontophoretic micropipette electrode was omega-dot glass filled with a nearly saturated solution of the drug dissolved in distilled water. The optic nerve-stimulating electrodes were thin platinum-iridium wire hooks connected to a stimulus isolation unit. Shocking pulses were 0.1–1 ms long and the voltage amplitudes were set to the level that gave the maximum size of antidromic compound action potential. Such stimulation causes retrograde propagation of an action potential to the eye. There the impulses invade collaterals of the lateral plexus and cause inhibition, which is assumed to have the same synaptic origin as light-evoked inhibition (Purple and Dodge, 1965). The perfusion system consisted of a double peristaltic pump, which fed in the perfusion fluid at the same rate it was withdrawn. This maintained the meniscus covering the eye slice at a constant height and added to the recording stability.

We recorded from single isolated eccentric cells near the top edge of the eye slice with an intracellular micropipette electrode or from an optic nerve fiber with an extracellular suction electrode, using conventional amplification. The intracellular electrode had 10–80  $\text{M}\Omega$  resistance; the suction electrode was a broken micropipette filled with ASW.

#### *Bathing Solutions and Pharmacology*

The ASW superfused into the chamber was prepared from the following component reagents: 480 mM NaCl, 10 mM KCl, 10 mM  $\text{CaCl}_2$ , 30 mM  $\text{MgSO}_4$ , 20 mM  $\text{MgCl}_2$ , 10 mM HEPES. All drugs were added directly to normal ASW

except in the preparation of higher concentrations of LSD. For concentrations of LSD  $>5 \mu\text{M}$ , an equivalent amount of water was evaporated from normal ASW equal to the volume of  $112 \mu\text{M}$  LSD added. The pH of all solutions was adjusted to 7.5 (pK of HEPES). The experiments were conducted at room temperature.

All drugs were obtained from Sigma Chemical Co., St. Louis, MO, except for LSD, which was obtained from the U. S. Food and Drug Administration/Drug Enforcement Agency (DEA). No differences in actions were observed between drugs freshly prepared during an experiment and those kept frozen and thawed when needed. 5-HT, whose potency noticeably decreased after several weeks in a refrigerated container, was the least stable of the drugs used.

## RESULTS

### *LSD and 5-HT Depress the Firing Rates of Optically Isolated Eccentric Cells*

Both LSD and 5-HT cause a decrease in the firing rates of optically isolated eccentric cells. Fig. 1 shows data from eccentric cells that were spiking at steady state rates under constant illumination. When LSD or 5-HT were added to the perfusion medium, the spiking rates were substantially reduced. We term these phenomena "LSD inhibition" and "5-HT inhibition." Most eccentric cells require 4 min of ASW superfusion to recover completely from the effect of 5-HT ( $N = 10$ ; see Figs. 1 and 3). Complete recovery from LSD requires a longer time; the control spiking rate may not be fully recovered even after 30 min of ASW superfusion ( $N = 6$ ; see Fig. 4).

Several lines of evidence indicate that the inhibition is not caused by reducing the light response of the cell. 5-HT- and LSD-induced inhibition could be evoked in the dark, if eccentric cells were depolarized and firing steadily because of injury or current injection. Furthermore, the light-to-spike transfer functions of single eccentric cells, measured during a sinusoidally modulated stimulus, do not change their amplitude or shape in the presence of LSD (Kass, 1981; to be reported fully in a subsequent paper) or 5-HT, which suggests that the drugs do not affect the light-transducing or spike-encoding mechanisms.

### *Loss of LSD Inhibition When Transmitter Release Is Prevented*

The inhibition of steady state firing by LSD is markedly reduced when the drug is added to a bathing medium high (60 mM) in  $\text{Mg}^{++}$  and low (0.1 mM) in  $\text{Ca}^{++}$  (high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW), conditions that are assumed to prevent or reduce transmitter release and which were verified in most cases to drastically reduce normal synaptic inhibition; 5-HT inhibition is not diminished under these same conditions. Fig. 2A shows intracellular recordings from an eccentric cell whose spiking was inhibited by antidromic stimulation of the optic nerve. Note that the inhibition was abolished after the eye slice was bathed in high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW for 10 min. Before, during, and after most superfusions, we monitored lateral

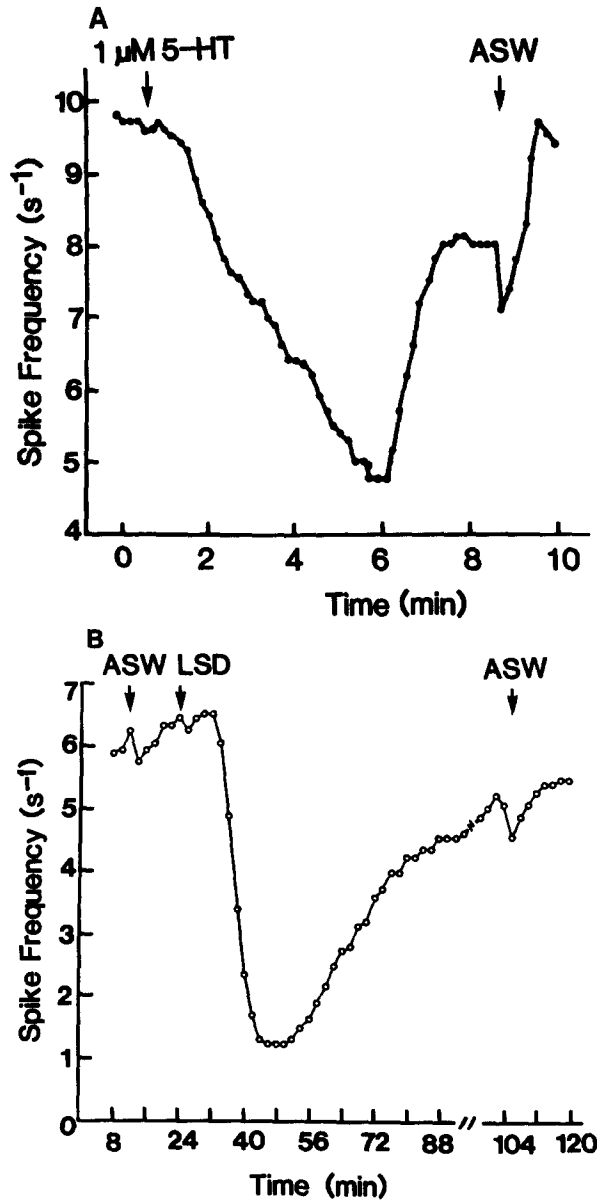


FIGURE 1. 5-HT and LSD effects on eccentric cell spike frequency. (A) 1  $\mu$ M 5-HT superfused over the retinal slice causes a decrease of the ongoing spike frequency (evoked by constant illumination). This depression of frequency reverses within a few minutes after an artificial seawater (ASW) wash. Note the substantial degree of "desensitization" to 5-HT that occurred before the ASW wash. (B) 6  $\mu$ M LSD superfused over the retinal slice causes a decrease of the extracellular spike frequency (constant illumination). This depression of frequency reverses after many minutes and several ASW washes. Full recovery is not shown here. (Simplified from Adolph and Kass, 1979.)

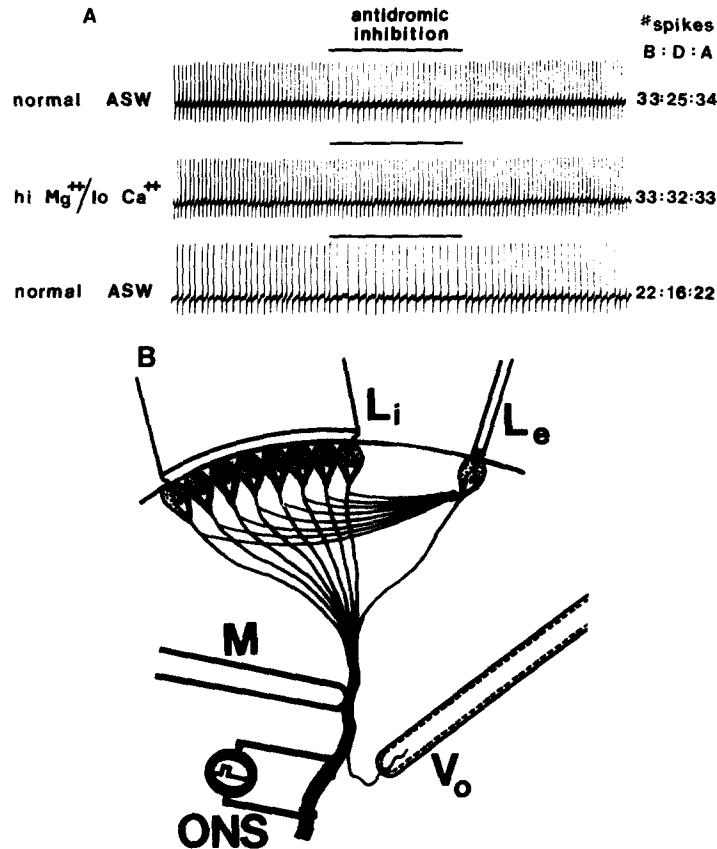


FIGURE 2. Loss of antidromic inhibition in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW. (A) The optic nerve was stimulated at 25 impulses/s for 10 s (indicated by bar), evoking a decrement in the ongoing spike activity (top trace). 10 min after the normal ASW was replaced by high  $Mg^{++}$ /low  $Ca^{++}$  ASW, the inhibition was markedly reduced (middle trace). 10 min after normal ASW was restored, inhibition reappeared (lower trace). The number of spikes per 10 s before (B), during (D), and after (A) antidromic inhibition are indicated to the right (B:D:A). Experiment 18F80. (B) Schematic diagram of lateral synaptic connections in the *Limulus* retina, illustrating stimulus and recording setup. Under constant illumination ( $L_e$ ), a suction electrode ( $V_o$ ) records optic nerve impulses from a single eccentric cell near the surface. Lateral inhibition is evoked by illumination ( $L_i$ ) of neighboring group of  $\sim 50$  ommatidia, or by stimulating the optic nerve (ONS). The compound antidromic action potential was monitored by a second suction electrode (M).

inhibition evoked by (a) illumination of facets neighboring the one whose eccentric cell activity is recorded, and (b) antidromic stimulation of a large bundle of optic nerve fibers (excluding the recorded fiber). Fig. 2B depicts the optical and electrical arrangement used for both of the experimental

methods for obtaining lateral inhibition. Lateral inhibition largely disappears within 10 min after normal ASW is replaced by high  $Mg^{++}$ /low  $Ca^{++}$  ASW ( $N = 6$ ). Similarly, it took  $\sim 10$  min in the experiment of Fig. 2A for lateral inhibition to return after the chamber was again filled with normal ASW ( $N = 4$ ). In all experiments ( $N = 12$ ), we allowed at least 20 min to elapse after high  $Mg^{++}$ /low  $Ca^{++}$  ASW substitution before testing the effects of LSD or 5-HT superfusion.

The 5-HT-induced inhibition of eccentric cell spiking is largely unchanged in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW ( $N = 2$ ), as is shown in Fig. 3. This result is expected if 5-HT is the lateral inhibitory trans-

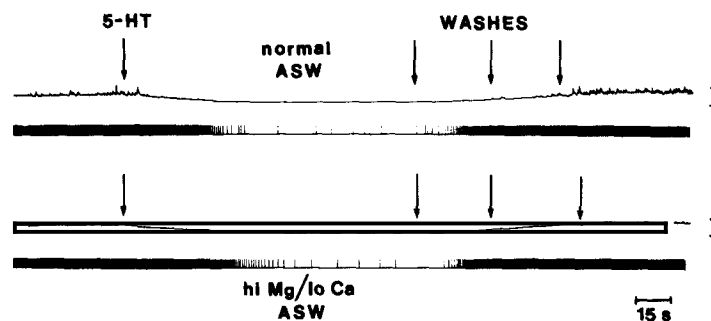


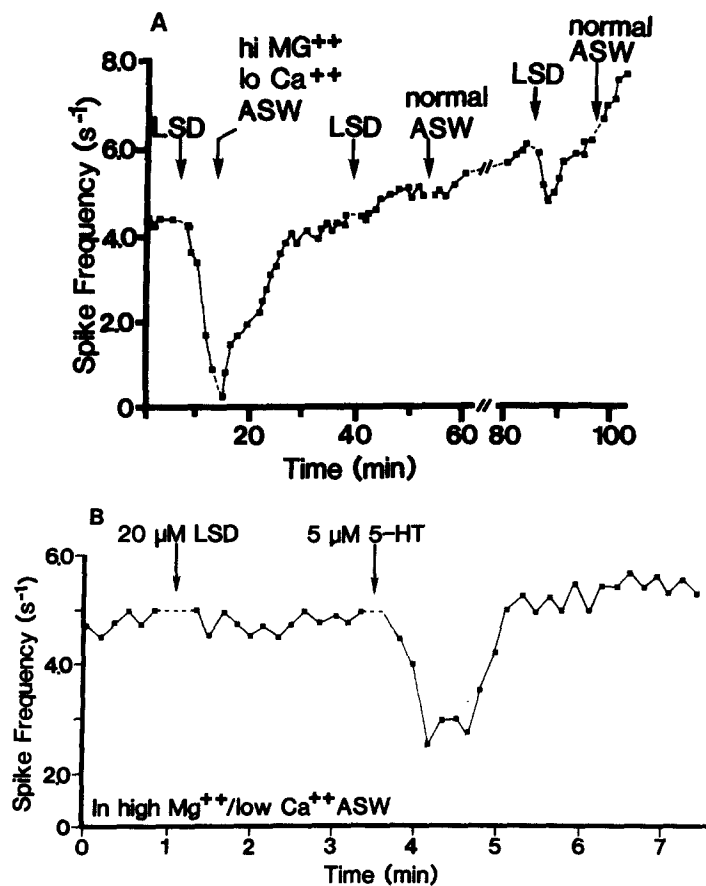
FIGURE 3. 5-HT-induced inhibition of eccentric cell spiking in high  $Mg^{++}$ /low  $Ca^{++}$  ASW. The output of the spike discriminator (bottom trace of each pair) was sent to a frequency-to-voltage converter whose output is shown in top trace; calibration bars indicate a constant 8 spike/s firing frequency. Top pair of records: in normal ASW (control), 5  $\mu M$  5-HT superfusion (arrow) causes the following depression of eccentric cell firing rate. The eye slice was rinsed three times (open arrows) with ASW high in  $Mg^{++}$  and low in  $Ca^{++}$ . Bottom record: after 20 min of 5  $\mu M$  5-HT superfusion in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW. The magnitude and reversibility of 5-HT inhibition in high  $Mg^{++}$ /low  $Ca^{++}$  ASW were similar to the controls. This indicates that 5-HT does not require normal synaptic release mechanism to be functioning; thus, its action is probably exerted directly on postsynaptic sites. Experiment 25A80.

mitter and only exerts direct action on the postsynaptic receptor. If LSD only acts postsynaptically, then its effect also should not be diminished in high  $Mg^{++}$ /low  $Ca^{++}$  ASW. However, LSD inhibition is markedly reduced under these conditions, as is shown in Fig. 4A ( $N = 2$ ). After superfusion of a bathing medium high in  $Mg^{++}$  and low in  $Ca^{++}$ , there was no depression of firing when LSD was applied. But in controls (normal ASW) before and after high  $Mg^{++}$ /low  $Ca^{++}$ , the eccentric cell spiking rate was depressed by 20  $\mu M$  LSD.

Subsequent LSD superfusions generally depress eccentric cell spiking rate less than the original application. This is indicated in Fig. 4A by the failure of the third LSD application (in normal ASW control) to depress

the spiking rate as much as the initial (control) application. The difficulty in restoring control conditions by washes after LSD applications necessitated using a modified procedure (Fig. 4B) to verify the experimental result above. In this experiment and others like it ( $N = 8$ ), we applied  $20 \mu\text{M}$  LSD only after the eye had been bathed in high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW for at least 30 min. This was done to maximize the potential for direct LSD action upon postsynaptic receptor. As shown in Fig. 4B, LSD inhibition was absent. Soon after LSD superfusion, 5-HT was applied to test whether the postsynaptic receptor was accessible to the superfusate. The consequent 5-HT inhibition of Fig. 4B demonstrates that the receptor was accessible to the superfusate, and hence to the prior application of LSD.

One-third of the latter experiments showed little if any inhibition from LSD superfusion, but did show strong 5-HT inhibition in the presence of high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW. Another third of the experiments showed an LSD-induced inhibition in the presence of high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW that was markedly smaller than the LSD-induced inhibition typically seen





with a normal ASW bath. Both of these classes of results support the idea that LSD-induced inhibition does not primarily occur through direct action on postsynaptic receptors, but rather via some indirect presynaptic mechanism. However, the remaining third of the experiments ( $N = 3$ ) showed LSD-induced inhibition in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW whose magnitude was in the range of LSD-induced inhibition typically seen in the normal eye (normal ASW superfusate). We cannot conclusively interpret these three latter experiments because (a) no control values for the magnitude of LSD inhibition (in normal ASW) were obtained and, therefore, no true comparison between the two conditions can be made, and (b) no measurement of the extent of synaptic blockade in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW could be made.

Thus, 5-HT inhibition of eccentric cell spiking is unchanged when high  $Mg^{++}$ /low  $Ca^{++}$  ASW is used to reduce transmitter release. In contrast, LSD inhibition is usually markedly reduced during these same conditions. Together, these results indicate that little or none of LSD's inhibiting effect is attributable to a postsynaptic action of LSD.

#### *Effects of LSD and 5-HT on Lateral Inhibition*

The effect of LSD on lateral inhibition is concentration dependent. Lateral inhibition is enhanced at low (1–5  $\mu M$ ) and suppressed at higher (5–20  $\mu M$ ) LSD concentrations. This is true for lateral inhibition evoked either by onset of a steady light illuminating neighboring facets ( $N = 14$ ; Fig. 5) or by antidromic optic nerve stimulation ( $N = 6$ ; Fig. 6A).

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FIGURE 4. (opposite) (A) Loss of LSD inhibition because of high  $Mg^{++}$ /low  $Ca^{++}$  ASW. Superfusion with 20  $\mu M$  LSD in ASW causes strong depression of spike frequency (for both A and B, the frequency was averaged over 10 s for each point). After a rinse in high  $Mg^{++}$ /low  $Ca^{++}$  ASW, the spike frequency gradually increased toward the initial rate. At 40 min, superfusion was begun with 20  $\mu M$  LSD in high  $Mg^{++}$ /low  $Ca^{++}$  ASW; no reduction of spike frequency was evident. After rinsing in normal ASW and during recovery for about 30 min, the spike frequency continued to increase (55–85 min). 20  $\mu M$  LSD in normal ASW was superfused again, causing a moderate depression of spike frequency. The third application of LSD (at 85 min) did not depress spike frequency as much as the initial application, but LSD applied in high  $Mg^{++}$ /low  $Ca^{++}$  ASW was completely ineffective. The illumination on the isolated eccentric cell was decreased by a 0.4 log filter at  $t = 70$  min (not shown). (B) Loss of LSD inhibition in presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW. The eccentric cell was bathed in high  $Mg^{++}$ /low  $Ca^{++}$  ASW for 30 min before superfusion with 20  $\mu M$  LSD in high  $Mg^{++}$ /low  $Ca^{++}$  ASW (there was no LSD superfusion before  $t = 0$ ). LSD did not cause a depression of spike frequency, but subsequent superfusion with 5  $\mu M$  5-HT caused marked reduction. The 5-HT response demonstrates that the synapse was accessible to the superfusate. The failure of LSD to depress the spike frequency suggests that this action of LSD is dependent upon ongoing transmitter release. Experiment 25F8.

In contrast, 5-HT in the superfusate suppresses lateral inhibition ( $N = 12$ ), a phenomenon termed "desensitization" by Adolph (1976). Suppression occurred at all superthreshold concentrations (0.1 to  $+25 \mu\text{M}$ ) of 5-HT superfused onto the retina. Fig. 6B shows an example of 5-HT-induced suppression of antidromically stimulated inhibition.

Plots analogous to Fig. 6, but with the abscissa giving the frequency of firing in a single inhibiting optic nerve fiber, allow determination of inhibitory strength and threshold (Hartline and Ratliff, 1957); the slope of the line would be the inhibitory coefficient and the  $x$  intercept would be the inhibitory threshold. Where shock of the whole optic nerve generates inhibition from all (or nearly all) neighbors of the fiber from

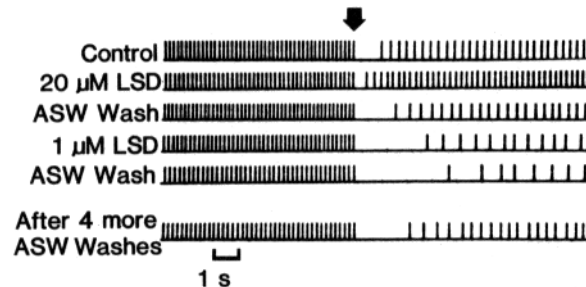


FIGURE 5. Concentration-dependent effects of LSD on lateral inhibition evoked by light. A steady light illuminated the facet whose eccentric cell's spikes were recorded. A shutter-controlled light was positioned over a neighboring group of ommatidia. The arrow indicates onset of lateral inhibition under each of the conditions stated on the left. Both transient and steady components of lateral inhibition were reduced after superfusion with  $20 \mu\text{M}$  LSD, and enhanced (compared with control) after washes and superfusion with  $1 \mu\text{M}$  LSD. The increased inhibitory strength during ASW wash immediately after  $1 \mu\text{M}$  LSD application suggests that enhancement of lateral inhibition in this experiment was maximal at an LSD concentration below  $1 \mu\text{M}$ . Five washes were insufficient to completely remove LSD and restore control conditions. Records are the output pulses from spike discriminator.

which impulses are recorded, the slope of the line well above all threshold frequencies is the sum of all inhibitory coefficients for the recorded fiber and the  $x$  intercept is a weighted average of inhibitory thresholds (Lange et al., 1966). From Fig. 6 it appears that low concentrations of LSD caused a decrease of the weighted average threshold, but did not greatly change the total strength (sum of inhibitory coefficients). In contrast, the high concentration of LSD and exogenous 5-HT both caused an alteration in the slope (and possibly the linearity) of the curve. Alteration of the slope or the form of the curve could be caused by uniform effects on each individual eccentric cell that affects the recorded optic nerve fiber, or by altering the population distribution of inhibitory thresholds and inhibitory coefficients. The similarity between the effects of 5-HT and

high concentrations of LSD may indicate that both effects have a common mechanism.

*Effects of Chlorimipramine on Eccentric Cell Spiking Rate and Lateral Inhibition*

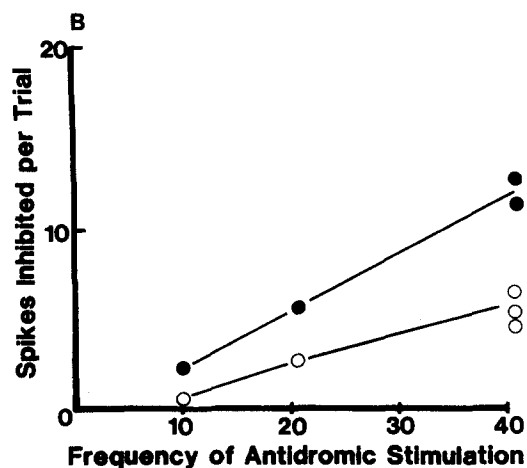
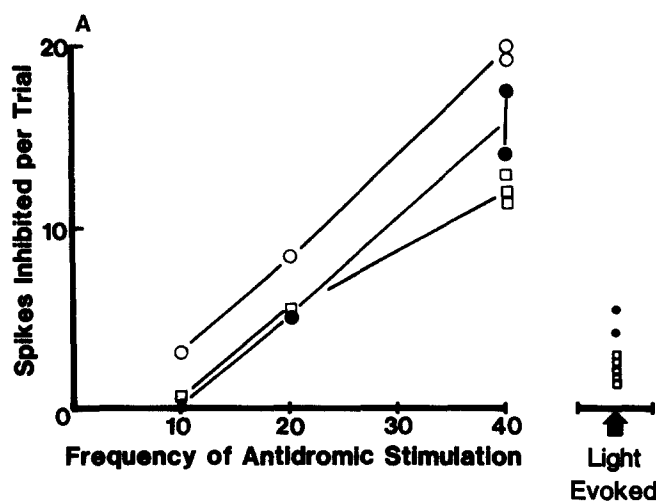
Chlorimipramine, a 5-HT uptake blocker in vertebrate (Ross and Renyi, 1972) and invertebrate (Gerschenfeld et al., 1978) preparations, causes a decrease in the eccentric cell firing rate ( $N = 10$ ; this is analogous to LSD-induced inhibition), enhances lateral inhibition at lower (1–20 nM) concentrations ( $N = 2$ ), and suppresses lateral inhibition at higher (0.1–20  $\mu\text{M}$ ) ones ( $N = 6$ ). Fig. 7 shows the depressive effect of (10  $\mu\text{M}$ ) chlorimipramine on eccentric cell firing rate. However, chlorimipramine did not significantly affect the light response of retinal cells ( $N = 2$ ) recorded intracellularly, even in external concentrations as high as 1 mM. Thus, the depression of the spiking rate induced by chlorimipramine appears to be due to eccentric cell hyperpolarization, an effect analogous to the hyperpolarization caused by 5-HT and LSD, as was shown by Adolph (1976).

Fig. 8 shows the effects of chlorimipramine on lateral inhibition. In this experiment, light-evoked lateral inhibition was first measured at many different levels of excitation by varying the light intensity illuminating only the facet of the test eccentric cell. Inhibition caused by a repeated test flash is plotted against the steady firing rate of the test eccentric cell. This experimental format was designed to minimize the problem of distinguishing drug-induced suppression of lateral inhibition from drug-induced depression of the test receptor's firing rate. It facilitated comparisons of the amount of inhibition in the absence and presence of chlorimipramine (see the legend to Fig. 8). After chlorimipramine was added in very low (1 nM) concentration, the magnitude of the inhibition decreased. This indicates that lateral inhibition was enhanced. When a higher concentration (0.5  $\mu\text{M}$ ) of chlorimipramine was added, the magnitude of lateral inhibition (as well as the ongoing spiking activity of the eccentric cell) decreased greatly. Chlorimipramine probably also decreased the firing rates of the eccentric cells in neighboring facets that provide the lateral inhibition; this would tend to decrease the strength of light-evoked inhibition on the test receptor. It is therefore likely that the enhancement of lateral inhibition by the low concentration of chlorimipramine was underestimated by this experiment, while the suppression of lateral inhibition caused by the higher concentration may have been exaggerated. In independent experiments ( $N = 3$ ) to test whether a high concentration (10  $\mu\text{M}$ ) of chlorimipramine suppresses lateral inhibition, antidromic stimulation of the optic nerve was the source of inhibition. During antidromic stimulation, the invasion of presynaptic terminals is presumably unaffected by postsynaptic inhibitory potentials or by direct drug-induced hyperpolarization of eccentric cells. Antidromically evoked lateral inhibition was depressed by high chlorimipramine concentrations;

therefore, the drug's suppressive action is exerted at the inhibitory synapse.

#### DISCUSSION

LSD is generally believed to mimic or antagonize 5-HT action at postsynaptic receptor sites in many vertebrates and invertebrates (Cooper et al., 1974). Our present results and those reported by Adolph and Kass (1979) suggest a different hypothesis for LSD action at the lateral inhibitory synapse in *Limulus*. At relatively low levels of LSD, partial blockade of presynaptic reuptake sites for the lateral inhibitory transmitter prolongs transmitter lifetime in the synaptic cleft and thereby enhances inhibition (Fig. 9B). In the presence of relatively high LSD, more uptake sites are blocked, which causes accumulation of the lateral inhibitory transmitter and, consequently, postsynaptic desensitization (Fig. 9C).



We refer below to "Tr," an abbreviation for "the *Limulus* lateral inhibitory transmitter." We distinguish between 5-HT and Tr in the discussion to emphasize the fact that the major conclusions do not depend on the identity of the natural inhibitory transmitter. However, there is evidence supporting the identification of Tr as an indoleamine. Our results neither detract from nor confirm that identification. We also wish to emphasize that this model for LSD action may be generalized to apply to synapses at which other biogenic amines are the transmitters, provided that presynaptic uptake is an important means of transmitter inactivation.

Below, we first discuss reasons for rejecting the view that LSD acts by blocking postsynaptic action of the inhibitory transmitter in *Limulus*. Then we discuss how our results and those of other investigators are in accord with our presynaptic uptake blockade hypothesis for LSD action. Finally, we discuss the possibility that LSD blocks a presynaptic autoreceptor for Tr (one that depresses further release of Tr), thus accounting for our results.

*Evidence against Direct Postsynaptic Action by LSD*

LSD-induced inhibition of eccentric cell spiking is markedly reduced under conditions that depress the release of lateral inhibitory transmitter.

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FIGURE 6. (*opposite*) (A) Concentration dependence of LSD action upon inhibition evoked by antidromic stimulation of the optic nerve. Ordinate shows the average number of spikes inhibited during 5-s trials of antidromic stimulation. Three different antidromic rates were tested during zero (control), low, and high concentrations of LSD in the superfusate. The ordinate was calculated by taking one-fifth of the difference between the total number of spikes obtained during five trials of antidromic stimulation (5 s/trial) and 25 s of uninhibited spike activity. The 1- $\mu$ M LSD curve was obtained after the curve for 20  $\mu$ M LSD. A decrement of 10 spikes/trial represents an  $\sim$ 30% inhibition of the background firing rate. More than the control number of spikes were inhibited at the low concentration of LSD, which indicates enhancement of lateral inhibition. Fewer than the control number of spikes were inhibited at a higher concentration of LSD during inhibition evoked by light and by 40/s antidromic stimulation. This indicates that the higher concentration of LSD suppressed lateral inhibition. Inhibition evoked by five repetitions of a 5-s light stimulus to neighboring ommatidia (shown on the right) was measured only for zero and high LSD concentrations. ●, before LSD; ○, 1  $\mu$ M LSD; □, 20  $\mu$ M LSD. (B) Effect of 5-HT on inhibition evoked by antidromic stimulation of the optic nerve. Ordinate as in A. Inhibition was measured during superfusion with 0  $\mu$ M (control) and 10  $\mu$ M 5-HT in the ASW. A decrement of 10 spikes/trial represents an  $\sim$ 30% decrease in the background firing rate. Fewer than the control number of spikes were inhibited in the presence of 5-HT at all antidromic stimulation rates, which indicates suppression of lateral inhibition. Same preparations as in A, after 1 h of ASW washing. ●, before 5-HT; ○, 10  $\mu$ M 5-HT.

In normal ASW perfusate, LSD exerts a strong depressive effect upon the firing rate (Fig. 1*B*). However, in the presence of high  $Mg^{++}$ /low  $Ca^{++}$  ASW, when normal synaptic transmission is demonstrably reduced (Fig. 2), LSD has a greatly reduced effect on spiking rate (Fig. 4*A* and *B*). In the control experiment, high  $Mg^{++}$ /low  $Ca^{++}$  ASW failed to prevent 5-HT from depressing the spike rate (Figs. 3 and 4*B*). These results support the inference that 5-HT exerts its action primarily at postsynaptic sites, but that LSD's direct postsynaptic action is, at most, minor. We infer that LSD's major effect on inhibition requires normal release of Tr from the presynaptic terminal.

The results cannot be explained by assuming indirect action of 5-HT

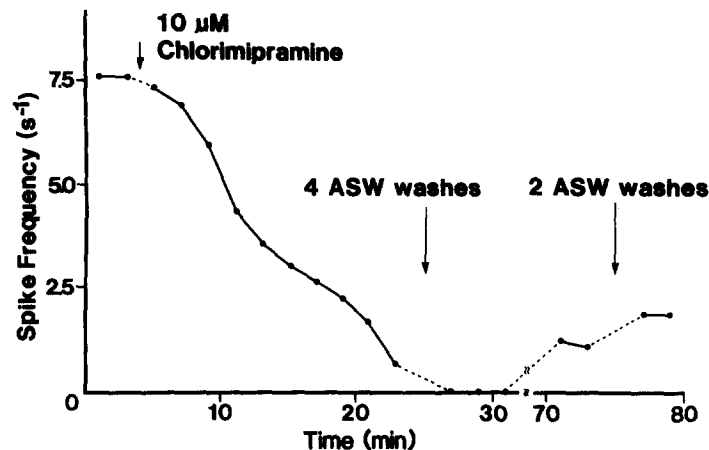


FIGURE 7. Effect of chlorimipramine on eccentric cell spike frequency.  $10 \mu M$  chlorimipramine superfused over the retinal slice causes a decrease of the ongoing extracellular spike frequency of an optically isolated eccentric cell under constant illumination. After 60 min and six normal ASW washes, the depression of frequency caused by chlorimipramine has only slightly recovered. Experiment 708.

or LSD at a different retinal synapse. The only other synapse in the lateral eye mediates centrifugal effects from a circadian clock in *Limulus* brain (Barlow and Chamberlain, 1980; Barlow et al., 1980; Fahrenbach, 1981). The neurochemical modulator released by efferent activity causes an increase of sensitivity of the lateral eye to light (Barlow et al., 1977) and affects spontaneous membrane noise of the reticular cells (Kaplan and Barlow, 1980). The substance in question appears to be octopamine (Kass and Barlow, 1980, 1981; Battelle et al., 1982); 5-HT can partially mimic efferent activity only when injected beneath the cornea of the lateral eye *in situ* in concentrations that are 200 times greater than those for octopamine ( $\sim 0.1$  mM; Kass and Barlow, 1982, 1983). Optic nerve shock does not evoke the effects of efferent activity after the lateral eye is excised (Kass, 1981; Barlow, 1983). Changes in visual sensitivity or

photoreceptor membrane noise after 5-HT or LSD superfusion (0.1 to +25  $\mu\text{M}$ ) are not obvious; this suggests that neither 5-HT nor LSD acts by mimicking the action of the efferent mediator at octopamine-like postsynaptic receptors.

The results obtained also cannot be explained by effects of the low

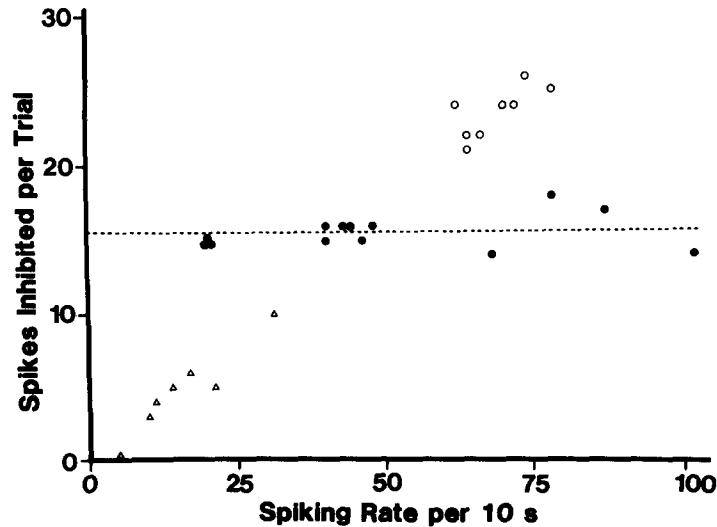
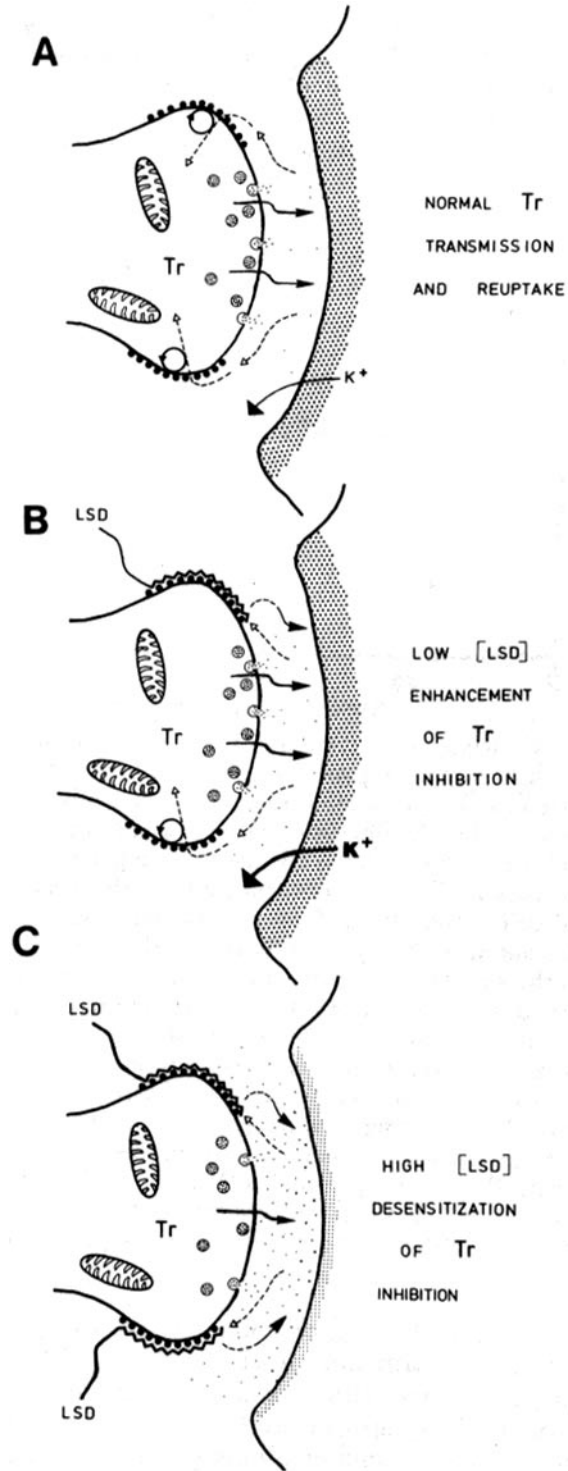


FIGURE 8. Concentration-dependent effects of chlorimipramine on lateral inhibition. Light impinging on a single extracellularly recorded eccentric cell was varied to obtain a wide range of baseline spike frequencies. Lateral inhibition was evoked by illuminating a group of neighboring facets for 10-s trials. Each filled circle shows the average depression of spike frequency during the 10-s inhibitory trials, plotted vs. the spike frequency of the uninhibited cell. After the highest rate of firing was achieved (far right), the stimulus intensity was left constant, and 1 nM chlorimipramine was added into the superfusate. Lateral inhibition was evoked (open circles) as the cell gradually decreased its rate of firing. Then 0.5  $\mu\text{M}$  chlorimipramine was applied and lateral inhibition was evoked (open triangles). The cell's rate of firing continued to decrease as a direct effect of chlorimipramine. In normal ASW, the absolute strength of lateral inhibition is relatively independent of the spiking rate (filled circles and broken horizontal line). 1 nM chlorimipramine caused an increase in the number of spikes inhibited compared with the control (normal ASW), which indicates an enhancement of lateral inhibition. 0.5  $\mu\text{M}$  chlorimipramine caused a decrease in the number of spikes inhibited, which indicates a suppression of lateral inhibition.

$\text{Ca}^{++}$  perfusate on transduction or spike generation processes, since the light-to-spike frequency transfer function (obtained by sinusoidal input-output experiments; Kass, 1981) is unchanged by the low  $\text{Ca}^{++}$ /high  $\text{Mg}^{++}$  ASW. Nonetheless, light-evoked and antidromically evoked inhibition are completely and reversibly abolished (Fig. 2A) by the high  $\text{Mg}^{++}$ /





low  $\text{Ca}^{++}$  ASW. The difference between the effects of the same conditions on the action of LSD and 5-HT are therefore attributed to the effects of the drugs on the lateral inhibitory synapse.

The postsynaptic Tr receptor's structural specificity appears to preclude its direct interaction with LSD. Adolph and Kass (1979) examined effects of many compounds structurally related to 5-HT for enhancing or antagonizing interactions with the putative 5-HT postsynaptic receptor for lateral inhibition. They found that a hydroxyl group in the 5 position on the indole nucleus is essential for obtaining inhibition. LSD does not have this hydroxyl group nor does its large side-chain, and other features of its structure make it unsuited for the structural specificity of the postsynaptic serotonergic-like receptor.

It remains possible to devise schemes whereby LSD's actions arise from entirely postsynaptic mechanisms. Each of these schemes requires that LSD be a complex neuromodulator of synaptic and 5-HT-induced inhibition at two distinct classes of sites. Consider one such scheme: at one site, LSD must potentiate action of Tr in order to account for LSD's enhancement of inhibition at low concentrations. This action could also account for LSD's depression of eccentric cell firing in normal ASW, and the absence of such depression in high  $\text{Mg}^{++}$ /low  $\text{Ca}^{++}$  ASW. At a second modulatory site or via an unknown intracellular feedback mechanism, LSD at high concentrations must depress the effect of Tr. Schemes such as this one or others that are easily constructed are more complex than postulated neuromodulatory mechanisms at other synapses (Kupferman, 1979), and lack any compelling evidence at present.

#### *Evidence Favoring the Presynaptic Uptake Hypothesis for LSD Action*

Enhancement of light-evoked and antidromically evoked lateral inhibition at low LSD concentrations and suppression at higher ones follow naturally from the premises of the presynaptic uptake blockade hypothesis for LSD action. If a low (1–5  $\mu\text{M}$ ) LSD concentration blocks some of the uptake receptors, then the Tr will not be removed from the synaptic cleft as quickly. If uptake is an important mechanism for inactivating Tr, this will have the effect of prolonging the average lifetime of Tr molecules. Thus, for the same rate of Tr release during prolonged inhibitory stimulation,

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FIGURE 9. (*opposite*) Action of LSD and lateral inhibitory transmitter (Tr), illustrating uptake blockade hypothesis. In *Limulus*, Tr is assumed to cause an increase of potassium permeability, resulting in membrane hyperpolarization. (A) Normal release and uptake of Tr. (B) Tr release with partially restricted uptake caused by presence of LSD in low concentration. The additional Tr in the synaptic cleft will lead to larger than the normal magnitude of inhibition. (C) Tr release with completely restricted uptake caused by the presence of higher concentrations of LSD. Note that accumulation of Tr in synaptic cleft is expected to cause a reduction of the  $\text{K}^+$  conductance increase; this is a result of postsynaptic desensitization to Tr.

the postsynaptic inhibition will be greater because the peak Tr concentrations will be greater and the inhibition will last longer.

At higher (5–20  $\mu\text{M}$ ) LSD concentrations, more presynaptic uptake sites will be blocked. The inhibitory transmitter will overwhelm its uptake mechanism when transmitter is released at a faster rate than it is taken up. This will lead to accumulation of Tr in the synaptic cleft and cause postsynaptic desensitization. The LSD-treated synapse will then become functionally “blocked,” in that more inhibitory transmitter released into the synaptic cleft will be relatively ineffective. Postsynaptic desensitization in response to repeated pulses of 5-HT does occur at the inhibitory synapse in the lateral eye. Furthermore, Adolph and Tuan (1972) and Adolph (1976) showed that 5-HT desensitizes light-evoked lateral inhibition. Indeed, pretreatment with LSD does functionally block or desensitize a subsequent 5-HT-induced inhibition, as has been demonstrated by Adolph (1976). Thus, desensitization provides a plausible mechanism for LSD’s blockade of inhibition at higher concentrations. Whether a particular concentration of LSD will enhance inhibition by prolonging Tr action or will depress inhibition by desensitization depends on the relative kinetics of the uptake, desensitization, and release mechanisms.

Tricyclic compounds with a tertiary side-chain amine (e.g., chlorimipramine, imipramine) have been shown to block the uptake of 5-HT into brain slices (Ross and Renyi, 1969; Carlsson, 1970; Shaskan and Synder, 1970). If LSD and chlorimipramine are both involved in blockade of presynaptic uptake of 5-HT (and presumably Tr), then one would expect to find similarities between their actions on eccentric cell firing rates and on lateral inhibition. Indeed, the presynaptic uptake blockade hypothesis generates qualitative predictions of the actions of chlorimipramine at the lateral inhibitory synapse. The eccentric cell spiking rate should be reduced and lateral inhibition should be enhanced by superfusion with sufficiently low chlorimipramine concentrations; lateral inhibition should be suppressed at higher superfused concentrations. These actions are demonstrated in Fig. 8. It is evident from the concentrations used in our experiments that chlorimipramine is  $\sim 100$  times more potent than LSD. At concentrations  $>0.1 \mu\text{M}$ , its depressive effects on eccentric cell firing rate and lateral inhibition are not reversible. As shown in Fig. 10, chlorimipramine lacks the 5-hydroxyl group on its indole nucleus, as does LSD. This leads us to expect that it should act not like 5-HT, but rather like LSD. It is a matter of conjecture whether the irreversibility of chlorimipramine action is related to its greater potency relative to that of LSD.

Biogenic amine transmitters are commonly believed to be inactivated by presynaptic uptake mechanisms (Rech and Moore, 1971; Cooper et al., 1974). At the lateral inhibitory synapse in *Limulus*, the receptors that mediate presynaptic uptake are much less specific than the postsynaptic receptors that mediate hyperpolarization. Nonspecificity of the uptake mechanism is suggested by Adolph and Ehinger’s (1975) finding that the

indoleamines 5,6-DHT and 6-HT were taken up into the eccentric cell at least as well as 5-HT. These same compounds were found by Adolph and Kass (1979) to be totally ineffective in causing depression of eccentric cell firing rate similar to that caused by 5-HT. Thus, whereas the receptor mediating inhibition is extremely specific for the 5-HT molecule, the presumed presynaptic uptake receptors appear nonspecific to a degree that might allow them to accept any indoleamine. It should be noted, however, that the uptake receptors probably do exhibit a high degree of specificity for hydroxylated indoleamines; the uptake receptors for 5-HT appear "nonspecific" only relative to the extremely specific postsynaptic receptors mediating inhibition. Since both LSD and chlorimipramine have an indoleamine nucleus as part of their structures, they may then bind to a significant degree to presumed presynaptic receptor sites, and consequently produce their effects, as documented in the results. Because

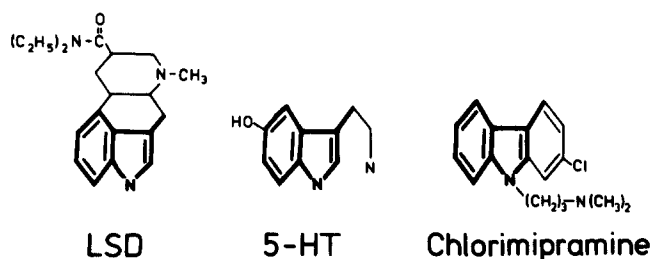


FIGURE 10. Structures of LSD, 5-HT, and chlorimipramine. The common indole nucleus is highlighted for emphasis. Note that neither LSD nor chlorimipramine contains a hydroxyl group in the 5 position of the indole group. Therefore, neither LSD nor chlorimipramine is expected to act directly on the postsynaptic 5-HT-like receptor. However, because of their indole groups, LSD and chlorimipramine may interact with the less structurally specific presynaptic uptake receptors for the lateral inhibitory transmitter.

neither LSD nor chlorimipramine has the 5-hydroxyl group, they would not be expected to interact directly with postsynaptic 5-HT-like receptors mediating inhibition.

#### *Presynaptic Autoreceptor Mechanism*

A presynaptic autoreceptor mechanism could offer an alternative to the uptake blockade hypothesis presented above. It is possible that presynaptic receptors for Tr exist at the lateral inhibitory synapse that are analogous to  $\alpha$ -adrenergic autoreceptors (Haggendahl, 1970; Starke, 1972) in other systems. Such autoreceptors provide a negative feedback action upon transmitter release. Thus, in *Limulus* lateral eye, one might hypothesize that some Tr, released by neural activity, combines with autoreceptors on the presynaptic membrane and depresses further release of Tr. LSD-induced inactivation of the postulated autoreceptors should then cause

an increase in the amount of transmitter released; LSD would thereby enhance lateral inhibition. Indeed, as we have shown (Figs. 5 and 6), low concentrations of LSD in the extracellular fluid do lead to enhanced lateral inhibition. The suppression of lateral inhibition by higher LSD concentrations (Figs. 5 and 6) can also be accounted for by an autoreceptor mechanism. The augmented Tr concentration anticipated because of blockage of the autoreceptors should lead to increased postsynaptic desensitization.

The similar effectiveness of the uptake blockade and autoreceptor blockade mechanisms in accounting for our major experimental findings leaves little basis for choosing between the two. Both hypotheses involve presynaptic modulation of synaptic transmission. Neither hypothesis requires that LSD exert direct inhibitory action on the postsynaptic membrane. The enhancement of lateral inhibition in the presence of low concentrations of LSD, and the suppression of inhibition by higher ones, would be expected on the basis of either hypothesis.

At present, however, we favor the uptake blockade hypothesis for several reasons. The actions of LSD on eccentric cell firing rate and lateral inhibition are closely mimicked by chlorimipramine (Figs. 7 and 8), a known uptake blocker in invertebrates. Furthermore, the presence of an uptake mechanism has been demonstrated at the lateral inhibitory synapse. A negative feedback autoreceptor mechanism has been demonstrated in some adrenergic synapses, and has been proposed for 5-HT in dorsal raphe serotonergic synapses in mammals (Haigler, 1981), but we are unaware of any similar mechanisms acting at biogenic amine synapses in invertebrates. A complicated scheme involving two modulatory effects of LSD on the postsynaptic response to Tr could also be constructed to account for our results. However, the absence of suitable modulatory examples in the literature leads us to favor the much simpler uptake blockade hypothesis.

#### *Implications for Mammalian Studies*

There are similarities between actions of LSD that are reported to occur in the mammalian central nervous systems and actions that occur at the lateral inhibitory synapse in *Limulus*. (a) In cat (see Aghajanian, 1972, for review) and rat (Aghajanian et al., 1972), LSD can "mimic" the inhibitory effects of 5-HT; in *Limulus*, both LSD and 5-HT depress the firing rates of eccentric cells (Figs. 1, 3, and 4). (b) In cat (Fox and Dray, 1979) and guinea pig (Kawai and Yamamoto, 1969), LSD can antagonize the inhibitory effects of iontophoresed or synaptically released 5-HT; in *Limulus*, LSD suppresses 5-HT-induced or synaptically mediated inhibition (Figs. 5 and 6). (c) In cat (Fox and Dray, 1979) and guinea pig (Kawai and Yamamoto, 1969), LSD can "facilitate" or enhance 5-HT inhibition; in *Limulus*, LSD enhances lateral inhibition (Figs. 5 and 6). (d) In cat (Fox and Dray, 1979) and guinea pig (Kawai and Yamamoto, 1969), the enhancement of inhibition occurs at lower concentrations of LSD and the

suppression of inhibition occurs at higher ones; at the lateral inhibitory synapse in *Limulus*, analogous concentration-dependent actions occur (Figs. 5 and 6). Many other findings concerning LSD's interactions at 5-HT receptors in the mammalian brain are also consistent with a hypothesis for LSD action in *Limulus* based upon presynaptic uptake blockade of 5-HT (Kass et al., 1979, 1980; Kass, 1981). In view of these similarities, further physiological and neurochemical experiments directed toward evaluating possible presynaptic modes of action of LSD in mammalian central nervous systems might at this point be fruitful.

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