# THE MECHANISM OF ACTION OF HALLUCINOGENIC DRUGS ON A POSSIBLE SEROTONIN RECEPTOR IN THE BRAIN

By J. R. Smythies, F. Benington, and R. D. Morin

Department of Psychiatry, University of Edinburgh, and the Neurosciences Research Program, Massachusetts Institute of Technology, Boston, Massachusetts; Department of Psychiatry, University of Alabama, Birmingham, Alabama



## I. Introduction: Characteristics of Hallucinogens

Lysergic acid diethylamide (LSD) is one of the most potent biologically active substances known, inducing its psychoactive effect at a dose of less than 1 $\mu$g/kg. Gaddum (1953) and Woolley and Shaw (1954) suggested that its potent antiserotonin properties (as determined on peripheral systems) might also be responsible for its central actions. The subsequent demonstration that 2-brom-LSD is just as active an anti-serotonin agent as LSD in peripheral systems, and yet is much weaker as a psychoactive compound, cast doubt on this theory. However, more recent evidence suggests that, after all, LSD does exert its central effects via the serotonin mechanisms in the brain. Freedman (1963) has shown that LSD raises the level of 5-hydroxytryptamine (5-HT) in the brain and decreases the level of its main metabolite, 5-hydroxy-

indoleacetic acid (5-HIAA). Diaz *et al.* (1968) have shown that LSD decreases brain 5-HT turnover rate. Aghajanian *et al.* (1968) have demonstrated that intravenous LSD, in very small dosage, markedly inhibits the firing of the 5-HT containing cells of the raphe nuclei. Neither of these results was obtained with 2-brom-LSD or other nonhallucinogenic relatives of LSD, and the latter result was obtained with mescaline and dimethyltryptamine (DMT) and in higher dosage (Fig. 1). LSD inhibits the effect of 5-HT in the isolated superior colliculus (Kawai and Yamamoto, 1968), and it inhibits the potential produced by 5-HT, and not by acetylcholine (ACh) in the cortex (Roberts and Straughan, 1967). LSD acts as an 5-HT agonist in the lateral geniculate system (Curtis and Davis, 1961). Pretreatment of animals with parachlorphenyalanine (PCPA), the specific 5-HT depleter, markedly potentiates the behavioral disruption produced by LSD (Freedman, 1969); pretreatment of animals and man with reserpine does the same, whereas prolonged administration of a monoamine oxidase inhibitor does the opposite. Marchbanks *et al.* (1964) have shown that LSD inhibits the binding of 5-HT by synaptosomes. Various studies have

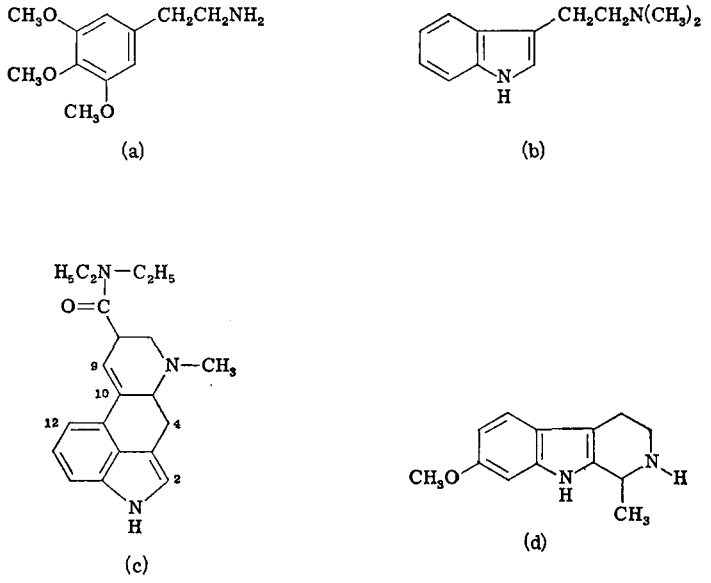



FIG. 1. The formulas of some typical hallucinogens: (a) mescaline, (b) dimethyltryptamine, (c) d-LSD, and (d) tetrahydroharmine.

indicated that at low doses LSD can excite rather than inhibit 5-HT mechanisms. Welsh and his co-workers (Wright *et al.*, 1962; Welsh, 1968) have tested a number of hallucinogens on the clam heart, where both 5-HT and dopamine are known to be transmitters (the former controlling the force of the beat, and the latter its rate). All hallucinogens proved to be 5-HT agonists in proportion to their effectiveness as hallucinogens, and none of them affected the response to catecholamines in any way. But what was remarkable in their findings was that LSD produced a *maximum* biological response at a concentration of $10^{-17}$ *M* (if given long enough (4 hours) to work). This strongly suggests that there are only a limited number of trigger zones in the cell that the LSD molecule has to find.

This great biological activity stands in marked contrast to the chemically inert nature of the LSD molecule. Its most remarkable property is its intense $\pi$ cloud energy contributed by the resonance of the conjugated structure of its four rings. Snyder and Merrill (1966) have demonstrated that LSD possesses a high HOMO (highest occupied molecular orbital) energy, and so the molecule is very lipophilic. The diethylamide side chain is very unreactive. The only points at which LSD can form hydrogen bonds are the indole nitrogen (and even this is not necessary since 1-methyl-LSD is an active hallucinogen) and the amide carbonyl oxygen which can act as an acceptor. However, its structure is stereospecifically defined, and the other three stereoisomers are quite inactive. Any change in the diethylamide side chain, removal of the methyl group or the double bond of the D ring leads to great loss or abolition of activity. The only known position where substitution is tolerated is at the 1 position (e.g., methyl, acetyl, and larger groups). Substitution at 2 by bromine or oxygen reduces or abolishes hallucinogenic activity. All this suggests that LSD must fit some receptor site so closely that it can maintain itself there tenaciously, mainly by weak interactions.

The mere inactivation, in the clam heart, of a few receptor sites for serotonin out of the large number of receptor sites on the surface of the muscle cell at the neuromuscular junction could hardly have much noticeable result. The trigger zone may be at a strategically more important site. Siegal and Salinas (1968) have recently reported that 5-HT inhibits RNA polymerase both *in vivo* brain slice and *in vitro* without interfering with its synthesis. They

also reported that 5-HT interacts strongly with nucleic acids. Furthermore several workers have suggested recently that the biogenic amines not only may act as ordinary "transmitters" whose only function is to depolarize or hyperpolarize membranes but that in addition they may cross into the postsynaptic cell there to act on some biochemical mechanism (Weiss, 1960; Costa, 1959; also see Salmoiraghi *et al.*, 1965). Kety (1969) has suggested that the biogenic amines may mediate control of protein synthesis; the latter is known to be concerned with the consolidation of the memory trace in the brain. Recent data (Barondes and Cohen, 1968; Flexner and Flexner, 1968; Hydén and Lange, 1968) indicate that memory depends on the initiation of protein synthesis at the time of the actual learning situation, or at the most within a very few minutes of it. Thus there may be some messenger from effective (reinforced) synaptic activity to the DNA/RNA system. The only candidates so far suggested for this role have been electrical fields to a repressor protein capable of changing its configuration in an electrical field (Hydén, 1968) and potassium ions acting on soluble RNA (John, 1967). However, Schmitt (1967) has written as follows: "The action of transmitters may not be limited to topochemical reactions on the external membrane of the neuron, as pictured in conventional receptor theory; they in fact may exert their effect intracellularly by repression or activation of gene expression."

Thus it is possible that 5-HT and norepinephrine (or their metabolites) could act as signals to nucleic acids. This would place the synthesis of new protein in the neuron directly under the control of specific transmitters. This new protein could alter the reactivity of the synapses concerned or even of the whole neuron, and this mechanism could provide an important mechanism of learning. There is considerable evidence that amine transmitters are concerned in emotional reactions in the brain and in reinforcement mechanisms so important for learning. Herskowitz (1967) reports that work on the neurons responding to dark-light schedules in the Californian sea have indicated that DNA-dependent RNA synthesis is linked with the long-term electrical activity of neurons. Neuhoff (1968) has reported that LSD (300 $\mu$g a day for 4 days) in rabbits caused a change in RNA base ratios in the hippocampal neurons (11% increase in cytosine, 4% decrease in guanine). There was also an over-all increase in RNA and of tyrosine-containing protein in these neurons.

### II. Specification of a Serotonin Receptor Site

It seemed worthwhile to investigate how serotonin could interact with the DNA/RNA system and how LSD and the other hallucinogens could interfere with this mechanism. A possible clue here is provided by the formula of the antibiotic mitomycin (Fig. 2). This is an analog of serotonin that prevents the separation of DNA strands on replication by forming a covalent link between two base pairs. The probable mechanism is as follows. The mitomycin molecule links two base pairs by disrupting the hydrogen bond between guanine and cytosine (probably involving the 0–6 position of guanine) and itself forming covalent bonds to the bases by means of its exceedingly active aziridine ring with one base and the active locus in the long side chain to the other. This prevents the separation of the DNA strands and leads to cell death. The number of cross-links produced is always very low—not more than one per $10^6$ or $10^7$ daltons (Waring, 1968). This suggests that it cannot attack any guanine-cytosine link but only special ones. Thus the site could normally be occupied by serotonin in a specific relationship to the DNA molecule that has some functional significance. Occupancy of the site by mitomycin, which can bond like 5-HT, introduces its highly reactive groups just in the right relation to the guanine-cytosine link so that its attack on this link is expedited. There are only three possible ways in which the mitomycin molecule can attack this link and thus, by derivation, there are three possible locations for the serotonin receptor site in relation to the DNA molecule.

(1) The mitomycin molecule can intercalate between the base pairs.

(2) It could attack the link from the side: This would locate the 5-HT receptor site in some protein molecule (possibly the protein moiety of RNA polymerase) wrapped closely around the

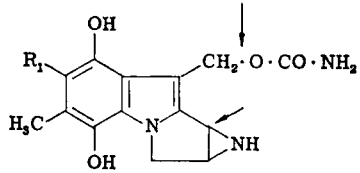



FIG. 2. The formula of activated mitomycin. $R_1$ can be $-OH$, $-OCH_3$, or $-NH_2$. The sites of cleavage are marked (Iyer and Szybalski, 1964).

DNA molecule. However, this approach is rather unrewarding as nothing is known about the protein at this site.

(3) A third possible site is at the very beginning of the DNA molecule. The first phosphate group may be anchored to a short additional segment of the ribose-phosphate chain, or alternatively to amino acid. However, for various reasons this proved unsatisfactory so we turned our attention to (1).

Important factors involved in the attachment of 5-HT to its receptor site are its lipophilicity, the energy of its $\pi$-cloud, steric factors, and its capacity to form hydrogen bonds (or similar electrostatic interactions). Serotonin possesses three sites for such bonding—the hydroxyl group, the indole nitrogen, and the primary amine group on the side chain. This suggests that at the 5-HT receptor there are three atoms so located and oriented that they can form H-bonds (or electrostatic interactions) with these three sites on 5-HT (Fig. 3). If the side chain is placed in the position shown in Fig. 3 and an external bonding chosen,[1] and if the "down" bonding of the freely rotatable ring hydroxyl (in the plane of the ring) is likewise selected, the three bonding atoms in the site will be arranged in a triangle with sides approximately $9.0 \times 8.0 \times 7.0$ Å in length (as measured from the midpoint of the bond); we can call these $O_1$, $O_2$, and $O_3$. The hydroxyl hydrogen bonds to $O_1$, the amine group to $O_2$, and the indole NH to $O_3$ (Fig. 3).

The objective now is to detail the specifications and mechanisms of action of the 5-HT receptor site such that blocking by the motley collection of molecules constituting the hallucinogenic drugs may be explained. After much work with molecular models we found that such a specification is possible based on the type of site described above. We will describe first the mechanism of a possible

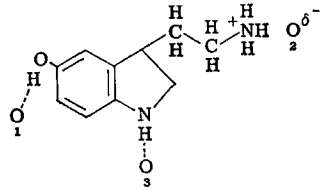


FIG. 3. Postulated orientation of 5-HT at its binding site.

[1] This configuration of 5-HT gives the closest "stacking" effect required for intercalation into a nucleic acid.

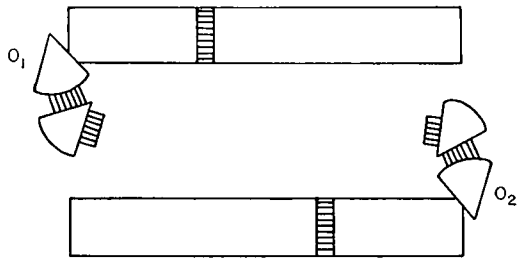



FIG. 4. Intercalation site in DNA with $O_1$ and $O_2$ shown in their hydrated state, viewed from the side.

reaction with DNA and then the mechanism of a similar reaction with helical double-stranded RNA. Possibly both these mechanisms may represent different biological functions of transmitters or their metabolites.

In the DNA molecule there are two atoms located in a position where they could hydrogen bond to molecules intercalated between the base pairs—namely the ring oxygens of deoxyribose on each side, one in the upper left-hand corner of the gap between the base pairs and one in the lower right-hand corner (Fig. 4) (Fuller and Waring, 1964). Because these are located in the hydrophilic portion of the DNA molecule it may be that these atoms would normally be hydrated. If water molecules are hydrogen bonded here with straight bond angles, two hydrogen atoms can be located facing each other approximately 8.0 Å apart (measured from the middle of the H-bond) (Fig. 4). If only one molecule of water is bonded this gives a distance of approximately 9.0 Å between the available H-bonds. The two H-bonds attached directly to the deoxyribose oxygens (without any intermediate water) would be approximately 10.5 Å apart (Fig. 5).

If a molecule of serotonin is intercalated at this site, there are four possible ways of doing it but only one way in which there is a maximal stacking effect (i.e., maximum contact between the $\pi$-clouds of serotonin and the base pairs above and below) and hydrogen bonding by its side chain N directly to $O_1$ and by its ring hydroxyl hydrogen to $O_2$ via water (Fig. 6). This locates $O_3$, to which the indolic N is postulated to bond, in some protein wrapped around the DNA molecule. The function of 5-HT at this site would appear to be to inhibit RNA polymerase by binding the two strands of DNA together and so preventing replication. The water mole-

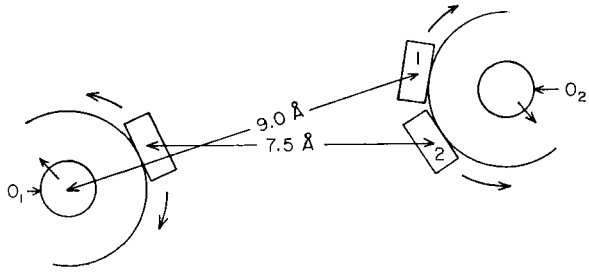



FIG. 5. Plan of the site viewed from above showing the range of horizontal rotation of the water molecules bonded to $O_1$ and $O_2$. The positions for bonding ot serotonin (1) and DOM (2) are shown by the water H bond, on $O_2$. On $O_1$ serotonin bonds directly to $O_1$ as shown and DOM to the water H bond in the position shown. 2,5-Dimethoxyamphetamine and mitomycin bond like DOM.

cules give a fair range of flexibility to the specification of the molecules that can bond at the site—for instance, both the 2,4 and the 2,5 methoxylated amphetamines can bind with good bond angles, but not the 2,3 nor the 2,6.

It is now possible to specify a central serotonin agonist. This is any compound that fulfills the steric requirements, that can be transported to the site, and that binds to $O_1$, $O_2$, and $O_3$. It is also possible to recognize two main types of antagonist:

A. Compounds binding to $O_3$ by an indolic N and failing to bond properly to $O_1$ and/or $O_2$.

B. Compounds bonding to $O_1$ and $O_2$ sufficiently strongly to resist displacement by 5-HT, but not to $O_3$. In this category we can recognize three subdivisions depending on the degree of hydration of $O_1$ and $O_2$ required for fit: B0—The antagonist connects directly to $O_1$ and $O_2$; B1—the antagonist connects directly to one oxygen and via a water molecule to the other; B2—the antagonist connects

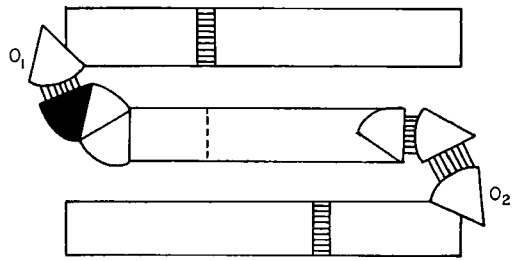



FIG. 6. Mode of bonding of serotonin at this site; black triangle = N.

to both $O_1$ and $O_2$ via water molecules. Using this classification we can now examine the hallucinogens.

## III. Specification of the Hallucinogens as Central Serotonin Antagonists

### A. Mescaline Analogs

Owing to the invaluable work of Shulgin *et al.* (1969) we now have available a quantity of structure-activity relationship data on the ring methoxylated phenylisopropylamines (amphetamines) in the human. The phenylethylamines themselves are too susceptible to attack by amine oxidase to give reliable data. Protection of the amine group from amine oxidase, however, by $\alpha$-methylation allows us to obtain a more reliable index of the activity of these compounds on the brain. Data from the rat (Smythies *et al.*, 1967b) are also useful once it is realized that the metabolism of these compounds is different in man and rat. The latter uses mainly 4-hydroxylation and the former conjugation. Thus a compound not substituted in the 4 position may be active in man and yet inactive in the rat, in which the compound is rapidly inactivated by 4-hydroxylation. Conversely, 4-methoxy compounds will tend to be more active in rat than man. An examination of the fit of these compounds into the site yielded the following results:

*Monomethoxy compounds.* The 2-substituted compound could not meet the requirements, and its *N*-methyl derivative is used therapeutically (Orthoxine). The 3-substituted compound has not yet been tested.

*Dimethoxy compounds.* Neither the 2,3 nor the 2,6 compounds could meet the criteria and should be inactive. The 2,5 compound can form good H-bonds (with the methoxy groups oriented facing in different directions) as can the 2,4 and the 3,5 compounds (in these the methoxy groups point toward each other). The 3,4 compound should act as the 4 (with possibly some steric hindrance). Table I compares the predictions that can be made from the hypothesis and the reported activity of these compounds.

It should be noted that to count as good binding the ring system of the molecule should be roughly where the ring system of 5-HT would be while occupying the site, i.e., sharing a maximum stacking effect.

*Trimethoxy compounds.* The effect of adding extra methoxy groups has two conflicting results. On the one hand, adding methoxy

TABLE I
COMPARISON BETWEEN PREDICTED ACTIVITY AND REPORTED ACTIVITY[a] OF DIMETHOXY COMPOUNDS

| Methoxy substitution | Predicted activity[b] | Reported activity[c] | Type of antagonist |
|---|---|---|---|
| 2 | 0 | ? | — |
| 3 | 0 | ? | — |
| 4 | + | 6 | B1 |
| 2,3 | 0 | ? | — |
| 2,4 | ++ | 5 | B1 and B2 |
| 2,5 | + | 8 | B2 |
| 2,6 | 0 | ? | — |
| 3,5 | ? | ? | ? |
| 3,4 | + | <1 | B1 |

[a] Activity in humans as reported by Shulgin and colleagues (1967).

[b] Each plus indicates one way of forming the specified bond.

[c] Activity is expressed in mescaline units, that is, 6 m.u. indicates that one sixth of the dose of the compound produces the same effect as a unit dose of mescaline.

groups increases the energy of the $\pi$-cloud, and it also makes it possible for certain molecules to fulfill the criteria in more than one way, which might be expected to increase activity; on the other hand, it also makes the factor of steric hindrance between one methoxy group and its neighbor more important. This would tend to decrease activity.

In the 2,3,4 compound the 2,4-type binding is entirely blocked by the presence of the 3 group, since to form it the 2- and 4-position groups must rotate with their methyl groups pointing directly at each other; this cannot occur if there is a methoxy group located in the 3 position. However, the 4-type binding should be possible. The inactivity of this compound may perhaps be partially explained by asymmetry of the $\pi$-cloud which interferes with effective stacking.

The 2,3,5 compound can form both the 2,5- and the 3,5-type binding. These may have opposite effects; moreover to form the 2,5-type binding the 2 group suffers some steric hindrance from the 3 group. Thus the molecule could be weakly active.

The 2,3,6 compound is equivalent to the 2,5 compound without any steric hindrance, and it should be active.

The 2,4,5 compound can form the 4-, the 2,4-, and the 2,5-type

TABLE II

COMPARISON BETWEEN PREDICTED ACTIVITY AND REPORTED ACTIVITY OF TRIMETHOXY COMPOUNDS[a]

| Methoxy substitution | Predicted activity | Reported activity | Type of antagonist |
|---|---|---|---|
| 2,3,4 | ? | 0 | B1 |
| 2,3,5 | + | 4 | B2 |
| 2,5,6 | + | 13 | B2 |
| 2,4,5 | +++ | 17 | B1 and B2 |
| 2,4,6 | +++ | 10 | B1 and B2 |
| 3,4,5 | + | 2 | B1 |

[a] See Table I footnotes. Again the fit is moderately close.

binding without any steric hindrance. It should therefore be very active. A compound with more than one way of binding should be more active than another with only one.

The 2,4,6 compound can form the 2,4-type binding in two different ways as well as the 4-type with no steric hindrance (the 2 and 6 groups are both freely rotatable); therefore it should be very active.

In the 3,4,5 compound, the 3,5-type binding is completely blocked by the 4 group. The compound could still form the 4-type binding, but the 4 group is not in an energetically favorable position. It should be weakly active.

Table II shows the comparison between the predicted activity and the reported activity.

The hypothesis also explains the activity of the methylenedioxy compounds. The methylenedioxy bridge places the oxygen atoms in

TABLE III

COMPARISON BETWEEN PREDICTED ACTIVITY AND REPORTED ACTIVITY OF METHYLENEDIOXY COMPOUNDS[a]

| Methylenedioxy-methoxy substitution | Predicted activity | Reported activity | Type of antagonism |
|---|---|---|---|
| 3–4 (=4) | + | 3 | B2 |
| 2,3–4 (=4 and 2,4) | ++ | 12 | B1 and B2 |
| 2,4–5 (=4; 2,4, and 2,5) | +++ | 12 | B1 and B2 |
| 2,3–4,5 (=4; 2,4; 2,5; and 3,5) | +++ | 12 | B1 and B2 |
| 2,3,4–5 (=4; and 3,5) | + | 5 | B1 |
| 3–4,5 (=4 and 3,5) | + | 3 | B2 |

[a] See Table I footnotes.

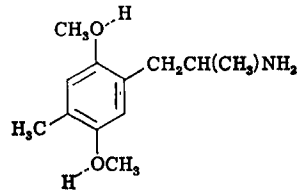



FIG. 7. Formula of DOM; possible H-bonds indicated.

the optimum position for bonding to $O_1$ and $O_2$ and avoids any steric hindrance. Table III shows the degree of correspondence between activity predicted by the hypothesis and the reported activity of the compounds (Shulgin *et al.*, 1969).

*Tetra- and pentamethoxy compounds.* In these, steric hindrance becomes a dominant factor and only the 2,4,5,6 compound can form H-bonds. However, the 2,3,4,5 compound is certainly active (6 m.u.) and the pentamethoxy compound is probably active (Smythies *et al.*, 1967b). The molecule is now too bulky to intercalate at all and it may act elsewhere.

*2,5-Dimethoxy-4-Methylamphetamine (DOM).* By far the most potent hallucinogen in this series is 2,5-dimethoxy-4-methylamphetamine (Fig. 7) which is some 100 times as potent as mescaline. As only a small proportion of amphetamine is metabolized by 4-hydroxylation in the human, the 4-methyl group may be important, not because it protects against catabolism, but because its steric hindrance ensures that the 5-methoxy group will take up the correct configuration to bond to $O_2$.

The hypothesis enables us to make some clear-cut predictions as follows:

(1) Type B1 antagonists should have their activity reduced by *N,N*-dimethylation and abolished by deamination. This is confirmed by the fact that *N,N*-dimethylmescaline is only about half as active as mescaline (Vojtechovsky, 1967). In the second category, Safrole

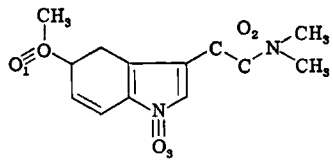



FIG. 8. Mode of binding of type A antagonists.

(Fig. 8) a constituent of nutmeg, represents a type B1 antagonist that has lost its amino group. This is inactive.

(2) Type B2 antagonists should be unaffected by *N,N*-dimethylation or deamination.

B. Tryptamine Derivatives

The active derivatives of tryptamine have an *N,N*-dialkyl (or equivalent) substitution. The dimethyl, diethyl, and dipropyl compounds are active, higher ones are not. Monosubstitution is only effective at the higher alkyl derivatives. The most active substitution is probably a pyrrole ring. *O*-Methylation, as in the case of bufotenin, increases activity. *O*-Methylserotonin has been reported to be hallucinogenic.

These compounds would appear to be type A antagonists, bonding to $O_3$ as serotonin does and then being unable to bond properly to $O_1$ and/or $O_2$ (Fig. 8).

*DMT, diethyltryptamine (DET), and dipropyltryptamine (DPT).* These all lack the 5-hydroxyl needed to bond to $O_1$ and the *N,N*-dialkylation will reduce bonding to $O_2$. Lipophilic attraction should help to locate the ring in the "proper" site.

*Psilocin.* This has the ring hydroxyl in the wrong place. Its greater activity than DMT may reflect the increased energy of its $\pi$-system imparted by the hydroxyl group.

*Bufotenin.* This has the hydroxyl in the right place and could bond to $O_1$ but very weakly to $O_2$. This compound has much too potent and toxic peripheral effects to allow it to be tested as a hallucinogen—but its *O*-methyl derivative is probably an active hallucinogen (Smythies *et al.*, 1967a).

The hypothesis would suggest that blocking the 1 position (e.g., by a methyl or a benzyl group) would abolish activity in a hallucinogenic derivative of 5-HT, as the molecule could no longer bond to $O_3$. This is confirmed by the observation that BAB is not a hallucinogen (Fig. 9).

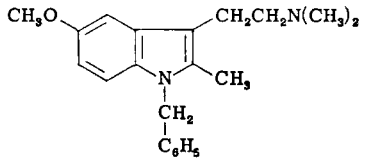



Fig. 9. Formula of BAB.

## C. Lysergic Acid Diethylamide

An examination of the possible mode of action of d-LSD on the postulated site yielded several observations of interest. The flat indole ring with its $\pi$-cloud enhanced by the resonance due to the double bond in the D ring is well suited to bind in the site by $\pi$-cloud stacking. This exposes the 1 position showing free under the edge of the superjacent base pair (Fig. 10). The methyl group on the D ring is wedged into an angle of the deoxyribose phosphate chain of the upper turn of the helix. But the diethylamide side chain provides the observation of greatest interest. Because of the skew in the D ring and the fact that no rotation is possible at the C—N diethylamide bond, the side chain is normally tilted with respect to the plane of the indole ring at the same angle as holds between the base pairs and the ribose phosphate backbone of the helix (Fig. 11). This makes it possible to orient the freely rotatable ethyl groups so that they lie snugly, each in contact with a deoxyribose molecule that occupies this portion of the helix with many close H—H contacts. This suggests that the final attachment is by van der Waals' forces here.

The closeness of the fit indicates that the only possible places for substitution on the molecule would be the 1 and 14 positions on one side of the slit-like site and the 7 on the other. This is consonant with the fact that considerable substitution is possible at 1 and none at 2 or 11. A dimethyl side chain would give fewer H—H contacts.

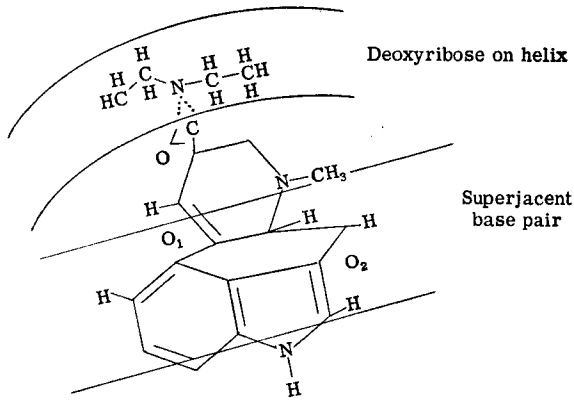



Fig. 10. One of the two possible ways that d-LSD can block the sites.

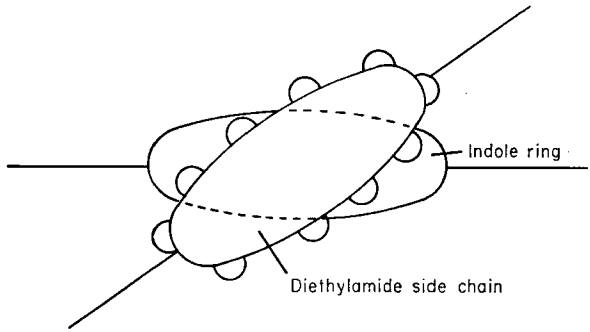



FIG. 11. "End on" view of d-LSD.

In the case of l-LSD and the two forms of iso-LSD these could intercalate with their indolic groups, but the position of the rest of the molecule would be different. Yielding and Sterglanz (1968) have reported that all four forms of LSD bind to helical DNA but not to RNA or denatured DNA. The exact mechanism has not been elucidated, but it does not seem to involve ionic binding to the phosphate groups of DNA. The biological specificity is presumably due to some special feature of the nucleic acid at the 5-HT receptor site, or in some associated mechanism offering a particular advantage of the d form over the other three stereoisomers. Thus d-LSD can be regarded as a sort of "plaster cast" of the site.

The action of LSD predicted by this model might depend on whether or not it were attached to $O_3$. If it were, it could act as an HT agonist, if it were not it could only be an antagonist. Thus the hypothesis would predict that 1-methyl- or 1-benzyl-LSD could not act as HT agonists (e.g., in the clam heart), although they could act perfectly well as antagonists. These two compounds were in fact found to be much weaker than LSD in the clam heart (Wright *et al.*, 1962).

### D. $\Delta^9$-TETRAHYDROCANNABINOL (THC)

An inspection of the models of the active constituents of hashish—THC—in comparison with an inactive congener—cannabinol—prepared by Helmut Neumann led us to link its action with this hypothesis. The active molecule consists of three ring systems: a flat benzene ring is attached to a complex two-ring system (Fig. 12) on one side and a five-carbon side chain on the other. The nature of the skew on the complex ring system plus the location

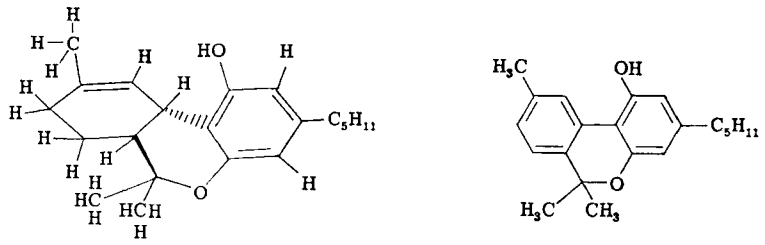



FIG. 12. Formulas of THC and cannabinol.

and orientation of the three associated methyl groups results in an arrangement that it would be difficult to deduce from an inspection of the ordinary line drawing of the formula alone. The complex ring system, plus the methyl groups, forms a smooth, elongated, regular, deltoid clump, studded with hydrogen atoms and tilted, with respect to the plane of the benzene ring, at the same angle that obtains between the diethylamide side chain and the ring system of LSD (which is the same as that between the base pairs and the helical backbone of DNA) (Fig. 13). If the molecule is intercalated, its benzene ring in the central position, its fixed complex ring system now lies along the same deoxyribose phosphate section of the helix that can be contacted by the diethylamide side chain of LSD with many close H—H contacts and possibility of van der Waals' interactions. The five-carbon side chain is now sticking out of the other side of the site and could take up a variety of positions. This is consonant with the fact that substitutions are

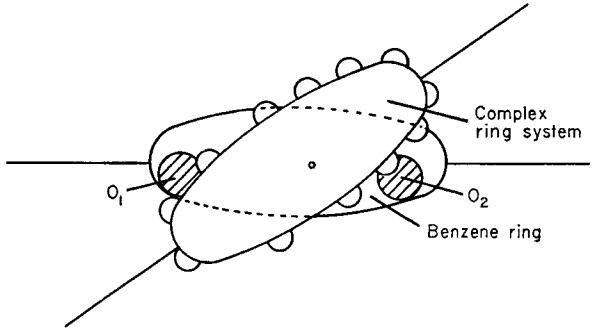



FIG. 13. "End on" view of THC.

tolerated here (e.g., dimethylheptylpyran, methyloctylpyran, and synhexyl are active), but none are tolerated in the complex ring system. The inactive compound cannabinol is composed of two benzene rings in the same plane (Fig. 12) which does not form the same limpet-like attachment to the site as THC. Thus THC appears to be a sort of caricature of LSD. The complex four-ring system of LSD is replaced by a single benzene ring, but the simple diethylamide side chain of LSD is replaced by a much larger, but functionally equivalent, ring system.

### E. Harmine

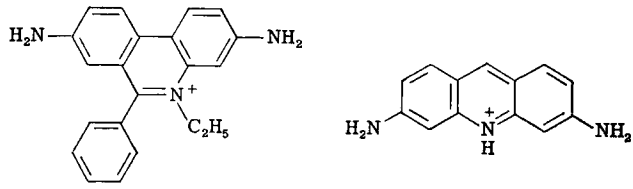
One difficulty here is that there is no certain evidence for establishing which of the various compounds in this series are hallucinogenic. It appears that a likely candidate is 1,2,3,4-tetrahydroharmine (Fig. 1). An examination of a molecular model of this compound indicates that it is functionally equivalent to 4-methoxyamphetamine in view of the relative positions and orientations of the methoxyl and the nonindolic NH groups. Thus it could act as a type B1 antagonist. Harmine and harmaline could act as type A antagonists.

## IV. Mitomycin

If a model of the molecule of mitomycin is inserted into the site (Fig. 2), it can bond either as the equivalent of a 2,4 or a 2,5 methoxylated amphetamine. In the latter case, its two reactive groups are located directly under the hydrogen bond between guanine and cytosine (N—O) that it is thought to attack. In Fig. 2 $R_1$ can be $OCH_3 > NH_2 > OH$ (in order of potency of resulting compound). This suggests this group is not being used for hydrogen bonding (if it were, the potency should be reversed).

## V. Type B0 Hallucinogens

It will be apparent that no type B0 hallucinogens have yet been described, that is, compounds capable of bonding to both $O_1$ and $O_2$ directly without a linking water molecule. The "widest" putative hallucinogen is harmaline, but this is not quite wide enough and its C ring N is in the wrong orientation to bond to $O_1$. However, two compounds that clearly could bond directly to $O_1$ and $O_2$ are ethidium and proflavine (Figs. 14 and 15), which are known to act as antibiotics by intercalation between base pairs. The fact that the




(a) (b)

FIG. 14. Formulas of (a) ethidium and (b) proflavine.

$NH_2$ groups in each compound are separated by the same distance, together with the fact that on intercalation both compounds can in fact bond in the model, does suggest that their intercalation is stabilized by H-bonding to $O_1$ and $O_2$ as was suggested by Fuller and Waring (1964). But there is no evidence that these compounds are hallucinogenic. However, one agent capable of producing a psychosis that fills the specification to be a type B0 hallucinogen is quinacrine. This is sterically quite similar to d-LSD with respect to the relation of the $\pi$-cloud and the diethylamide side chain.

## VI. Generalization of the Hypothesis

Two separable aspects of this hypothesis should be borne in mind—(1) the identification of the receptor site in terms of three atoms ($O_1$, $O_2$, and $O_3$) whose only specifications are their relative location, their capacity to form hydrogen bonds, and their bonding angles, and (2) the further identification of these atoms. The particular identification suggested above may be supplemented by others. The similarity between $O_1$ and $O_2$ and the deoxyribose oxy-

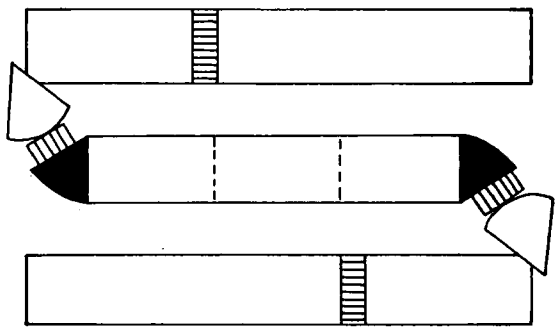


FIG. 15. Fit of proflavine into site.

gens of DNA (as described) may be no more than an unremarkable coincidence. The real $O_1$, $O_2$, and $O_3$ may belong to a similar site in membrane protein with some helical structure concerned with opening or closing ionic channels, or with some helical RNA. There may even be serotonin receptor sites in more than one such locus. The matter can only be settled by further experiment in such fields as electron microscope autoradiography and nucleic acid chemistry. The reactions generally termed "hydrogen bonding" in this presentation may also include electrostatic interactions with the same steric requirements—for example, a protonated amino group could react with an O bearing a negative charge. However for our purpose the precise nature of the bonding is less important than the stereochemistry.

## VII. A Possible Role of RNA

Investigations using a model of helical RNA kindly loaned by Dr. W. Fuller indicate that the main stereochemical relationships described above between 5-HT and its central antagonists and DNA hold as well for RNA. The extra hydroxyl group is so located that it can also form an H-bond to the primary amino group of 5-HT. The intercalation site in helical RNA (A configuration) has a different shape from that of DNA in B configuration. The former is a rhomboid and the latter a rectangle (viewed from the side). This entails that $O_1$ and $O_2$ are about 1.5 Å closer together in RNA than in DNA. Thus 5-HT could bond in the intercalation site in helical RNA without a water link and moreover it can form no less than four hydrogen bonds (NH to $O_1$: NH to O of ribose hydroxyl: OH to $O_2$: H of ribose hydroxyl to O of 5-HT OH), and maximum $\pi$-cloud overlap. Likewise type $B_1$ hallucinogens such as 4-methoxy amphetamine can bind in helical RNA with no water link, and type $B_2$ hallucinogens need only one water link. The hydroxyl groups in the groove of the RNA helix would appear to offer less strong attachment to the ethylamide side chain of LSD and the complex ring system of THC.

Morgan and Austin (1968) have shown that RNA is present in synaptosomes in two forms. One is mitochondrial RNA and the other is located either in membrane or in the cytoplasm and has properties akin to ribosomal RNA. However, it has not yet been established on which side of the synaptic cleft this latter type of

RNA is located. Ribosomes have not been located in the axon terminal, but they could exist incorporated in the membrane in such a manner that they would not be seen by electron microscopy. On the other hand, the RNA may be located in the postsynaptic region in the form of the subsynaptic ribosomes described by Bodian (1966) in the spinal cord and that may be associated with the spine apparatus. In either event, the function of 5-HT and NE could be to inhibit or initiate the synthesis of membrane protein (or proteins) at the level of RNA that would alter the electrical characteristics of the synapse so that learning would result: ". . . the existence of subsynaptic ergastoplasm in the adult indicates a continued role of the adult neuron in adaptive adjustments of synapses, such as formation of new contacts" (Bodian, 1966).

Furthermore, if RNA is present and actually incorporated in the subsynaptic membrane, part of it may be available to access from the outer surface of the cell so that 5-HT and NE could react with it without actually crossing the membrane and thus affect ionic channels or charge transfer properties of the membrane.

Evidence that LSD binds to nucleic acids by intercalation has been presented by Wagner (1969), Yielding and Sterglanz (1968), and Smythies and Antun (1969).

Further reports that RNA is present in membrane have been given by Kaspar and Kashing (1969), King and Fitschen (1968), Takagi and Ogato (1968), and Tulegenova *et al.* (1968). A further stereochemical analysis has been made (Smythies, 1969a,b) of the relationship between drugs known to act on neurons and RNA. This has led to the general hypothesis that the channels conducting ions through neuronal and muscle membranes, as well as those carrying amines into synaptic vesicles are composed of helical RNA (or ribonucleoprotein). A segment of double-stranded helical RNA orientated vertically through the membrane would form a potential channel capable of transporting $Na^+$ ions into (or $K^+$ ions out of) the neuron. When the base pairs are hydrogen bonded they would form an internal shutter closing the channel. If the hydrogen bonds were disrupted, the two RNA strands would separate thus opening the channel. Hoffman and Ladik (1964) point out that nucleic acids are normally insulators, but if they lie in an electric field orientated parallel to their long axis, and a powerful electron acceptor or donor intercalates at one end, the charge transfer resulting (the addition or removal of an electron) would supply sufficient energy

(12.4 eV) to disrupt the base pair hydrogen bonds. The charge transfer sets up a migration of $\pi$-electrons and the terminal base pairs become polarized and repel each other. This could set up a cooperative disruption of the rest of the hydrogen bonds joining the base pairs and so the channel would open.

A study of the molecular configurations of acetylcholine, its agonists and antagonists, as well as NE, histamine, and glutamate, and their agonists and antagonists indicates that they all have the required stereochemical structure to bond to helical RNA. All the molecules in these groups have the common property of possessing charged atoms 4–5 Å apart with the correct bond angles to bind in the way described. Thus the general hypothesis suggests that the receptor sites on the neuronal membrane for all transmitters may be made up of a portion of the helical RNA that itself makes up the main part of the channels for conducting ions through membranes. The attachment of the transmitter leads to a charge transfer process that disrupts the hydrogen bonds joining the base pairs and the channels opens. The factors that specify which particular transmitter is active at the site could include the following: (1) the particular base pairs involved, (2) the amount of RNA left unmasked by the protein moiety of ribonucleoprotein or other molecules, (3) steric hindrance imposed on binding by the same moieties, and (4) the sense in which the helix is wound. As an example under (3), d-LSD as well as its three inactive stereoisomers all bind equally well to native DNA. The factor making only the d-LSD active may depend on steric factors imposed by some of the molecules, e.g., prostaglandin (see below) bound to the RNA.

*A role for the prostaglandins.* The prostaglandins are thought to play an auxillary role in transmitter function without being transmitters themselves. Molecular models indicate that prostaglandins with fully staggered side chains bear a close stereochemical relationship to helical RNA. Two prostaglandin molecules can bond to a four base-pair segment of RNA. One limb of each prostaglandin molecule binds along one ribose-phosphate chain; the other binds along the outer base-pair (of the four) to the other side. Each molecule can form no less than five hydrogen bonds (in the case of 19-hydroxy-prostaglandins) to elements in RNA (COOH to ribose OH; 9-O to ribose OH; 11-OH to guanine NH; 15-OH to cytosine O; 19-OH to ribose hydroxyl). The two molecules form a highly lipophilic rhomboid-shaped ring around the primary receptor site (the

bonding sites on the two middle base pairs of the four). Without the prostaglandin molecules, many molecules of the transmitter (such as ACh) would be wasted by bonding to the many phosphate and ribose anionic sites in the normally highly hydrophilic surrounds of the receptor site. With the two prostaglandin molecules in place, these are blanketed off and the transmitter is limited to the effective bonding site. On this hypothesis prostaglandins should only be relevant to minor-groove transmitters (such as ACh and glutamate). They should not be necessary for adrenergic sites in the major groove. The upper prostaglandin molecule can only bind to a CG base pair and the lower one to a GC pair.

It is also possible to demonstrate that reserpine can form a very close attachment to helical RNA (but not DNA). The lipophilic tetrahydroharmine moiety could intercalate between base pairs and bond like 5-HT to which it is sterically equivalent. The complex ring system terminating in the trimethoxybenzene moiety now fits neatly down the minor groove of the helix with two additional hydrogen bonds to the hydroxyl groups of ribose to give a total of four hydrogen bonds, multiple weak interactions and the strong $\pi$-cloud interaction for binding. If the RNA was forming a channel as described, reserpine binding in this location and manner would clearly block it mechanically. Likewise, yohimbine could also bind to helical RNA but with an interesting difference. Yohimbine is a truncated stereoisomer of reserpine such that reserpine could only bind to helical RNA wound left-handed and yohimbine could only bind to helical RNA wound right-handed. Thus it is possible that the RNA in channels carrying ions are wound right-handed (and thus are blockable by yohimbine) and those carrying amines into synaptic vesicles are wound left-handed (and thus blockable by reserpine).

It is further possible to demonstrate that ouabain, which blocks $Na^+$ channels, could physically block an RNA based channel by forming five hydrogen bonds to three adjacent base-pairs. Furthermore veratridine, which potentiates the transport of $Na^+$ across membrane, can form no less than 12 hydrogen bonds to unpaired RNA, and thus could prevent the RNA from reconstituting the helical form and would thus hold the channel open. The stereochemical details of this mechanism have been detailed elsewhere (Smythies, 1969a,b). Likewise tetrodotoxin has a similar array of oxygen and hydroxyl groups in the precise steric configuration

required to bind to single stranded RNA, only the radius of the circle around which they are aligned is much smaller than is the case for veratridine. This suggests that tetrodotoxin may react with an acutely flexed portion of single stranded RNA or would block a channel running down the middle of a piece of helical RNA with disrupted base pairs.

Molecular models indicate that the following putative transmitters can bind only to particular groupings of base-pairs, which therefore can specify the receptor site of the prostaglandin-RNA complex.

(1) *Muscarine.* The active stereoisomer can bind only to a GC:GC combination in the narrow groove (ring O to guanine NH; $N^+$ to cytosine O) with an additional bond from its hydroxyl group to the adjacent prostaglandin carboxy OH.

(2) *Nicotinic site.* If both nitrogen atoms of nicotine are protonated this will bind only to a CG:GC combination (to both cytosine Os).

(3) *Acetylcholine.* In the muscarinic site this binds like nicotine (noncarbonyl O to guanine NH; $N^+$ to cytosine O; carbonyl O to prostaglandin carboxy OH). In the nicotinic CG:GC site, it binds differently to nicotine, i.e., by its carbonyl O to the guanine NH and $N^+$ to cytosine O.

(4) *Glutamate.* This can bind to a GC:CG combination (O to guanine NH in each case).

(5) *Glycine* can bond across any single base pair (O to NH; $NH_2$ to O).

(6) *Adrenergic site.* 2-Substituted phenothiazines like chlorpromazine and perphenazine can intercalate between base pairs only from the major groove, since the intercalation site, seen from above, is trapezoidal in shape. This locates the long phenothiazine side chain (with its charged groups 3 Å apart in the fully staggered form) running down the floor of the major groove binding to successive NH or O groups which are themselves located 3 Å apart. Since phenothiazines are mainly antiadrenergic in their action, this suggests that the adrenergic receptor site may also be in the major groove. A good site is provided by a UA:UA base pair combination. The ring hydroxyls bond to the two uridine Os and this locates the $\beta$-hydroxyl and amino groups correctly to bond to the two double-bonded oxygens of two successive phosphate groups.

## VIII. Testing of the Hypothesis

The advantage of this hypothesis is that it postulates alternative but precise mechanisms of the mode of action of serotonin and the interactions of this amine by the hallucinogens. This specificity enables us to make quite specific predictions that can easily be tested by experiment.

1. Hallucinogens may protect RNA polymerase against inhibition by 5-HT.

2. Both should also bind to DNA (or helical RNA).

3. *N,N*-dimethyl derivatives of type B1 antagonists should be prepared and tested for activity (e.g., 4 and 3,4 substituted amphetamines). They should be less active. Deaminated derivatives should be inactive.

4. 1-Methyl and 1-benzyl derivatives of type A antagonists should be inactive.

5. Other substituents besides the methoxy group capable of hydrogen bonding could be tried in the amphetamine series such as $NH_2$ or $S—CH_3$. *p*-Amino-*N*-methylamphetamine has been reported to be hallucinogenic (Usdin and Efron, 1967). It has already been shown that the methyl group, incapable of H-bonding, is ineffective by itself.

6. THC should inhibit central 5-HT mechanisms as does LSD. Freedman (1969) has already shown that it increases brain 5-HT levels.

7. LSD (and other hallucinogens) as well as 5-HT should offer protection against attack by mitomycin on DNA.

8. The hypothesis that mitomycin and serotonin act at the same site or sites can be tested by a variety of methods.

9. Ribonuclease may depolarize neurons.

These predictions can be tested by a variety of techniques. New putative hallucinogens can be tested on humans, or in suitable animal tests (Smythies *et al.*, 1967b). If the rat is used for testing derivatives of amphetamine with an unsubstituted 4 position, these derivatives should be protected from hydroxylation by methylation, otherwise misleading results will be obtained. These compounds can also be tested as central 5-HT antagonists since the hypothesis equates central 5-HT antagonism and hallucinogenic activity. The interaction among 5-HT, hallucinogens and nucleic acids, and

enzymes associated with nucleic acids, as well as with various antibiotics, should be further explored.

## IX. Summary

1. It is proposed that serotonin acts at a site in the brain in which nucleic acid is involved. The action may involve RNA in the membrane or DNA or RNA inside the cell. Helical RNA provides a better stereochemical "fit" than does DNA.

2. Serotonin may intercalate between base pairs and further attach by hydrogen bonding to the two sugar ring oxygens (one via a hydroxyl ion) that appear at the site from each side, as well as to a third atom located in some other molecule (perhaps protein) in close apposition to the nucleic acid. In RNA no water link is necessary.

3. Using this model it is possible to define the specifications of a central 5-HT antagonist and it is possible to show that this can account for the known structure-activity relationship data for the hallucinogenic drugs (central 5-HT antagonists).

4. There are a number of interesting chemical relationships between 5-HT and some antibiotics known to act on nucleic acid—e.g., mitomycin, vinblastine, violacein, chloroquine, and quinacrine.

5. The function of RNA in membrane may be concerned with local protein synthesis, charge transfer, or ionic channels. The function of serotonin at its ribonucleoprotein receptor site may be to initiate or modulate these processes. It is also possible that 5-HT (or its metabolites) has an additional intracellular site of action on RNA or even DNA in the axon terminal or postsynaptic site. Other transmitters such as NE may have similar sites of action.

6. The hypothesis is highly specific and is capable of undergoing experimental tests, a number of which are suggested.

7. The specification of possible receptor sites for other transmitters on RNA is given and an auxillary role for the prostaglandins has been suggested.

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*Note added in proof:* In helical RNA the 4-substituted amphetamines can bind like 5-HT to $O_1$ and $O_2$. However, there is no obvious fit for 2,5 compounds. Hence, the methoxylated amphetamines remain a puzzle, particularly as we have now shown that *N,N*-dimethyl-DOM is quite inactive and must therefore use its N group to bind. We have also shown that the 2,5 compound has a higher native fluorescence than the 2,3 or 2,4. Therefore, these groupings may act partly sterically and partly by modulating the quantum chemical properties of the molecule.