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# Hallucinogenic Drug Research—If So, So What?: Symposium Summary and Commentary

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FREEDMAN, D X *Hallucinogenic drug research—if so, so what? Symposium summary and commentary* PHARMACOL BIOCHEM BEHAV **24**(2) 407–415, 1986 —The conference brought a critical focus both on older findings and new strategies in hallucinogenic drug research as well as on behavioral and electrophysiologic tools that correlate with neurobehavioral and neurochemical events A number of unsolved problems to be investigated were noted The sensitizing effects of both mescaline and LSD on other inputs at motor nuclei or at limbic sites, and effects on afferent processes were examples of important new directions as were drug discrimination tests that can determine the relative roles of aminergic systems in drug effect Fixed ratio operant schedules can differentiate serotonin blocking agents, detect active indole psychotomimetics and demonstrate temporal and tolerance parameters relevant to drug induced neurochemical changes Data and critique as well as pharmacokinetics no longer support the presynaptic-disinhibition model of LSD effects, nor is LSD induced "slowed turnover" of serotonin seen as an accurate description of neurochemical changes The distinct nerve ending biochemical changes after LSD are reviewed Subcellular compartmental analyses require a radical revision of the picture of the ratio in the nerve ending of cytoplasmic and vesicular amine as well as factors normally regulating accessibility of amine to intraneuronal MAO

Indole psychotomimetics Psychedelic drugs LSD Mescaline Drug discrimination Fixed ratio schedules of reinforcement 5HT 5-HIAA Tryptophan LSD tolerance NE MAO Autoreceptor Raphe Nerve ending vesicle

ONE can want to know about a drug, or employ a drug to know about something—as a "tool," in Sparber's terms to probe known or hidden biochemical and biobehavioral processes Both purposes have actuated studies with those indole or phenylethylamine psychotomimetics that produce "psychedelic" effects in the human and are related by cross tolerance [25]. Certainly one impetus behind the study of LSD as a paradigmatic "hallucinogen" was to gain a perspective on pathophysiological sequences underlying the reliable *array* of drug induced effects in the human, components of which are encountered in various clinical disorders ranging from the acute initial phase of pyschoses [11] to temporal lobe epilepsy, panic disorder, and even the tic syndrome [31] Whether we analyze molecular structures or neurochemical regulations and receptor changes in roaches, rodents, feline or canine preparations, monkeys or humans, or work with gut, fundus, vas deferens, or spinal, subcortical, or cortical neural systems and tissues, use electrophysiological monitoring of nuclei or single cells or employ behavioral assays or analytic techniques (ranging from drug discrimination tests through operant conditioning) or observation of psychomotor performance—whatever system is used with LSD, not every measure or event needs to be (or even conceivably could be) directly relevant to hallucinogenesis

Given the range of relevant neuronal aggregates and neurobehavioral systems entailed *and* their incompletely known intrinsic regulatory mechanisms, it is little wonder that one may have to construct different test systems to analyze different issues in the sequence of events related to the mode of action and determinants of drug effects Given the fundamental lessons of pharmacology, different dose response curves and drug interaction characteristics (for both putative agonists and antagonists) in every test system have to be generated Finally, we can anticipate that at some point our test system may well be limited in terms of explanatory power and may pose puzzles that divert us from our original purpose, e g , I view certain of my own studies as more revealing of nerve ending serotonin mechanisms [42,47] and of indole psychotomimetics than of necessary hallucinogenic mechanisms in general Thus, if any set of observations can truly be established to be reliable (and the history of investigation of hallucinogenic drugs indicates this is *not* a trivial task!) we then have to ask If so, so what? What is the next step and why? What have we explained and what do we next need to know?

## THE SYMPOSIUM IN PERSPECTIVE

We can gain a clearer grasp from this symposium of "what is so" than is usually the case White elegantly summarizes and demonstrates (and Sparber cogently argues) that the sites, receptors, processes, experimental design and

study of drug interactions specifically relevant to LSD effects require systematic analysis on the relevant sets of behavioral, neurochemical, subcellular, receptor and electrophysiologic measures as well as incessant search for the exceptions that may disprove a "rule " Having reviewed studies of LSD and congeners at least a dozen times over the past twenty years [2, 24, 26, 27, 28, 29, 30, 31, 33, 37, 40, 43], I find these verities are often obscured by an impulse to epitomize one or two test systems as reflecting *the* essential mode of action of all hallucinogenic drugs (at best an unlikely contingency) Lack of precision in referring to "low or high" dosage, to pre and post synaptic events, to "receptors," or "*the* behavioral effects of LSD" or "slowed turnover" too often has lead to unwarranted comprehensively conclusory leaps rather than to salient experimental design or candid assertion of "working hypotheses "

In this symposium one encounters circumspection and precision with respect to what is and is not being sought and explained This reflects a distinct impetus both of new research and critical appraisal in this topic area over the past decade A fairly comprehensive range of topics—including some that are infrequently noted—and new data and clarifications are to be found

This is evident in Trulson's review of the highly productive work with combined electrophysiologic and behavioral measures in cat He provides new data on dopamine agonist induced shifts in raphe unit response to LSD and the association and dissociation of these maneuvers with respect to the cat limb-flick response Events beyond the firing of LSD induced inhibition of the raphe are linked to behavioral effects Both he and Sparber demonstrate a blockade of different LSD effects with mianserin Trulson sharply focuses (and others concur) on the compelling rationale for a "post synaptic" rather than the so-called "pre synaptic" model (i e , the raphe inhibition by LSD and concommitant "release" of tonic inhibition on post synaptic elements) as accountable for behavior changes I will return to this topic, but having strongly made the same case eight years ago [42]—albeit with less accumulated evidence—only with some weary reluctance! In agreement with White and myself [30,31], Trulson notes that we should "abandon" the search for "the one and only animal model" and elucidate components of the LSD response He notes that it is the integration of the various models that will lead to an understanding of the neural basis of hallucinogenic experience This is not a trivial but a useful guiding principle—absent startling new evidence—for most research in this topic area

Highly significant are those electrophysiologic studies that bear on the shifts in sensorimotor reactivity induced by LSD (Larson, McCall, White) Larson's work at the dorsal root relevantly pertains to afferent information processing Within the central nervous system McCall (extending work begun with Aghajanian) saliently shows the sensitizing or facilatatory effect of hallucinogens on *other* inputs at motor nuclei This is a behaviorally meaningful effect and a site where both mescaline and LSD finally most clearly converge within the CNS I am unaware of the effects, if any, of tolerance dosage regimens or amine depletions in these electrophysiologically defined systems but antagonists are reportedly effective Some of the noted variability and oscillations in mood and perceptions—an *intrinsic* LSD effect in the human [25,31]—and the sensitivity to environmental input (lowering of the "sensory gates") may be explicated by this action I have elsewhere speculated on this, but the overall balance in feedbacks between sensory input and motor response must be somehow shifted with hallucinogens [31,42] and "dehabituation" or an enhanced response to customary inputs (the familiar appears to be novel) is a cardinal feature of the psychedelic experience [25] This excitomodulatory effect (and the role of ongoing neural activity in determining a neuron's responsivity to LSD) has now been preliminarily noted by White in the limbic system—sites of inestimable importance for hallucinogenesis That 5-HT or NE mediated functions ("receptors") are "sensitized" and so demonstrably changed by drugs—as implied by twenty-five years of data—should now stimulate studies at the molecular level as well as a search for similar effects at afferent sites and perhaps a search for clues to processes in tolerance

The symposium also presented grounds to think about a generally overlooked observation of the past and to attend to the role of tryptamine and its receptors (Martin and Sloane) Without speculating on their relevance for more rostral events, they have demonstrated caudal facilitatory tryptamine and inhibitory 5-HT systems Especially relevant are some interactions in dog and human The LSD tolerant animal is tolerant to tryptamine but one wonders for example, if the reverse could be demonstrated Given the exquisite importance of temporal parameters for establishing tolerance [21, 23, 25, 31, 32, 35] and the importance of drug half-life [30, 31, 37, 38, 65], this might not be possible with a rapidly metabolized agonist With respect to mydriasis, given its centrality [23, 25, 31] as a correlate of the onset, offset, intensity of and tolerance to LSD induced mental effects—the question arises as to whether the tryptamine blockade by chlorpromazine was complete or simply attenuating on this measure Clear cut systematic observations of neuroleptic pretreatments on the LSD induced "trip" and mydriasis are lacking (or difficult to retrieve), further tryptamine infusions in humans or animals might now be feasible if systematic data are not in hand Whether intravenous tryptamine reliably and regularly produces psychedelic effects (in more than one subject) I cannot clearly grasp It is, however, puzzling that in their useful monkey model, Schlemmer and Davis see no effects of tryptamine and apparently none were observed in the cat limb-flick behavioral model Differences in tryptamine binding sites and 5-HT receptors are now being actively explored in both peripheral and central systems [12, 14, 15] so that we may anticipate a clearer picture of the role of this amine and its utility as a tool in investigating hallucinogenesis

Domino has presented preliminary new evidence that opiate receptors and neuropeptides might be a useful avenue for further research and Larson points to evidence that Krivoy's old observation that LSD spares substance P has new relevance With respect to Domino's investigations, powerful new 5-HT blocking agents relevant to opiate systems seem to be in hand [64] One is incidentally reminded that Gaddum thirty years ago had originally classified one set of peripheral 5-HT receptors as morphine reactive

The power of the drug discrimination (DD) behavioral model as White has used it is remarkable in differentiating the relative roles of 5-HT and dopamine systems in the behavioral effects of LSD and lisuride Subsequent biochemical and electrophysiological data extend the predictions of behavioral analysis White concludes his comprehensive study and lucid review by emphasizing the strategy (analagous to that with LSD and BOL) of searching for mechanisms upon which LSD is active but the nonhallucinogenic lisuride is less so The powerful direct effects of lisuride on dopamine

and NE systems, its prepotent effect at the raphe but the greater effects of LSD on other 5-HT systems as compared to lisuride may help to bring into focus the relative roles of different amine systems and cellular sites in specifically hallucinogenic drug actions I doubt we will have a confident basis from which to deduce receptor linkages to drug effects without such patient, systematic analyses

The utility of the FR operant model in dissecting relevant processes (while relatively overlooked), has long been productively studied by Sparber He again aptly demonstrates its value and differentiates the blocking effects of methysergide and mianserin He notes dose effects and the fact that "blocking" agents may have complex and partial agonist actions and on different receptor systems as is also evident in recent studies [61] The neat work on the functional pools of 5-HT affected by LSD is congruent with our previous observations [42,47] In our hands the FR not only classified psychotomimetics as to potency [5], but demonstrated the expectable tolerance and cross tolerances [6], correlated with a relevant period of neurochemical changes (see below), and nicely demonstrated enhancement of LSD effects [4, 7, 9, 10] The system responds to lisuride and quipazine but analysis does lead to interesting inferences about receptor functions Finally, the enhanced reactivity to noxious input of the locus coerulus in the presence of LSD is of interest [3] Sparber's observation that the initial excitatory effects on sympathetic arousal with LSD imply a possible role of 5-HT blocking agents in anxiety disorder might be tested in this paradigm, their mode of action is, however, likely complex [17]

Schlemmer and Davis describe another behavioral model (limb-jerk) and note the utility of the subhuman primate model to test drug effects and interactions—especially in the current absence of human experimentation It is to be hoped that this model can also test for mydriasis They briefly remark on a point worth elaborating—it is that in the search for pretreatments that truly block rather than attenuate response there is much to be done Conceptually, whether one can stop the initiation and/or the subsequent unfolding of the array of effects or simply attenuate certain effects is very important to determine I have extensively discussed this under the rubric of tolerance [31] and cross tolerance as well [30] In the human with a "bad trip," chlorpromazine produces effects of its own and dampens but does not stop the march of the unfolding events once the trip has started Long ago the blockade of LSD effects *only* by a very small dose of chlorpromazine—not larger than 30 $\mu$g/kg—was demonstrated in rat on FR in two laboratories [4,63], this overlooked dose effect is still unexplained The monkey model might test this and also clinically useful benzodiazepines

The study of enhancements and blockade requires focus and I cannot prejudge how each behavioral model is likely to be useful Michael Davis *et al* [18] recently emphasized the challenge to study blockade specifically relevant to hallucinogenic effects, noting that blockade of LSD induced motor effects (as in the 5-HT syndrome) may not be relevant since most of the motor syndromes can be elicited in transected spinal preparations Nevertheless, *chronic* monoamine oxidase inhibition known to attenuate—perhaps block—LSD effects in the human is now shown to do this in the limb-flick [57] and 5-HT syndrome models Such pretreatment could conceivably produce a pan monoaminergic subsensitivity [31] so that dissection of which receptor systems are entailed (and in what sequence) remains a challenge that animal models may yet solve Whether effects of MAO inhibition are seen in the excitomodulatory systems studied by McCall and White would be of interest Similarly, Horita and Hamilton's old observations [55] in rabbit—but not rat—of dopamine mediating "excitability" (but not pyrexia) induced by LSD require a fresh look in current test systems Disentangling attenuation, blockade, and cross tolerance in the monkey might—absent human studies—clarify the utility of many of the animal observations and receptor studies

Finally, Nichols has long demonstrated how a phenylethylamine configuration may be conformed to elicit a 5-HT receptor response As molecular studies of receptor sites and structures advance, his various structure activity studies and analyses of molecular properties and potencies of hallucinogens will be increasingly utilizable The potency of certain substituents on the ergoline structure is demonstrated herein and new active compounds, apparently more potent than LSD, are identified Given the range of models we have seen in this symposium, those new compounds should be tested and might thereby provide tools for finding which actions and effects are essential for producing or releasing the train of effects we call psychedelic The recurrent need for clear cut studies in the human surely is evident with Nichol's report

This conference has, then, brought some older observations into focus and clearly outlined many current trends in hallucinogenic research This is not the format to attempt a comprehensive review of the current status of LSD research (attempted in somewhat obsessive detail elsewhere [30, 31, 37]) Jacobs has recently pulled together his own views and the evidence for the post synaptic effects of LSD [56,57] The recent volume edited by Jacobs [56] has many valuable reviews, including M Davis *et al* 's [18] comprehensive review of mechanisms mediating the startle response and exploratory behavior (not addressed herein) and the neurochemical basis for a number of the unconditioned behaviors presented in the symposium (for example limb-flick, limb-jerk, the 5-HT syndrome) Pavlovian paradigms, also worthy of attention [53] are briefly cited in Appel and Rosecrans's review of drug effects on conditioned behaviors [8]

Obviously, at some point a comprehensive and critical review of the current status of LSD acceptor sites and 5-HT and catechol receptors will be valuable With new powerful 5-HT antagonists [17] appearing almost monthly and with new 5-HT receptor subtypes being defined [66] it is presently difficult confidently to ascribe physiologic function and precise relevance for LSD's hallucinogenic and other behavioral effects to particular receptors The lack of pure and thoroughly analyzed blocking agents (e g , for 5-HT$_1$ sites) *and* the intrinsic complexity of different receptors for 5-HT are probably accountable Thus, interesting arguments for the role of 5-HT$_2$ receptors and effects on drug discrimination paradigms have been advanced [45] but may, as the authors acknowledge, be premature Their link to human subjective effects [8,17] is a "hot" working hypothesis but the search for exceptions and other complexities (such as no tolerance on DD) warrant suspended judgement—or at least limit conclusory clarity

Michel Hamon notes interesting new observations relevant to the 5-HT nerve-ending autoreceptor where LSD acts [51] With respect to chronic dosage there has been the observation of reduction in 5-HT receptors, but Trulson cautions that with a specific tolerance dosage for the cat behavioral response this is not observed [69] We have recently noted a reduction in LSD binding specifically at the 5-HT$_2$

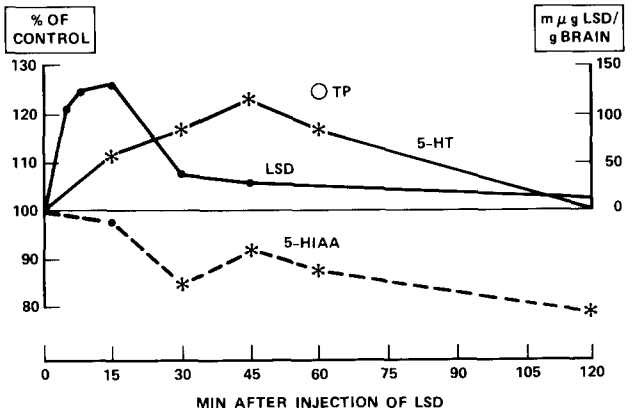


FIG 1 Characteristic clearance of drug in rat brain after IP LSD 520 μg/kg, the time course of 5-HT and 5-HIAA changes and peak elevation of tryptophan are represented (see e g , [36,65]) See text for discussion

receptor [13] after tolerance dosage schedules in rat Yet all such studies are in my view but a sketchy beginning, further, whatever time dependent processes or mechanisms are "uncoupled" from "effector systems" during tolerance, more than receptor numbers or affinity are likely, in my view, to be entailed A variety of techniques, including autoradiography and coupled in vitro and in vivo studies [19, 20, 59], are promising, but apart from an established close linkage of many 5-HT and stereospecific LSD acceptor sites [58] and their regional specificities [51,59] definition and especially the functional linkages of receptors are a part of the research agenda of the future We may be faced with intrinsic complexity and the need for molecular and ion channel studies to define satisfactorily unique and meaningful LSD effects [37]

## RETROSPECT AND PROSPECT

However the data are marshalled, the evidence is overwhelming of a linkage of LSD to 5-HT systems Relative roles of other amine systems are important to dissect and evaluate (e g , [37]) but most observers would agree these are subtle and far more intricate to disentangle Some of the agonist LSD effects on dopamine systems are evident only with perturbed systems (lesioned or drug pretreated animals, e g ) My own observations in the human, for example, might argue for a dopamine antagonist rather than agonist effect of LSD—at least with respect to the extrapyramidal effects elicited by LSD 48–72 hours after reserpine pretreatment [23,31] The effects on NE metabolites of different indole psychotomimetics or mescaline sharply differ [46,68] We have long wondered why drugs with "stronger" effects on NE release and synthesis than LSD's are *less* potent psychotomimetics and what the relative "balance" of HT and catechol systems implies for potency [24, 31, 37, 68] In any event, in assessing the differential roles of monoamines, investigators are not choosing a "favorite" amine or seizing upon a novel observation (e g , the prepotent effects of LSD but not other psychoactive indole amines at pituitary dopamine receptors [60]) simply out of boredom with the perplexities in defining mode of hallucinogenic drug action! Rather, these various complicated systems still require the search for clues for relevance to mental effects

Because some of the work with which I was associated initially established a linkage of 5-HT and psychotomimetic indole amines in brain, I've been asked briefly to highlight aspects of that work In addition, there are first of all several parameters of LSD research to be touched upon—simply in the hope that they may, if kept in view, be useful for assessing or designing research Finally I cannot—in this company—fail to comment on the issue of post synaptic effects and the issue of their direct or "disinhibitory" mediation by LSD

### *The Pre-Synaptic "Model"*

Fundamentally, it has always seemed to me to be clear (though forgotten) that one cannot have an LSD "trip" without LSD! Indeed within broad limits, increased dosage above a threshold level leads to a more intense LSD "trip"—hardly thereby sparing post synaptic sites from LSD Clearly, the dosages necessary for the unfolding of a predictable and dose responsive duration of behavioral effects in the human (or animal) are far greater than the maximal concentration necessary for the high affinity binding of LSD or the threshold dose for raphe inhibition Accordingly, we must conceive of processes beyond the initiation of the LSD raphe effect that are set into motion [37,42]

Without doubt the exquisite sensitivity of the raphe to LSD (lisuride, however, later found to be even more potent) and the remarkably accurate classification of the relative hallucinogenic potencies of indole psychotomimetics (with respect to high sensitivity at the raphe but relatively less at some post synaptic sites) initially made the "release" of post synaptic elements from tonic raphe inhibition attractive to consider as a major mechanism accountable for LSD's effects Even so, one would expect the greatest "pure" disinhibitory effects to be manifested at the lowest LSD concentrations (with perhaps *only* the raphe responding) This would have to occur at the farthest point in the time course after the clearance of LSD and not initially (e g , see Fig 1) Of course, a *relative* difference in pre and post synaptic sensitivity to LSD clearly did *not* mean no effects at post synaptic sites [43,57], rather, these have been amply demonstrated as have excitatory or modulatory responses at cellular sites Without extending the arguments here, it should be noted that there are both behavioral and biochemical effects of LSD that can occur not only dissociated from raphe inhibition but even in the absence of the raphe Thus the FR effects still occurred with LSD after raphe lesions [9] and so too, for the nerve ending 5-HT and 5-HIAA changes [42,50]

The component of behavioral change that raphe nonfiring might relate to remains unclear but surely worth exploring Conceptually "disinhibition" has always loomed as a general explanation in neuropsychiatry for the release of "primitive" behaviors I have elsewhere [31, 37, 42] listed effects found in the human that "ought" to be demonstrated in animal models—for example, tolerance Yet, on reflection, those "requirements" require some modification! Thus, the fact that tolerance or the antagonisms one observes in the human do not occur at the raphe merely tells us to look elsewhere for accountable mechanisms, the neurobehavioral consequence of raphe inhibition by hallucinogens remains to be defined Similarly, even though humans cannot detect an effect of LSD after four daily doses, the lack of tolerance with DD in animals could be in accordance with the observations that negatively reinforced behaviors and parasympathomimetic effects also show no tolerance in the rat [31, 35, 42] Again, we confront the puz-

zle of what behavior or receptor systems are linked to or analagous with the processes that lead reliably to tolerance to subjective effects (and mydriasis) in the human

### *LSD Half-Life and Clearance*

A striking correlation, insufficiently incorporated into research designs, is that half-life of LSD correlates in the human with termination of the "TV show in the head" Similarly, half-life in the rat (about 45 minutes) marks the termination of acute behavioral effects and initial biochemical changes (Fig 1) Half-life is dose dependent and species specific (rapid in mouse and slower in cat and monkey and about 4 hours in the human) These facts should be considered when comparing dose and effect across species

For the onset of effects in the human [25,31], route and rate of administration are important After an oral or slow intravenous dose, effects will be seen in 20 minutes, more rapidly with fast injection and within 1 to 2 minutes after intrathecal injection In rat, brain concentrations peak quickly (Fig 1), correlating with the onset of behavioral effects [65] Yet as later noted, the threshold dose to *initiate* an LSD response is clearly influenced by factors *other* than the absolute values of peak drug levels—primarily by reduced 5-HT levels [4, 7, 9]

Clearance from the rat brain during which acute behavioral effects appear, regularly follows the thousand fold higher plasma values of LSD These and other considerations [31,38] might lead to the hypothesis that the unfolding "march" of effects seen over hours in the human is dependent on the clearance of drug from the tissues of various neural systems (which, in turn, are modulated by aminergic and other interactions) In any event, both the initiation of LSD effects and its clearance are useful parameters for further study with respect to the onset and sequencing of drug effects in animal models

Finally, I have pointed out that the last half of the ten to twelve hour LSD effect in the human is an overlooked but significant phase It commences after the 4 hour "TV show" (when mydriasis is diminished but pupil size has not yet returned to normal) and is a different "model psychosis" than the psychedelic phase [25, 31, 37] There is a distinct period of paranoid ideation, ideas of reference and heightened self-centeredness (not unlike certain aspects of amphetamine psychoses) This "second phase" of the LSD experience—its neurophysiology, neurochemistry and pharmacology—has not yet been sufficiently explored in animal models

### *The First Sixty Minutes in Rat*

In Fig 1, several typical effects found after doses of 520 μg/kg of LSD in rat brain are represented The curves shift to the right with higher doses [65] and to the left with doses as low as 130 or 80 μg/kg, or with tolerance dosage schedules [36,37] We initially began to map out the temporal sequences of observable and conditioned behavioral effects, in part because of an interest in tolerance which requires such careful mapping With positively reinforced rope-climbing behavior [35] and later with FR schedules, *both* the dose and the time for combined behavioral and neurochemical analyses were indicated Thus 130 μg/kg appeared to be a reliable threshhold dose for the array of effects on measurable changes (heart rate [35] and EEG [40]) and observable changes (piloerection, hind-limb ataxia, etc ) Parasympathomimetic effects (bradycardia in the unrestrained rat, salivation) and failure to groom—unlike the other effects which last about 45 minutes—last twice as long, cholinergic effects do not show tolerance

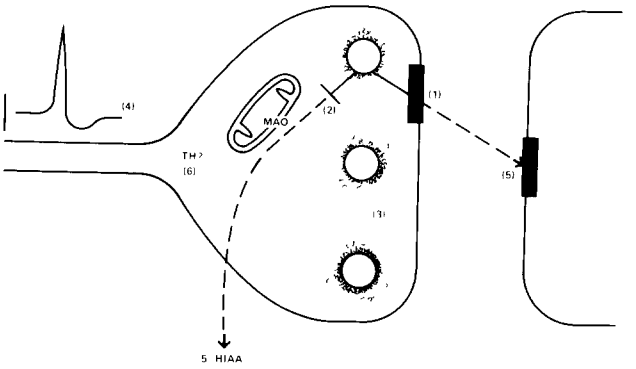


FIG 2 Some sites and mechanisms of LSD action See text for discussion

As LSD leaves intracellular compartments [38] and reaches half-life values at 45 minutes (when there is the peak retention of 5-HT) we see the termination of the excitatory and FR effects After 45 minutes there is a *shift* in the 5-HT and 5-HIAA curves—they become parallel in a declining mode (very much as is observed when the changes following chlorimipramine are mapped [42,49]) Further, this second phase of "reduced turnover" may last for 2 or more hours

The effects of LSD are stereospecific and do not occur with nonpsychoactive LSD congeners [22, 24, 39] With subcellular fractionization, the 20% or so 5-HT increase in whole brain was confined largely to the particulate fraction (after a 100,000 × g spin) [22,39] Later, with osmotic disruption of nerve endings, we defined increases of 50% or more which were confined to the vesicular subfraction [42,47] The LSD effect was originally characterized as enhancing the binding of newly formed 5-HT to intracellular storage sites and without direct effects on 5-HT enzymes [22] A small decrease in NE during this time period was also observed [24] and later the decrease in 5-HIAA [65] and tyrosine [67] and an increase in brain tryptophan [36,48] The effects on 5-HT and 5-HIAA appear to occur with indole psychotomimetics (DMT, psilocybin) but not with DOM or mescaline [41], (the claim that *all* psychotomimetics do this was reinvestigated in the strain of rats used in that report and could not be replicated [42]) The 5-HT increase does not occur after various methods of decarboxylase inhibition or during the period of chlorimipramine induced feedback inhibition of synthesis [42]—very much in agreement with Sparber's current observations The LSD effect is thus on newly synthesized 5-HT and the increase is dissociable in many tests from the regularly observed initial drop in 5-HIAA

I note these various features because the events and mechanisms entailed are not correctly summarized [37] simply as "slowed down turnover," as has often been inferred Rather the signals and discrete processes entailed within the first 45–60 minutes require analysis (see Fig 2) For example, the peak brain tryptophan increase at 60 minutes (Fig 1) shows tolerance, as do blood corticosterone elevations, but both initial effects are abolished by adrenalectomy [48] (an interdependence requiring investigation) Hamon, in an excellent recent review [51], notes that

the slowed conversion of labeled tryptophan to 5-HT (which can begin as early as 18–21 minutes) does not occur in slices treated with LSD or in cortex after transection—implying a long loop feedback (to raphe or nerve-ending?) for this effect The 5-HT and 5-HIAA effects of the first 45 minutes however, occur after raphe lesions [42,50] or adrenalectomy or hypophysectomy [48]

One final note on "turnover " With tolerance dosage, the effects on indoles [36] in the first sixty minutes seem "speeded up" and of shorter duration but similar in pattern, the 5-HIAA effect may also be diminished in magnitude What, however, is lacking is a mapping after the first 60 minutes of subsequent changes over the next 24 hours Thus measures taken 18 hours after multiple LSD doses showed "increased turnover" [51]—but are *not* matched against "periods of change" (e g , from 3 to 6 to 18 to 24 hours) after the *first* dose Thus the sequence of changes and possible causal interactions regulating turnover cannot yet be temporally defined or stipulated for tolerance I have recently [31] described possible "rebound" effects by mapping mydriasis 24 hours after the initial and subsequent doses in the human [23], these rebound phenomena may possibly be sought in measures of turnover What we really seek of course, are the unknown and essential regulatory processes that these neurochemical measures reliably but elusively reflect!

*Some Sites of LSD Action*

Figure 2 implies an axone with a nerve ending and post synaptic site To summarize what appeared to be the best established effects of LSD, 6 sites or possible mechanisms are briefly noted

(1) LSD inhibiting the release of 5-HT [51,54] probably acts at an autoreceptor The drug clearly enhances retention of 5-HT [70]

(2) There is a brief period of inaccessibility of intrasynaptosomal 5-HT to MAO—leading to a 5-HIAA decrement No partial MAO blockade (unlike with psilocybin) can be demonstrated with LSD [16,22] The 5-HIAA decrement is a regularly observed phenomenon and is found after various pretreatments We even saw it giving LSD in the first hours after reserpine when 5-HIAA levels are markedly increased [47], as well as many days after a raphe lesion [50] My favored hypothesis is that the brief protection from MAO after LSD may be a biophysical effect of the drug on soluble macromolecules which normally have a "carrier" function [30,36]

(3) The shaded portion around the vesicles indicates what we have called the "juxtavesicular" cytoplasmic fraction where 5-HT can be "held" if not bound [42, 47] In brief, findings after osmotic disruption of the nerve-ending fraction indicate that the normal picture of the serotonin nerve ending—a large proportion of amine in vesicles to be then readily released across the nerve membrane to the synaptic cleft and on to the post synaptic receptors—may not be the case Rather, approximately 80% of the 5-HT is held in a soluble binding compartment and only 20%–30% in the vesicular subfraction (which shows a large increase after LSD) The crude 100,000 g spins [22,44] had given us a different picture of a nerve ending, with "particulate " increases after LSD but a normal ratio of 75 25 of particulate to supernatant amine This picture has to be revised The soluble juxtavesicular materials along with the vesicles are in fact within that crude particulate fraction, subfractionation then reveals the strikingly different picture with about a fourfold greater concentration of amine in an extravesicular compartment After reserpine—indeed even as long as two weeks following such pretreatment [47]—the 5-HT retention after LSD occurs in the juxtavesicular, rather than in the still depleted vesicular fraction

Given the initial huge increases of 5-HIAA in the first few hours after reserpine (followed thereafter by almost unmeasureable levels of the metabolite), the ample capacity of MAO to metabolize available 5-HT is shown [42,47] But one has then to question what normally governs the accessability to MAO of the HT in the large cytoplasmic compartment containing the amine Simplistically, if the large juxtavesicular retention in the normal nerve ending were not somehow regulating accessability to MAO, the 5-HT should be normally mostly "flushed down the drain" much as initially occurs post reserpine! I've privately mused on this so long as to suspect the hypothesis, if publically posed, can readily be perceived by others to have a simple answer! In any event, different "pools" of 5-HT are evident in compartmental analyses

(4) The classic LSD effect on the pacemaker raphe soma and on the propagation of action potentials following LSD has been established by Aghajanian's laboratories These do not seem linked to the first three mechanisms discussed, they operate in the absence of the raphe soma

(5) Less 5-HT is released, so perhaps more LSD can more readily attach to post synaptic receptors Could the closely adjacent LSD and 5-HT acceptor sites be affected by small changes in concentrations of drug and amine in the synaptic cleft? Allosteric changes, postulated by Giarman and myself [43] might be effected in this environment Changes in the events beyond the receptor, such as in adenylate cyclase have also been described [36,51]

(6) Finally, the slowed conversion by tryptophan hydroxylase of tryptophan to 5-HT and the question of the indirect "long loop" feedback signal has been discussed LSD might act as a "false transmitter," signaling less need for 5-HT synthesis [33] Methiophepin, a 5-HT antagonist, counteracts the action of LSD on release, it acts at the autoreceptor and also stimulates synthesis, probably by a direct action on the hydroxylase [52] The signals regulating these events require further investigation, since neither the noted accumulation of tryptophan at 60 minutes or its slowed down conversion to 5-HT are yet fully explained

*Prospects for Future Research*

These seem both rich and exciting How all the indole psychotomimetics affect each of the steps discussed above isn't completely known Different effects on 5-HT disposition by mescaline and DOM indicate that some of the neurochemical effects are unique to LSD and psychoactive indoles and do not explicate the cross-tolerances or—taken alone—define what the linkage is to specific hallucinogenic mechanisms

Some years ago, Aghajanian and I noted the then current difficulty of estimating the significance of biochemical findings viewed in isolation from the cellular systems that link chemical events to physiological and behavior effects [1, 2, 33] With the exciting range of studies underway it would be useful in the future to keep a distinction between the "languages" of electrophysiology and behavioral analyses "Receptor effect" detected or elicited by electrodes, deduced by binding parameters or physiological measures, or inferred by

drug probes (putative agonists or antagonists) or by neurochemical effects can be readily blurred if we do not specify the different relevant procedures It is difficult—at least for me—to understand what is an agonist and an antagonist "receptor" [57] in brain unless we are clearly speaking of physiologically established functions. The receptor and cellular events that occur at somatodendritic synapses on the raphe may be somewhat different than at other cellular junctures In any event, I prefer to envisage the "source cells" for 5-HT in the brain and various nerve ending and post synaptic events as different units for analysis Aghajanian [56], with membrane analyses of conductance, is beginning to define differences between 5-HT and LSD effects at the raphe soma We should anticipate studies at the nerve ending and post synaptic elements at the molecular and ion channel levels that will further clarify the actions of these drugs Someday we may better understand how it is that very different behavioral, CNS or even invertebrate receptor systems (e g , clam heart) or those of liver fluke or thoracic ganglia of the roach [62] can rank order these drugs in terms of their potency of interactions—or even according to their class [31] On what basis do such differential effects occur? And I have elsewhere [31,37] indicated some systematics (which White has specified in this symposium) as useful for bringing some order—perhaps light—into this complex field!

Finally, in my view the most striking implication of the role of 5-HT lies in the observation of the enhanced potency of LSD in animal or human after the depletion of 5-HT Indeed with only a 15% depletion of 5-HT in rat the threshold dose of LSD is lowered as much as fourfold [4] In the human, the effect is more intense 48–72 hours post reserpine, and somewhat prolonged as well [23] The fourfold lowering of a threshold dose of LSD is also observed after raphe lesions or PCPA (and not after NE synthesis inhibition), yet brain and plasma levels of drug differ with each procedure [31,34] Along with the observation of chronic monoamine oxidase inhibition diminishing the effects of LSD, the evidence seems to be that very small changes in the pharmacodynamics of 5-HT can enhance hallucinogenically relevant reactions to, and influence the intensity of, some of the component events within the LSD response Speculations over the past thirty years that excesses or deficiencies of amine at receptor sites were related to LSD effects have in some senses been specified Changes in binding and release of amines and other substances at receptor sites along with the study of the relationships and drug induced "imbalances" of afferent and efferent systems may—in their specifics—clarify how we can begin to construct a picture whereby cellular assemblies provide the elusive links of mind and body These are the (simple!) challenges we undertake when we embark on neurobehavioral investigations with hallucinogens That is a "trip" which, in perspective, has just begun

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