Neuroscience & Biobehavioral Reviews, Vol. 5, pp. 197–207, 1981. Printed in the U.S.A.

Speculations on the Mechanism of Action of Hallucinogenic Indolealkylamines

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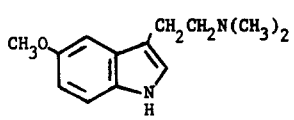
Received 1 December 1980

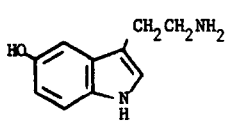
GLENNON, R. A. AND J. A. ROSECRANS. *Speculations on the mechanism of action of hallucinogenic indolealkylamines*. NEUROSCI. BIOBEHAV. REV. 5(2) 197–207, 1981.—In this review we attempt to develop a fluid theoretical model which is being used as a strategy-base for future experimentation. The first two sections (A and B) describe how we have conducted our research, and present the perspective value of each. This is important because the research strategies developed in these laboratories over the last 5 years combine *in vitro* and *in vivo* pharmacological technqiues as a means of understanding mechanisms of drug action. Sections C and D attempt to describe how we interpret our data and how we have utilized these data to formulate hypotheses concerning drug mechanisms. The last section of this review sets forth our own ideas on how we believe hallucinogenic agents produce their effects and presents some original data, which we feel, allows us to develop the overall hypotheses presented.

Hallucinogens Indolealkylamines Mechanism of action

THIS review does not presuppose that indolealkylamine (e.g., LSD, tryptamine) hallucinogens/psychotomimetic agents interact with only a singular neurotransmitter system or at only an individual brain site. Indeed, as investigations in this area progress, one is confronted with a plethora of data which are, at times, very difficult to interpret and even more difficult to attribute to a single neurotransmitter/brain site. Nevertheless, investigations of this sort must begin at some starting point and should have a working hypothesis. Examination of the structure of the hallucinogen 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT), as well as those of related compounds, reveals an obvious similarity with the structure of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). Hence, a starting point, and a



5-OMe DMT



5-HT

working hypothesis, that 5-HT might be involved in the mechanism of action of various indolealkylamine hallucinogens. We take this opportunity to describe some of our work in this area.

A. RECEPTOR MODELS FOR STUDYING HALLUCINOGENIC AGENTS

Early investigators recognized the structural similarity between 5-HT and tryptamines and began studying the agonistic and antagonistic effects of tryptamines shortly after the discovery of 5-HT. Many of the *in vitro* preparations used in these studies were eventually found to suffer from a number of short-comings, e.g., the presence of more than one type of receptor, poor responsiveness, lack of reproducibility of results, inconvenience of procuring or preparing tissue/organ sample, lack of sensitivity [8]; see Offermeier and Ariens [32] for a critical appraisal and comparison of several preparations. (This is not to imply that useful *in vitro* models are unavailable.) Nevertheless, numerous tryptamine analogs were found to act as 5-HT antagonists in such preparations. It was even postulated that LSD and hallucinogenic tryptamine analogs might act by serving as 5-HT antagonists at various brain sites [26,48]; these suggestions were supported, for the most part, by antagonism studies in the above mentioned *in vitro* assays [8]. A major finding which stymied, or at least impeded, additional *in vitro* studies was that 2-bromo LSD (BOL) was at least equivalent in potency to LSD as a 5-HT antagonist and yet was either behaviorally inactive or was found to produce behavioral

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effects which were qualitatively dissimilar to those of LSD [12,38].

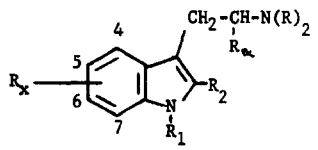
More recently, microiontophoretic and brain homogenate binding studies suggest that LSD and other tryptamine analogs might function as 5-HT agonists [4], i.e., these compounds might mimic the effect of 5-HT. Haigler and Aghajanian [24], for example, have found that LSD acts selectively on serotonergic receptors of the midbrain raphe to inhibit tonic raphe firing. This decrease in firing releases from inhibition those structures receiving serotonergic projections. In addition, several reports have appeared describing the binding of 5-HT to crude brain membrane preparations, at two binding sites, with distinct dissociation constants [5, 9, 11, 34, 44]. It has been suggested that these high affinity and low affinity binding sites represent two types of 5-HT receptors. Furthermore, the lack of modification of binding after presynaptic fiber degeneration would indicate that these receptors are probably post-synaptic [5,9]. LSD and certain related tryptamines possess high binding affinities for these 5-HT binding (receptor?) sites and Peroutka and Snyder [34] have recently reported that LSD binds equally well to both 5-HT_1 and 5-HT_2 sites. Freedman and Halaris [11] have most recently reviewed much of the data implicating a serotonergic involvement in the mechanism of action of LSD.

The possibility that hallucinogenic tryptamines might act as 5-HT agonists was also entertained as far back as twenty years ago and was the subject of experimentation using various *in vitro* methods [8]; for example, Vane [13] and Offermeier and Ariens [33] studied the effects of a large series of substituted tryptamine analogs employing an isolated rat stomach fundus preparation. Vane noticed certain peculiarities in the results and suggested that a diffusion barrier prevents 5-HT (and several other compounds) from penetrating into the cell [43]. Those compounds which enter the cell are rapidly oxidized by intracellular monoamine oxidase [25]. Barlow and Khan [3] noted differences in the agonistic effects produced by certain tryptamine derivatives in the rat fundus as compared with the rat uterus preparation. Although unable to account for these differences, they suggested that all of the tryptamines might not be acting in the same manner or at the same receptors. Determining affinities via an agonistic method Offermeier and Ariens [33] reported the affinity of psilocin (4-OH DMT), for example, to be approximately one-fifth that of DMT (subsequently, using the pA_2 method, we have found 4-OH DMT to possess five times the affinity of DMT [17]; this represents a twentyfive-fold difference depending on the method employed). The possibility was often raised that there may be more than one type of "tryptamine-like" (5-HT or otherwise) receptor present in the isolated rat fundus preparation (RFP). In 1968 Winter and Gessner [47] reported that the rat fundus preparation possesses two tryptamine-like contractile receptor-types, 5-HT receptors and phenoxybenzamine-resistant tryptamine (PRT) receptors. The existence of PRT receptors in the isolated RFP has since been confirmed by others [10]; on the other hand, using different incubation times, Green *et al.* [23] have been unable to find evidence for PRT receptors. 5-HT itself interacts almost exclusively (>98%) with 5-HT receptors of the RFP [47]; blocking experiments have shown that 5-HT is capable of protecting the 5-HT receptors from irreversible inhibition by phenoxybenzamine [10,47]. Compounds such as tryptamine [47], N,N-diethyltryptamine (DET) [47], N,N-dimethyltryptamine (DMT) and 5-OMe DMT (Glennon, unpublished data) can interact in an agonistic manner with both types of receptors. These compounds, while capable of protecting the 5-HT receptors from phenoxybenzamine, will still elicit a contractile response when 5-HT receptors are irreversibly blocked. In addition, fundus strips that are fully contracted to maximal doses of 5-HT will further contract if exposed to high enough concentrations of, for example, 5-OMe DMT. Bufotenine (5-OH DMT), on the other hand, is 5-HT-like and has very little effect on PRT receptors (unpublished observation). It might also be mentioned, though much more data are necessary before any final conclusions can be drawn, that tryptamine derivatives which are not very hydrophobic (i.e., not very lipid soluble), such as 5-HT and bufotenine, tend to interact only with 5-HT receptors while compounds which interact with the PRT as well as 5-HT receptors are somewhat more hydrophobic. These results, which support Vanes findings, lead to speculation that PRT receptors are either deep within the cellular structure (as opposed to 5-HT receptors which, Vane has concluded, are on or near the cell surface) or are otherwise enshrouded in a hydrophobic region. It even may be speculated that PRT receptors are simply 5-HT receptors which are in a different environment than "ordinary" 5-HT receptors, or, that 5-HT and PRT receptors bear a functional relationship as do 5-HT_1 and 5-HT_2 receptors; PRT receptors deserve further study. In addition to the presence of PRT receptors, α-adrenoceptors, etc. . . ., there is evidence that certain agents can produce indirect effects on the RFP; dopamine, for example, appears to contract the fundus strip by releasing 5-HT [40]. With these factors in mind, utmost caution should be used when comparing and interpreting agonistic data (e.g. equipotent molar ratios, pD_2 values, ED_{50} values) derived from the isolated rat fundus preparation at least until the extent of PRT receptor participation has been determined.

In light of the existence of PRT receptors, it was felt that information might be gained by a re-examination of a series of tryptamine analogs in a manner which would preclude, or at least minimize, PRT receptor involvement. It has been established that the agonistic effect of 5-HT in the RFP is mediated only by 5-HT receptors [10,47]. Because most tryptamine derivatives are partial agonists, i.e., mixed agonist-antagonists, the effect of these agents on 5-HT receptors can be measured by their ability to block the action of 5-HT at its own receptors. In this manner, receptor affinity (pA_2 values) can be determined by the method of Arunlakshana and Schild [1]. Most of the examined tryptamine analogs behave as competitive antagonists as determined by the slope of their Schild plots or by double-reciprocal plots. Being partial agonists, these agents will occasionally produce an agonistic response of their own if sufficiently high concentrations are used, however, these high concentrations are avoided. Thus, using the RFP, 5-HT receptor affinity data (pA_2 values) have now been determined for a large series of tryptamine analogs [17]. Such data have allowed the formulation of structure-activity relationships (SAR) and have enabled us to determine the influence of structural modification on affinity. Some of the findings are as follows: (a) N,N-dimethylation of the terminal amine generally halves affinity, (b) N,N-diethylation further reduces affinity and, for the one case examined (5-OMe DPT), disubstitution of the terminal amine with *n*-propyl groups results in a still further reduction in affinity, (c) methylation at either the indole nitrogen or at the 2-position has no effect on the affinity of DMT, (d) methoxylation of tryptamine and/or DMT can either increase or decrease affinity; affinity decreases in the order 5-OMe>4-OMe>6-OMe>7-OMe, (e)

TABLE 1

pA_2 VALUES FOR SOME REPRESENTATIVE TRYPTAMINE ANALOGS*



	R_1	R_2	R_α	R	R_x	pA_2	Designation
(±)	H	H	Me	H	5-OMe	7.66†	(±)-5-OMe α-MeT
	H	H	H	H	5-OMe	7.54†	5-OMe T
	H	H	H	Me	5-$OCOC(CH_3)_3$	7.42	5-PB
	H	H	H	Me	5-OH	7.41	5-OH DMT (Bufotenine)
	H	H	H	Me	5-OMe	7.08	5-OMe DMT
	H	H	H	Et	5-OMe	6.94	5-OMe DET
	H	H	H	Me	4-OH	6.84	4-OH DMT (Psilocin)
	H	H	H	H	4-OMe	6.58†	4-OMe T
	H	H	H	n-Pr	5-OMe	6.53	5-OMe DPT
S(+)	H	H	Me	H	H	6.46	S(+)-α-MeT
	H	H	H	Me	7-Me	6.29	7-TMT
	H	H	H	H	H	6.27	T (Tryptamine)
(±)	H	H	Me	H	H	6.25	(±)-α-MeT
	H	H	H	Me	4-OMe	6.17	4-OMe DMT
	Me	H	H	Me	H	6.04	2-TMT
	Me	H	H	Me	H	6.02	1-TMT
	H	H	H	Me	H	6.00	DMT
	H	H	H	Et	H	5.79	DET
	H	H	H	Me	6-OMe	5.77	6-OMe DMT
R(−)	H	H	Me	H	H	5.49	R(−)-α-MeT
	H	H	H	Me	7-OMe	5.33	7-OMe DMT
(±)	H	H	Et	H	H	5.23	(±)-α-EtT
	H	H	H	Me	7-OH	4.88	7-OH DMT

*pA_2 values, for the most part, are taken from published literature [13, 15, 18]; pD_2 for 5-HT is 7.45 (n=104).

†pA_2 value not previously reported; pA_2 value followed by standard deviation and number of Schild plots are: 7.66 (±0.11) n=3; 7.54 (±0.06) n=2; 6.58 (±0.09) n=3. Neither (+) nor (−) tryptophan interact with the 5-HT receptors in a competitive manner; i.e. slopes of Schild plots are −0.58 and −0.56, respectively.

with respect to 4-OMe DMT and 5-OMe DMT, O-demethylation of the methoxyl groups, to give psilocin and bufotenine, respectively, enhances affinity while O-demethylation of 7-OMe DMT reduces affinity, (f) α-methylation of the side chain of tryptamines has little or no effect on affinity when racemates are examined. It should be mentioned that LSD, as well as some tryptamine derivatives, do not appear to behave as competitive antagonists in this preparation and, hence, pA_2 values can not be determined for these compounds. Table 1 lists the pA_2 values for a representative number of tryptamine derivatives, including several which have not been previously published.

Several questions of prime importance now arise: (a) do the 5-HT receptors of the RFP display stereospecificity, (b) is there a similarity between these peripheral 5-HT receptors and central 5-HT receptors, and (c) is there any relationship between receptor affinity and behavioral activity?

Only one chiral tryptamine analog has been examined thus far, however, based on the results of this single compound, the RFP 5-HT receptors do display stereoselectivity with respect to affinity [13]. The affinity of S(+)-α-methyltryptamine is approximately twice that of its racemate and ten times that of its enantiomer R(−)-α-methyltryptamine. (This finding of stereoselectivity is supported by other work we have done on non-tryptamine analogs [15].)

Comparing the agonistic activity of a series of tryptamine analogs on the 5-HT receptors of the isolated RFP, Green *et al.* [23] have noted a parallelism with the ability of these same compounds to displace LSD binding by rat brain homogenates. Comparing bufotenine, 5-OMe DMT, psilocin, DMT, tryptamine and quipazine, we have found [16] that their pA_2 values strongly correlate with the concentration (I_{50} values) necessary to inhibit specific (^{3}H)-5-HT binding to calf brain homogenates as determined by Whitaker and Seeman [44]. Though both investigations employed a rather limited series of compounds, trends suggest that similarities exist between the 5-HT receptors of the isolated RFP and central (post-synaptic?) 5-HT binding sites.

It has yet to be determined whether or not a relationship exists between 5-HT receptor affinity and hallucinogenic potency (primarily due to the paucity of human hallucinogenic data!). Once again, though, certain trends are evident. With

respect to hallucinogenic activity, the potencies of the following compounds are listed in descending order: 5-OMe α-methyltryptamine>5-OMe DMT>psilocin (4-OH DMT) >α-methyltryptamine>DMT [29]. The 5-HT receptor affinity of these agents decreases in the same order. There are, however, several inconsistencies. Bufotenine possesses twice the affinity of 5-OMe DMT but is generally regarded are behaviorally-inactive. Although the RFP 5-HT receptor affinity of tryptamine is identical to that of α-methyltryptamine and twice that of DMT, tryptamine is also considered non-hallucinogenic. At this point, we must be reminded that ours is an *in vitro* assay; to this extent, factors such as metabolism and distribution which may be of paramount importance *in vivo* are not necessarily of major consequence in this isolated preparation. The lipid solubility of bufotenine, as determined by chloroform/buffer partition coefficient, is less than one-fiftieth that of 5-OMe DMT. Dependent to a large extent on the dose administered, only a small amount of bufotenine penetrates the blood brain barrier, as determined by Sanders and Bush [39] and by Gessner and Dankova [13]. There is evidence, however, that bufotenine is an intrinsically active compound which simply does not reach the brain. For example, Mandel [30] and Geyer *et al.* [14], have found bufotenine to be at least equiactive with 5-OMe DMT when administered to animals via intraventricular injection. Newborn chicks have a poorly developed blood-brain barrier and Rauzzino and Seifter [35] have noticed that in these animals, bufotenine has an effect similar to that of other hallucinogens, such as DMT. Gessner and Dankova [13] have administered the more lipid-soluble acetoxy analog of bufotenine to mice; presumably brain tissue esterases hydrolyze the ester to result in an *in situ* synthesis of bufotenine. Measuring LSD-like activity as a function of brain concentration, the following order of potency was observed: bufotenine (5-OH DMT)>5-OMe DMT >DMT. Finally, when administered directly into the dorsal raphe nucleus, employing indwelling cannula as previously reported [31], we have found bufotenine to be at least as active as 5-OMe DMT in producing disruption (Minnema, Rosecrans and Glennon, unpublished observation).

Tryptamine, lacking both an N-alkyl or an α-methyl group perhaps undergoes oxidative deamination by monoamine oxidase more rapidly than some of the other tryptamines. Both N,N-dimethylation and α-methylation of tryptamine result in compounds which are behaviorally active in man. It thus appears that 5-HT receptor affinities, alone, are not sufficient to account for the hallucinogenic potential of tryptamine analogs. Factors such as lipid solubility (ability to penetrate the blood-brain barrier) and rate of metabolism must be taken into account [17].

Human hallucinogenic data on tryptamine analogs is relatively scarce. Although the literature abounds with behavioral data on tryptamine, employing a wide variety of animal species and a diversity of behavioral paradigms, few studies, indeed, have examined sufficient numbers of compounds to make valid comparisons between affinity data and behavioral activity. There was a need, therefore, to screen a large number of compounds in a relatively reliable behavioral model in order to obtain the necessary data.

B. A BEHAVIORAL MODEL FOR STUDYING HALLUCINGOENIC AGENTS

Recent developments in approaches to studying the mechanisms of hallucinogenic drugs has provided needed information as to how these substances may be acting at CNS neurones; paramount amongst these approaches has been the ability to study the discriminative stimulus (DS) properties of psychoactive drugs in experimental animal subjects. Within this paradigm, which is essentially a drug detection procedure, animals are trained to discriminate between non-hallucinogen (vehicle or saline) and hallucinogen-induced drug states using behavioral techniques. More specifically, food-deprived rats are trained to press one of two levers for food, under one drug state, while on different days, the same animals are trained to press the opposite lever in the non-drug state. Rats learn to associate one lever with LSD, for example, and the opposite lever with saline such that LSD becomes the stimulus that enables the animal to differentiate between which lever will deliver reinforcement on a given day. Generally, animals learn the discrimination within 30–40 sessions under each drug or non-drug condition (see Fig. 1 for an example) such that animals trained to discriminate 5-OMe DMT will make 85–100% of their responses (lever-presses) on the 5-OMe DMT-correct lever during a 2.5 minute extinction session (i.e., where animals are not rewarded for lever-pressing) while the same animals will emit only 5–15% of their responses on the same 5-OMe DMT-appropriate lever when administered saline.

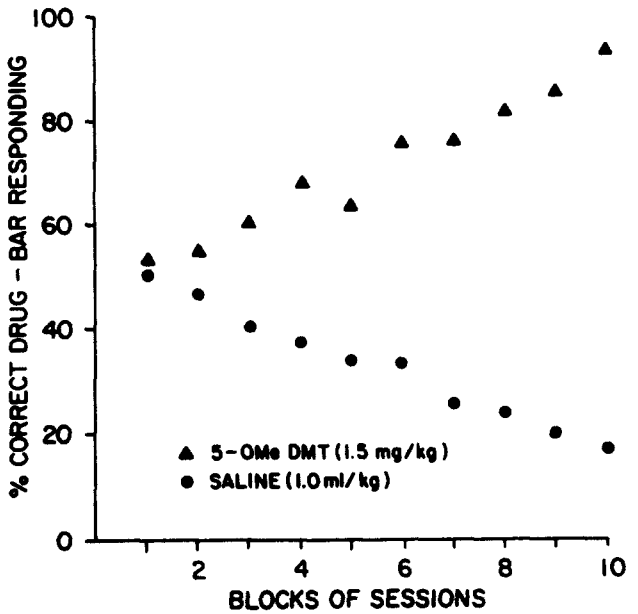


FIG. 1. Discrimination training curve for rats trained to discriminate 5-OMe DMT from saline. Rats trained to discriminate 5-OMe DMT responded 90±% on the 5-OMe DMT lever after receiving 1.5 mg/kg 5-OMe DMT while they responded 10% on the same lever after receiving saline.

The usefulness of this procedure in determining mechanisms of drug action is contingent upon the animal subjects' learning and retaining the acquired DS. Animals learn the DS and reach a stable level of discriminability within twelve weeks and are able to maintain a high level (80–90% discriminability) of precision for at least eighteen months. Studies involving chemical or neuropharmacological manipulations as a means of dissecting out specific phar-

macological mechanisms are conducted during non-reinforced test sessions interspaced between training sessions needed to maintain a given hallucinogen as a DS. These test-sessions are presented every third to fifth session in between either two drug or non-drug training sessions [36].

Advantages of this Pharmacological Model

Because of its stability, sensitivity and specificity, the DS model has several advantages over other *in vivo* pharmacological techniques used to study mechanisms and sites of hallucinogen action. For example, the DS procedure is not measuring disruptive or other pharmacological effects other than the ability of the rat to detect a given agent. In addition, rats generally become behaviorally tolerant to the disruptive effects of a given drug on operant behavior so that experimental results are not influenced by changes in rates of behavior. Furthermore, tolerance to the DS effect does not readily occur, thus allowing one to study the chronic effects of a given hallucinogen using the same experimental subject repeatedly [7,36].

The model is also specific within pharmacological class. Thus, hallucinogens don't generalize with other drugs such as the opiate agonists, sedatives or CNS stimulants [36]. In fact, as we learn more about this procedure we become continually more amazed at its specificity. At this point it seems to us that an animal can learn to discriminate very specific chemical structures and will not generalize to compounds of similar structure unless they have very similar mechanisms of action. This suggests that the DS effect of a specific chemical entity is the result of a specific drug-receptor interaction and that for two drugs to produce similar DS effects they need to mutually act at similar receptor sites.

Lastly, these procedures are quite sensitive in that the doses serving as stimuli are generally below the level at which they affect other pharmacological measures or neurochemical events. This is not to say that other parameters are not effected, but that the effects observed, at the doses employed, are below the detection level with our current methodologies. For example, morphine produces a potent DS effect at 3 mg/kg but evokes only a small increase in tail-flick latencies at this dosage, at least when given SC to the rat.

Overall, the DS model provides us with a rather unique procedure whereby we can evaluate how and where hallucinogens are producing their effects, with the added advantage that the model appears to be a subjective pharmacological measure (in the rat) quite analagous to when humans are administered a given hallucinogen.

Approaches to Studying Mechanisms of Hallucinogenic Drug Action

With this paradigm two overall strategies can be used to study drug mechanisms. First, neuropharmacological techniques involving the lesioning (chemical or electrolytic) or the stimulation of specific brain sites electrically, or, the application of agents (agonist or antagonists) to various brain sites can be used to either mimic or alter a given hallucinogen's ability to serve as a DS [31,36]. Such studies can provide much information as to site and mechanism of a centrally-mediated DS induced by any hallucinogen. It should be understood that the data collected at this point in time suggests that the DS properties of drugs are elicited via some central mechanism. Thus, drugs not entering the brain do not readily exert DS control of behavior [36]. In addition to these central approaches one can attempt to antagonize peripherally or centrally-generated cues by the use of peripherally administered antagonists. For example the 5-OMe DMT-induced DS can be antagonized by the putative 5-HT antagonist pizotyline (BC-105) [19]. Thus, one can begin to evaluate mechnism of action by the selective use of pharmacological antagonists.

The second overall strategy involves drug transfer or generalization experiments. The DS-model by its very nature makes it a suitable technique by which to compare new compounds to those already demonstrated to exert DS control of behavior. Thus, a suspected hallucinogen can be administered to a group of rats trained to discriminate a given hallucinogen to determine if the new agent will produce DS control similar to that exerted by the training drug.

From our experience, we observe one of three results from such studies: (1) the rat indicates that an unknown compound (challenge drug) is similar to the drug-DS to which it was trained and will make 70–100% of its responses on the drug-correct lever (Fig. 2); (2) the DS of a different drug will generalize only partially with the training drug (Fig. 3 indicates that the LSD DS generalize only partially with cyclazocine), with the percent responding on the drug-correct lever rarely exceeding 60%; (3) and lastly, animals will perceive a given drug more like saline than like the training drug, for example as with pentazocine when given to LSD-trained rats (Fig. 3). This approach can provide much information as to whether a new compound has a pharmacological spectrum similar to the training drug and gives some indications as to how the drug may be acting. However, there are two important considerations in interpreting such data. First, even though a given drug may not substitute for the training drug, one cannot conclude that the unknown agent is not affecting receptors similarly until some pharmacokinetic data is obtained. For example, the drug may not enter the brain. Thus, this approach provides whole animal data which may differ, from *in vitro* studies because of metabolism and drug dispositional factors. Secondly, one should be very cautious in concluding that because a drug transfers (generalizes) to another drug it may have a similar pharmacological spectrum. There are examples of non-hallucinogenic drugs which generalize with LSD ([7, 36, 46] and unpublished observations). Therefore, conclusions, such as the suggested hallucinogenic potency of a given agent, must be tempered until additional information is obtained in human subjects. (For a detailed discussion of drugs as discriminative stimuli, see, for example, reviews by Colpaert and Rosecrans [5] and by Rosecrans and Glennon [31].)

C. THE COMBINED-MODEL APPROACH FOR STUDYING HALLUCINOGENIC AGENTS

We now have at hand two models with which to study the mechanism of action of behaviorally-active substances: the receptor model and the behavioral model. The data obtained from the combined models should be complimentary. Because the receptor model appears to minimize the effects of lipid solubility [17], a given compound may be found to possess a high affinity for 5-HT receptors; on the other hand, if this compound does not enter the brain, generalization with 5-OMe DMT should not be observed in the behavioral model. Theoretically, all factors being equal (or at least inconsequential with respect to behavioral effects), there should be somewhat of a parallelism between the two models

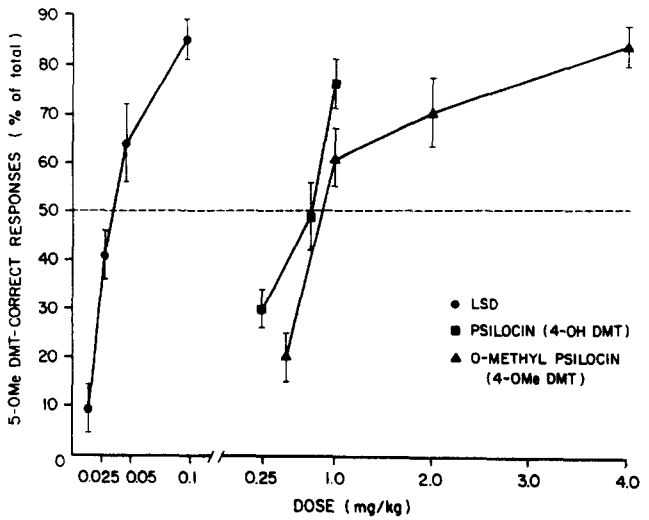


FIG. 2. Generalized responding to various psychoactive agents administered to rats previously trained to discriminate 5-OMe DMT (1.5 mg/kg) from saline. Each point represents the mean responses of six animals.

if the behavioral activity involves a serotonergic mechanism. Animals trained to discriminate 5-OMe DMT (1.5 mg/kg) from saline were challenged with each of eleven tryptamine analogs in a dose-related manner. The generalization dose was found to be correlated (Fig. 4) with pA_2 ($r=-0.88$, $n=12$) [21]. The compounds used in this initial study were chosen because, intuitively, they should possess roughly similar lipid solubilities and routes of metabolism. In other words, these compounds constitute a matched set. The results suggest that 5-OMe DMT will generalize more readily to tryptamine derivatives which display a high 5-HT receptor affinity; conversely, compounds with a lower affinity are less active. As with human data, not all tryptamines will be active in this combined model. Three types of exceptions can be envisioned and those are discussed below (the term "activity", as used below, refers to the ability to generalize):

Compounds with a high pA_2 but with low activity (i.e., activity is lower than expected based on pA_2 value):

Compounds which might deviate dramatically, with respect to route of metabolism, rate of metabolism, lipid solubility, etc. . . ,from the bulk of compounds in this series will be found in this category. Bufotenine and tryptamine, as mentioned earlier, are not usually considered behaviorally-active when administered in the usual manner. No generalization was observed when tryptamine hydrochloride was administered to the 5-OMe DMT-trained rats; at doses of up to 60 μmol/kg ($n=16$) maximal correct-lever responding never exceeded 19%. 5-Methoxytryptamine (5-OMe T), the N-demethylated analog of 5-OMe DMT, possesses a high 5-HT receptor affinity *(vide supra)*. Because the affinity of 5-OMe T ($pA_2=7.54$) is more than twice that of 5-OMe DMT ($pA_2=7.08$), a high degree of activity might be anticipated. However, like tryptamine, 5-OMe T lacks both an N-alkyl or an α-methyl substituent and, as with tryptamine, no generalization is observed. Administration of 5-OMe T to 5-OMe DMT-trained rats reveals that maximal correct-lever responding occurs at 3.5 μmol/kg (24%), at doses above this, disruption of behavior occurs ($n=21$).

Yet another compound which is less active than expected is 5-pivalylbufotenine (5-PB) [20]. Although generalization occurs between 5-PB (which possesses twice the 5-HT receptor affinity of 5-OMe DMT) and 5-OMe DMT (20), the ED_{50} for generalization is 25 μmol/kg. Being a highly lipid-soluble compound, 5-PB may become highly protein bound *in vivo* and, as a consequence, extensive hydrolysis of the

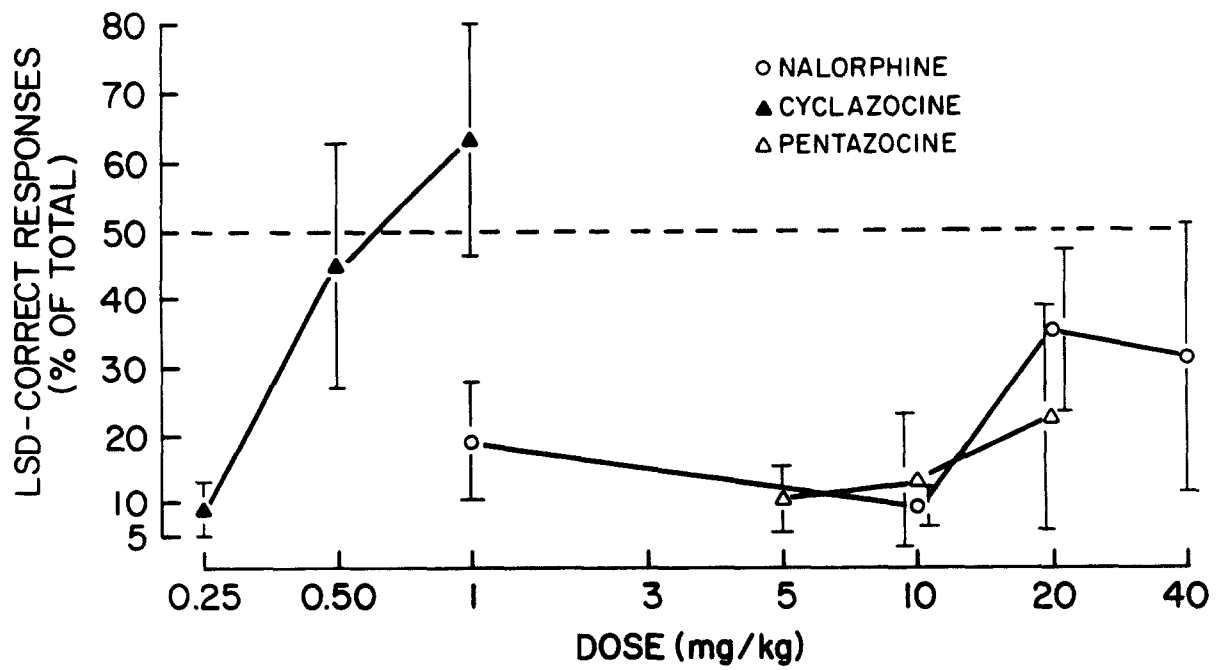


FIG. 3. Generalization studies of discriminated responding to narcotic antagonists in rats trained to discriminate LSD (96 μg/kg) from saline. Data obtained during 2.5 minute test sessions. With permission, Hirshhorn and Rosecrans [27].

ester linkage may occur prior to penetration of the blood-brain barrier.

Compounds with a low pA_2 but with high activity (i.e., activity higher than expected based on pA_2 value):

Certain compounds may possess a low affinity but yet be behaviorally-active, i.e., generalization may occur at a dose lower than anticipated. One explanation is that *in vivo* these compounds might act via a slightly different mechanism (e.g., via release of 5-HT). To date, however, no examples have been found that fall into this category.

Special Cases

There are several cases where there may be no direct relationship between pA_2 and behavioral effects. If a compound is not a competitive antagonist of 5-HT in the RFP, a pA_2 value can not be obtained. For example, because of its apparent non-competitive nature, a pA_2 value can not be obtained for LSD in the isolated rat fundus preparation yet behaviorally, LSD is very active and does generalize with 5-OMe DMT [19]. On the other hand, neither 6-methyl-N,N-dimethyltryptamine nor 5-methoxy-6-methyl-N,N,dimethyltrypamine (also not competitive antagonists) generalize with the 5-OMe DMT stimulus.

Another type of problem is encountered with 5-methoxy-N,N-diisopropyltryptamine. Although generalization has been observed (ED_{50}=2.7 μmol/kg; n=18), this latter compound produces an agonistic response in the RFP (by interaction with 5-HT and/or PRT receptors) and, hence, precludes determination of a reliable pA_2 value.

D. EXAMINATION OF OTHER PSYCHOACTIVE AGENTS

The combination of the receptor and behavioral models should prove useful in studying the mechanism of action of hallucinogenic agents, however, the use of these models should not be limited only to the study of indolealkylamines. We have recently reported that phenalkylamine analogs, depending upon the presence and location of certain aromatic substituents, also possess varying affinities for the 5-HT receptors of the RFP [18,22]. Furthermore, we have suggested that these phenalkylamines may represent a series of compounds whose activity varies on an "amphetamine-like to serotonergic continuum" [18]. That is, compounds at the low end of the affinity scale (e.g., amphetamine, pA_2=5.27) should not generalize with 5-OMe DMT while compounds at the opposite end of the scale, such as 2,5-DMA (2,5-dimethoxyphenylisopropylamine, pA_2=6.83) and 2,5-dimethoxy-4-methylphenylisopropylamine (DOM, "STP", pA_2=7.12) should. The results of initial studies indicate that there is no generalization between amphetamine and 5-OMe DMT (unpublished observation) while generalization does in fact occur between both 2,5-DMA or DOM and the 5-OMe DMT stimulus [19,21]. Because the behavioral effects produced by phenalkylamine analogs may possess a stimulant component, the relationship between pA_2 and generalization dose may not be as significant (or as evident) as with the tryptamine analogs. Nevertheless, such studies should prove quite informative. Most recently, Sloviter *et al.* [41] have shown that hallucinogenic indolealkylamines, as well as substituted phenylisopropylamines, may act via serotonin receptor activation. The role of serotonin in the mechanism of action of hallucinogenic phenylisopropylamine derivatives deserves further attention.

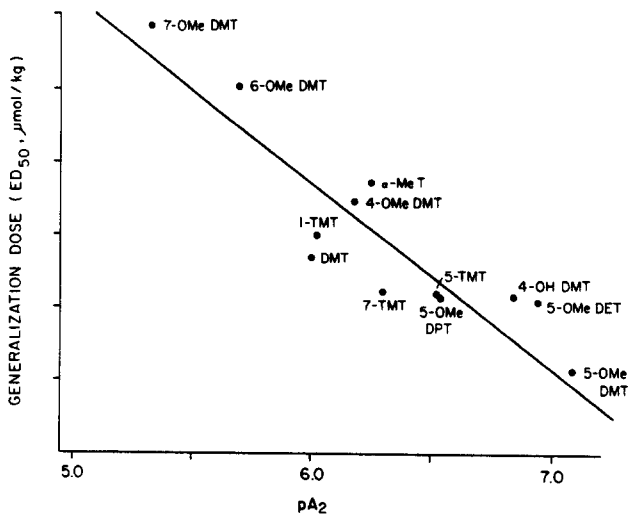


FIG. 4. Relationship between generalization dose and rat fundus 5-HT receptor affinities (pA_2 values) for a series of tryptamine derivatives.

E. DEVELOPMENT OF A THEORETICAL MODEL FOR FUTURE RESEARCH

While these investigations have clearly shown a correlation between the effect of various indolealkylamine hallucinogens on 5-HT receptors and their ability to exert discriminative stimulus control of behavior, many questions remain to be answered. For example, research conducted in these and other laboratories [6] suggest that several of these hallucinogens, in addition to possessing a serotonergic component, may exert some of their effects by acting on dopamine neurons.

To emphasize these complexities, a separate series of investigations were conducted involving three psychoactive agents: quipazine (a purported 5-HT agonist) [45], (+)-amphetamine and (±)-cathinone (Fig. 5). Our interest in these compounds stems from our earlier studies in which we showed 5-OMe DMT to generalize with DOM [19]; the present study was directed toward demonstrating possible interactions between dopaminergic neurons and hallucinogenic indolealkylamines. In addition cathinone, which is one of the active constituents of the plant drug KHAT, has been of special interest because of its possible abuse potential [42]. Three groups of animals were trained to discriminate either (+)-amphetamine, quipazine or 5-OMe DMT, respectively, from saline. Generalization did not occur when quipazine was administered to the amphetamine-trained animals but a partial generalization (47%) was observed when amphetamine was given to quipazine-trained animals. Analogously, 5-OMe DMT generalizes in the quipazine-trained animals but the administration of quipazine to 5-OMe DMT-trained animals results only in partial generalization (47%) (Fig. 6). Furthermore, generalization occurs when cathinone is administered either to the amphetamine-trained (86%) or quipazine-trained (76%) animals, but does not occur when administered to the 5-OMe DMT trained animals (Figs. 5 and 6). These results suggest that either 5-OMe DMT (or quipazine) is acting via a mechanism in addition to its 5-HT mechanism or that different types of 5-HT mechanisms (re-

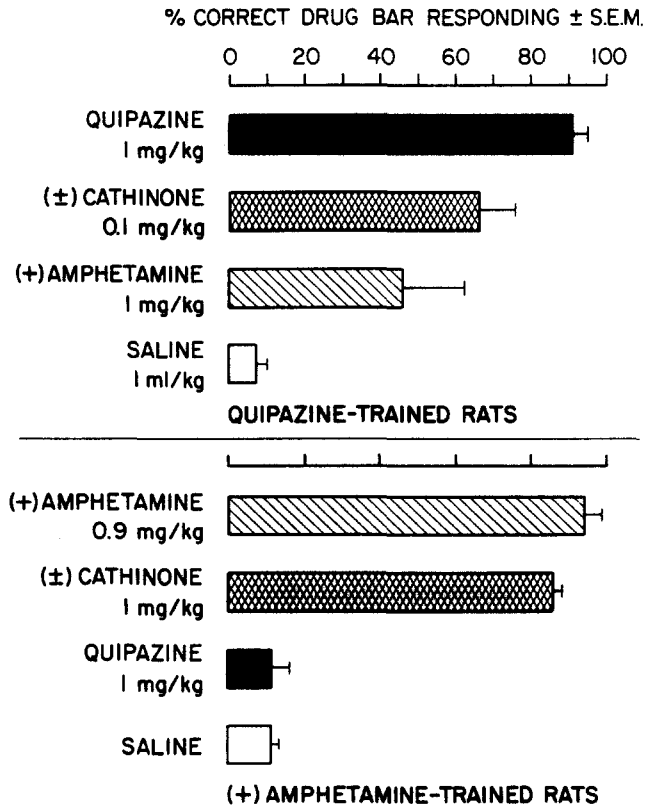
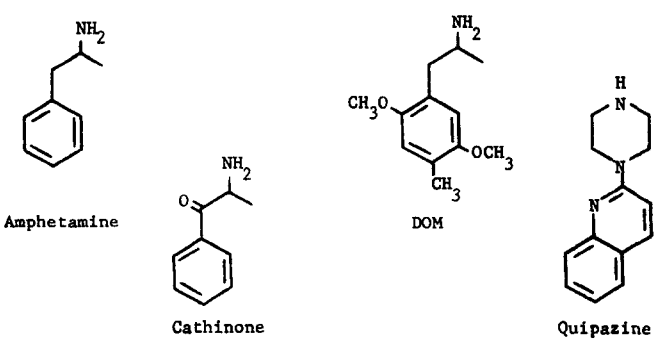


FIG. 5. Generalization of discriminated responding to various psychoactive agents in rats trained to discriminate either quipazine (upper frame) or (+)-amphetamine (lower frame) from saline. All data represent the mean responses of 8–10 rats and were obtained during 2.5-minute non-reinforced test sessions.



ceptors) might be involved in the mechanism of action of 5-OMe DMT versus that of quipazine. Interestingly, quipazine and LSD, and LSD and 5-OMe DMT were the only drugs to mutually generalize to each other [19,45]. Furthermore, quipazine does not appear to produce a hallucinogenic response in man, but does show some similarities with (±)cathinone.

When taken in its broadest view, these findings, while extremely diffuse, may in fact, be quite ordered. Before attempting to put these pieces together we should first appreciate the complexity of the brain and the interactions between various neurotransmitter systems. At one point in

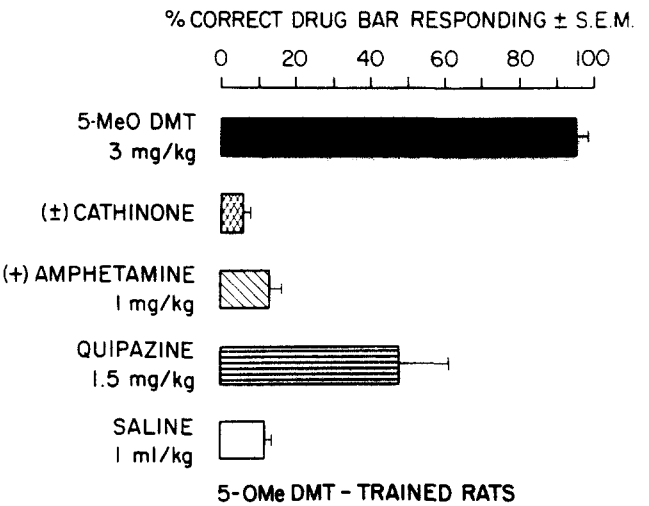


FIG. 6. Generalization of discriminated responding to various psychoactive agents in rats trained to discriminate 5-OMe DMT (3.0 mg/kg) from saline. Data represent the mean responses of 8–10 rats and were obtained during 2.5-minute non-reinforced test sessions.

time it seemed very clear that each amine projection system, whether it be serotoninergic, dopaminergic or noradrenergic, was a system unto its own. With time, however, it has become more apparent that these systems are not independent systems but are all interrelated [37]. Likewise, a given psychoactive drug does not effect only one pathway but may affect others indirectly or by its own action at some receptor site. In the case of the indoleamine hallucinogens, they seem to be acting on one or more sites located within either the dorsal or medial raphe projection systems (Fig. 7). The views of some investigators is that the psychopharmacological effects of these agents are mediated via an agonist or antagonist receptor-interaction at some cell body, or, are acting by affecting serotonin postsynaptic receptors further upstream in some forebrain area. We should understand that these 5-HT projections systems not only impinge on 5-HT receptors but also impinge upon dopamine neurons as well. Furthermore, the postsynaptic sites of these projection systems may also influence the cell bodies of the same system or of other amine-containing neuron pathways by feed-back loops. Thus, the possible sites at which a psychoactive drug could act seem to be numerous.

What we propose is that these psychoactive drugs interact with more than one amine system, but that one system predominates in terms of an animals' ability to discriminate a given chemical structure (or related structure). That is, rats, and probably humans, use one of these amine systems as a means of detecting a drug even though that drug may be affecting other systems as well. For example, the (+)-amphetamine discriminative stimulus seems to be mediated, centrally, through some catecholamine pathway (DA and/or NE), but it also affects 5-HT systems as evidenced by its partial generalization with quipazine (this is depicted as a simple schematic in Fig. 8). Thus, a rat trained to discriminate quipazine may recognize amphetamine somewhat like quipazine because of this 5-HT effect. (±)-Cathinone generalizes completely with (+)-amphetamine but generalizes

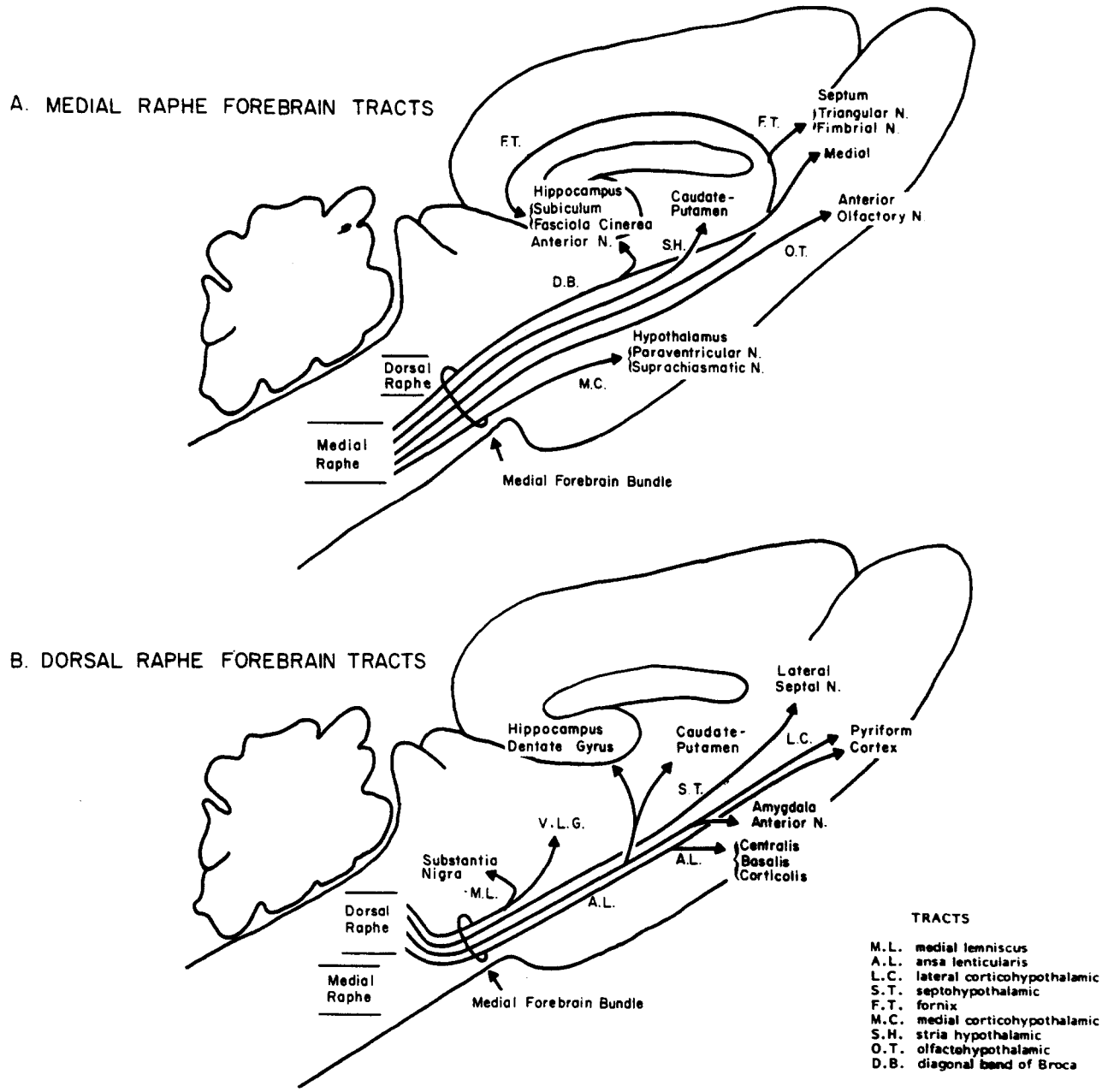


FIG. 7. A diagramatic representation of 5-HT projection systems originating from either the dorsal or medial raphe nuclei. Note that post synaptic 5-HT sites seem to be located on several areas rich in NE and DA receptors, i.e. substantia nigra, cuadate putamen, hypothalamus and hippocampus. For a better understanding of these relationships see Rosecrans *et al.* [37].

with quipazine more than did (+)-amphetamine. Thus, cathinone may provide us with an unusual example of a compound with a more equally distributed mechanism of action which crosses over both 5-HT and catecholamine neurons. Quipazine shares many properties with LSD, but LSD also appears to have a dopaminergic component as evidenced by the fact that apmorphine will generalize partially with LSD. (+)-Amphetamine, even though it shares many properties with apomorphine, generalizes with apomorphine only partially (Minema and Rosecrans, unpublished observations), and may represent another subtle difference between these two agents and how they effect dopamine and/norepinephrine neurones. (Generalization between apomorphine and (+)-amphetamine has been a controversial issue; not all laboratories have demonstrated that these two agents produce similar cues. Ho and Silverman [28], for example, present data both for partial and complete generalization between these two agents.) Finally, 5-OMe DMT, which generalizes completely with LSD, generalizes only partially with quipazine, but not at all with amphetamine or

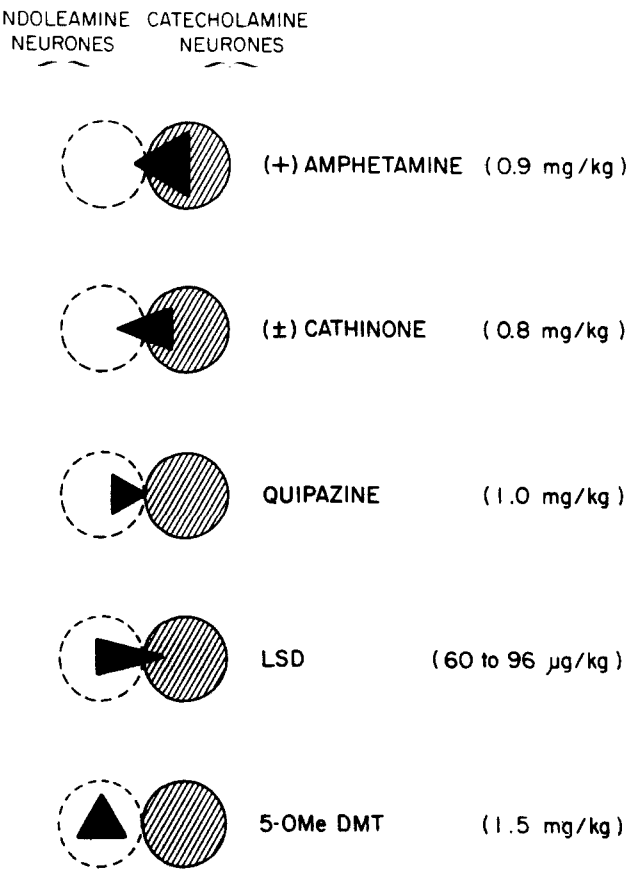


FIG. 8. A diagramatic representation of the relative effects of various psychoactive agents on either or both indoleamine and catecholamine neurons. The triangles are intended to roughly represent the proportionate effect of each agent on these neuronal systems.

cathinone. This would place 5-OMe DMT at the other end of the continuum going from the catecholamine extreme to the 5-HT extreme. In fact much data has been generated which suggests that 5-OMe DMT is acting much like 5-HT with minimal effects on other biogenic amine pathways.

Thus, while the picture is quite complex, there does seem to be some order to how these drugs may be producing their affects at brain receptors. As more data are obtained, we will begin to increase the length of continuum of drug effects, and maybe then, will we be able to make some sense out of these data, especially in terms of how the brain is functioning and how drugs may be producing their respective psychopharmacological effects in human subjects.

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