INVOLVEMENT OF DOPAMINE AND 5-HYDROXYTRYPTAMINE NEURONAL SYSTEMS IN THE BEHAVIORAL EFFECTS OF HALLUCINOGENS

By

Randall Lee Commissaris

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology and Toxicology

1981

ABSTRACT

Involvement of Dopamine and 5-Hydroxytryptamine Neuronal Systems in the Behavioral Effects of Hallucinogens

by

Randall Lee Commissaris

The effects of a number of hallucinogens and non-hallucinogenic agents were examined in rats performing on a fixed ratio-40 (FR-40) operant schedule for food reinforcement. Many psychoactive agents disrupt this behavior. However, hallucinogens disrupt FR-40 responding uniquely, characterized by periods of non-responding or "pausing" interspersed between periods of responding close to the control rate. A number of non-hallucinogenic psychoactive drugs examined disrupted this behavior with a pattern of slowed and erratic intrasession response rates rather than pausing. The development of a 10-second pause interval counter allowed for the quantification of this dose-dependent "pausing" produced by the hallucinogens. Since the non-hallucinogens examined in these early studies produced increases in "pausing" only at doses which decreased response rates dramatically, the pause interval counter was used to classify agents as similar or dissimilar to hallucinogens.

The neurotransmitter basis for the "pause" effect of the hallucinogens was explored. Studies employing the neurotoxin 6-hydroxydopamine, the catecholamine synthesis inhibitor α-methyl-p-tyrosine,

the neuroleptics haloperidol and chlorpromazine and the dopamine releasing agent d-amphetamine were conducted. These treatments failed to alter the pause-inducing effects of 2,5-dimethoxy-4-methylamphetamine (DOM) or d-lysergic acid diethylamide (LSD), suggesting that the "pause" effect does not appear to be mediated via brain catecholamine mechanisms.

A second line of investigation into the neurotransmitter basis for the effects of the hallucinogens centered on the role of 5-hydroxytryptamine (5-HT). Initial studies were conducted employing the intraventricular administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) or systemic administration of the 5-HT synthesis inhibitor p-chlorophenylalanine to decrease whole brain 5-HT concentrations. In these studies, depletion of whole brain 5-HT potentiated the effects of LSD, DOM and mescaline equally. These studies implicated 5-HT neurons in the mechanism of action of the hallucinogens and suggested similarities in this mechanism.

In an effort to localize the site of the hallucinogen-5-HT neuronal interaction, local injections of 5,7-DHT were made into nucleus accumbens and septal nuclei. These failed to alter the "pause" effect of DOM, LSD or mescaline. Injection of 5,7-DHT into the medial forebrain bundle caused only moderate depletion of 5-HT in forebrain areas, slightly potentiated the FR-40 disruption by LSD, attenuated somewhat the influence of DOM, while not altering the "pause" effect by mescaline.

A number of drug interaction studies were carried out using putative 5-HT agonists and antagonists. Initial studies employing these

agents alone revealed two important findings: 1) the putative 5-HT agonists quipazine and m-chlorophenylpiperazine (MCPP) produced a dose-dependent disruption of FR-40 behavior characterized by "pausing", and 2) the putative 5-HT antagonists methergoline and cinanserin alone actually decreased the "pausing" observed in control sessions. Thus, "pausing" produced by the hallucinogens may relate to 5-HT agonistic properties of these agents. The "pausing" produced by MCPP or quipazine was found to be additive to the effects produced by DOM. Pretreatment with cinanserin or methergoline shifted the dose-response curves of indolealkylamine (LSD-type) hallucinogens about 2- to 4-fold to the right. These 5-HT antagonists (particularly methergoline) caused a much greater shift to the right in the dose-response curves for the phenethylamine (DOM-type) hallucinogens, however. Reducing the methergoline pretreatment from 1.0 mg/kg to 0.1 mg/kg decreased the extent of the shift in the DOM dose-response curve for disrupting FR-40 responding. Methergoline antagonized the "pause" effects of quipazine in a manner similar to that observed with DOM.

The results support a role of brain 5-HT receptors in the pattern of disruption of FR-40 responding induced by the indole and phenethylamine hallucinogens. They further suggest that the two classes exert their actions on 5-HT neuronal functions by somewhat different mechanisms.

ACKNOWLEDGEMENTS

The author extends his sincere thanks to Dr. Theodore M. Brody, Dr. Walter K. Beagley and Dr. Gerard L. Gebber of his graduate committee for their support and constructive comments throughout the course of these studies. The author extends a special thanks to Dr. Kenneth E. Moore of his committee for his assistance throughout these studies, particularly in their early stages. The author is grateful for the assistance and consultation of a number of individuals throughout these studies, in particular Richard Alper, Lisa Bero, John Cordon, Russell Owen, Charles Rewa and Barbara Stetler. Lastly, the author sincerely thanks Dr. Richard H. Rech for his advice, encouragement and support throughout the course of these studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

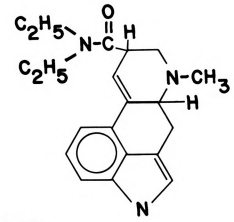
LIST OF FIGURES (continued)

GENERAL INTRODUCTION

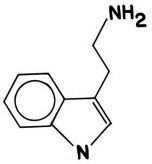
Hallucinations often occur in persons with certain mental disorders. They can also be drug-induced. The precise mechanism(s) for the production of hallucinations from either cause is unclear, although interactions with dopamine (DA) and/or 5-hydroxytryptamine (5-HT) neurons in the brain have often been proposed (see reviews by Brawley and Duffield, 1972; Jacobs, 1978).

The most specific types of hallucinogens are divided into two general classes based on their chemical structures. Indolealkylamine hallucinogens, of which d-lysergic acid diethylamide (LSD) and N,N-dimethyltryptamine (DMT) are members, resemble the indolealkylamine neurotransmitter 5-HT (Figure 1). 2,5-Dimethoxy-4-methylamphetamine (DOM) and mescaline are members of the phenylethylamine hallucinogen class; members of this class resemble the catecholamine neurotransmitter DA (Figure 2). Because of the similarities between the structures of the hallucinogens and these neurotransmitters, it is tempting to speculate that the effects of the indolealkylamine and phenylethylamine hallucinogens are mediated through 5-HT and DA neuronal systems, respectively. However, considerable behavioral experimental evidence has suggested that agents of both classes produce effects related to both 5-HT and DA neuronal interactions (Brawley and Duffield, 1972; Jacobs, 1978).

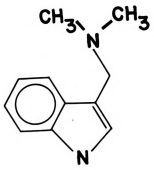
Figure 1. The chemical structures of the indolealkylamine hallucinogens d-lysergic acid diethylamide (LSD) and N,N-dimethyltryptamine (DMT) and the indolealkylamine neurotransmitter 5-hydroxytryptamine (5-HT).



LSD



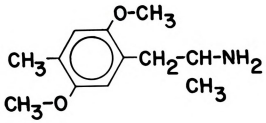
5-HT



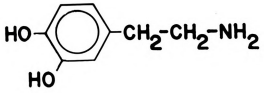
DMT

Figure 1

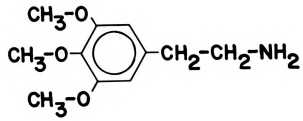
Figure 2. The chemical structures of the phenethylamine hallucinogens 2,5-dimethoxy-4-methyl-amphetamine (DOM) and mescaline and the catecholamine neurotransmitter dopamine (DA).



DOM



DA



MESCALINE

Figure 2

In addition to the phenethylamine and indolealkylamine hallucinogens, atropine-like anticholinergic agents have been reported to produce hallucinations in man (Byck, 1975). The effects of these agents differ from the "classical" hallucinogens in that the atropine-like compounds produce hallucinations characterized by confusion, delusions and memory disturbances. The effects of these atropine-like agents will not be examined in these studies. Drugs in other classes may also induce hallucinations under certain conditions. However, their primary pharmacology generally focuses on some other effects on bodily functions; therefore, they will not be considered here.

I. 5-HT Neurons and the Effects of Hallucinogens

A. Anatomy of 5-HT Neurons (Figure 3)

Cell bodies of 5-HT neurons in the brain are located primarily in the dorsal and median raphé nuclei of the brainstem (Cooper et al., 1974). Axons from many of these cells form a major portion of the medial forebrain bundle (MFB; Lorden et al., 1979), which projects to many areas of the brain receiving 5-HT input.

B. Hallucinogen Interactions with 5-HT Neurons

Alterations in the activity of 5-HT neurons and receptors have been implicated in the mechanism of action of hallucinogens. Studies demonstrating the antagonism of 5-HT-induced contractions of gut strips by LSD in vitro led to the early hypothesis that LSD produced hallucinations by acting as an antagonist of 5-HT receptors in the brain (Gaddum, 1953; Wooley and Shaw, 1954). Since 5-HT is generally regarded as having an inhibitory modulating effect on many

Figure 3. Anatomical distribution of 5-HT neurons. Mid-sagittal diagram of the rat brain illustrating the location of 5-HT neurons and their projections (from Cooper et al., 1978).

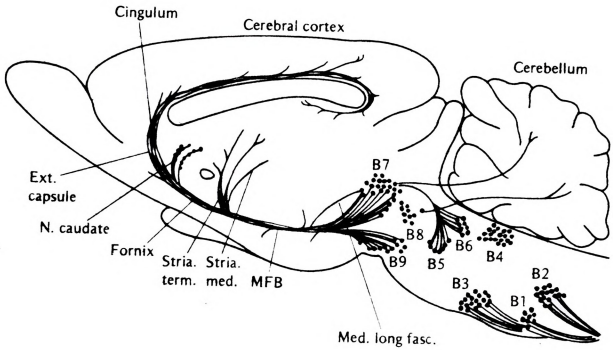

Cingulum
Cerebral cortex
Cerebellum
Ext.
capsule
N. caudate
Fornix
Stria.
term.
Stria.
med.
MFB
B7
B8
B9
B6
B5
B4
B3
B2
B1
Med. long fasc.

Figure 3

areas of the brain (Aghajanian and Wang, 1978; Bloom et al., 1972; Haigler and Aghajanian, 1977), the putative 5-HT antagonist could produce hallucinations via disinhibition. Recent evidence, as discussed below, has failed to provide much support for this hypothesis.

Microiontophoretic and electrophysiological studies by Aghajanian and co-workers (Aghajanian et al., 1970, 1972, 1975; Aghajanian and Haigler, 1974, 1975; DeMontigny and Aghajanian, 1977; Haigler and Aghajanian, 1973, 1977) have suggested that LSD is an agonist at 5-HT receptors on both the cell bodies of neurons in the raphé nuclei (autoreceptors) and on postsynaptic sites at the projections of these neurons. The effects of systemically administered LSD are greatest at these autoreceptors, where they inhibit 5-HT neuronal activity (Aghajanian et al., 1975; Aghajanian and Haigler, 1974, 1975; DeMontigny and Aghajanian, 1977; Haigler and Aghajanian, 1973, 1977). Brom-LSD, a non-hallucinogenic analogue of LSD, is devoid of this action on 5-HT autoreceptors (Aghajanian, 1976). Many other hallucinogens have been shown to activate, albeit indirectly, the 5-HT autoreceptors to decrease 5-HT neuronal activity (Aghajanian et al., 1970; Haigler and Aghajanian, 1973). It has been proposed, therefore, that the effects of hallucinogens are due to activation of 5-HT autoreceptors which inhibits 5-HT neuronal activity and disinhibits other areas of the brain.

Recent reports by Trulson and co-workers, however, have suggested that activation of 5-HT autoreceptors may not be responsible for producing the behavioral effects of hallucinogens. Chronic administration of LSD has been reported to produce rapid tolerance (3-5

days) to the behavioral effects of LSD and other hallucinogens (Freedman et al., 1963; Appel and Freedman, 1968; Winter, 1971; Rech, 1975). Trulson et al. (1977a) found that chronic administration of LSD to rats in doses which have been reported to produce dramatic tolerance as measured by a number of behavioral tests did not change the effects of LSD on the activity of single cells in the midbrain raphé nucleus. These studies have been repeated in unanesthetized, freely-moving cats (Trulson and Jacobs, 1978), in which it was reported that the aberrant grooming patterns produced by LSD dissipated over the course of repeated administrations of the drug (tolerance). However, the effects of LSD to inhibit raphé unit activity in these same animals persisted. These data suggest that the behavioral effects of hallucinogens may not be mediated solely by the activation of 5-HT autoreceptors. The results of work by Rogawski and Aghajanian (1979) have further impugned the raphé inhibition hypothesis as the theoretical basis for the mechanism of action for the hallucinogens. These latter investigators found that the non-hallucinogenic LSD analogue lisuride inhibits raphé neuronal activity similar to LSD. Unfortunately, in these studies the effect of lisuride on the activity of post-synaptic 5-HT neurons was not examined. This is a critical omission since the raphé inhibition hypothesis was based on the relative potency of LSD for these two sites (autoreceptors vs. post-synaptic receptors).

Behavioral studies by Minnema et al. (1980) have cast even further doubt on the raphé inhibition hypothesis. These investigators, using a drug-discrimination technique (described below), have

found that administration of LSD directly into the median/dorsal raphé produces surprisingly weak discriminative effects in subjects trained to discriminate systemic LSD from saline. Presumably, if raphé autoreceptor activation is sufficient for the discriminable effects of LSD, then extremely low doses would be required by this local application. In defense of the technique, similar studies employing the administration of morphine have shown that minute quantities of morphine placed into the periaqueductal gray area of the brain (presumed site of morphine action) in subjects trained to discriminate systemic morphine from saline produces lever-pressing almost exclusively on the morphine-bar (Krynock and Rosecrans, 1979). These reports also suggest that the raphé inhibition hypothesis for the behavioral effects of hallucinogens is questionable.

Considerable evidence has accumulated to suggest that hallucinogens act as postsynaptic agonists at 5-HT receptors. Much of the behavioral evidence for the 5-HT agonistic actions of hallucinogens comes from stimulus control experiments in the laboratories of Appel, Rosecrans and Winter (Cameron and Appel, 1973; Winter, 1974; Schecter and Rosecrans, 1972). In a two-bar test cage rats were trained to discriminate between the effects of a hallucinogen and saline. When a hallucinogen was administered, bar pressing on bar A produced food reinforcement; when saline was administered, bar pressing on bar B produced food reinforcement. Rats trained on this task will perform at a level of at least 95% correct responses. To summarize the results of many recent studies, rats trained to discriminate the effects of an hallucinogen (of either class) from those of saline generalize universally to all phenethylamine and indolealkylamine

hallucinogens examined but not to the non-hallucinogens d-amphetamine, apomorphine and methylphenidate (Glennon et al., 1979, 1980; Schechter and Rosecrans, 1972; Winter, 1975, 1978, 1980; Silverman and Ho, 1980; Kuhn et al., 1978; Shannon, 1980; Järbe, 1980; Tilson et al., 1975a; Browne and Ho, 1975). These effects are selectively blocked by the putative 5-HT antagonists cinanserin, methysergide, methiothepin and BC-105, but not by the DA antagonists butaclamol and haloperidol (Glennon et al., 1979; Winter, 1969, 1978, 1980; Kuhn et al., 1978; Silverman and Ho, 1980; Browne and Ho, 1975).

Studies by Andén et al. (1968; 1971; 1974) have shown that the exaggerated extensor reflex in reserpine-treated rats (specific for 5-HT agonists) can be produced by the hallucinogens LSD, DMT, DOM and mescaline. Joseph and Appel (1977) reported that the behavioral effects of LSD on operant responding are potentiated following depletion of 5-HT produced by either p-chlorophenylalanine (PCPA; 5-HT synthesis inhibitor) or intraventricular administration of 5,7-dihydroxytryptamine (5,7-DHT), an agent which selectively destroys 5-HT neurons in the brain. Since recent reports have demonstrated that "denervation supersensitivity" occurs in the 5-HT system following 5,7-DHT administration (Nelson et al., 1978), these data also may support a 5-HT agonist theory for the mechanism of action of LSD. Thus, there is considerable evidence to propose that hallucinogens may act as agonists at 5-HT receptors in the brain.

Hallucinogens have also been reported to increase responding that is normally suppressed by punishment (Schoenfeld, 1976). This

influence is not nearly as prominent as with the classical "anti-anxiety" agents of the benzodiazepine and barbiturate classes, but the effects are reproducible. These effects have been suggested to relate to interactions of hallucinogens with 5-HT neurons (Schoenfeld, 1976).

Further evidence for the 5-HT agonistic actions of hallucinogens comes from studies with the putative 5-HT agonist, quipazine. This compound produces head twitches in mice similar to LSD (Vetulani et al., 1980) and has been shown to produce disruptions of complex operant behavior in rats (multiple schedule: fixed ratio-15, fixed interval-60 seconds) similar to the disruptions produced by LSD (Poling and Appel, 1978). In addition, stimulus control experiments by Kuhn et al. (1978) have indicated that the cue produced by hallucinogens generalizes to quipazine. Quipazine has also been reported to produce stimulus cues; similarly, this cue has been shown to generalize to hallucinogens and to be antagonized by the 5-HT antagonists BC-105, cinanserin, cyproheptadine, methiothepin and methysergide (White et al., 1977; Winter, 1979).

In summary, evidence from a number of studies has suggested that hallucinogens of both the phenethylamine and indolealkylamine classes produce effects that are mediated via 5-HT neuronal systems.

II. DA Neurons and the Effects of Hallucinogens

A. Anatomy of DA Neurons (Figure 4)

In contrast to the 5-HT neuronal system, in which axons radiate out from the cell bodies in the raphé nuclei to innervate nearly all areas of the brain, the DA neuronal pathways are composed

Figure 4. Anatomical distribution of DA neurons. Mid-sagittal diagram of the rat brain illustrating the location of DA neurons and their projections (from Cooper et al., 1978).

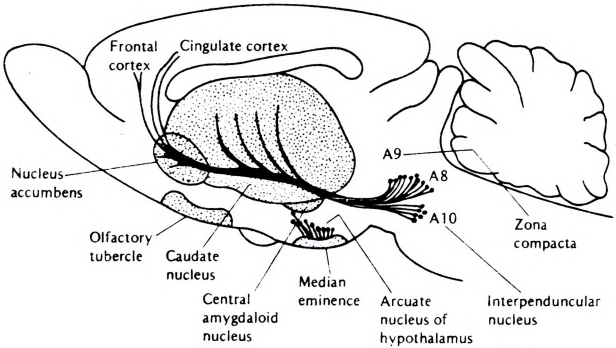

Frontal cortex
Cingulate cortex
Nucleus accumbens
A9
A8
A10
Zona compacta
Olfactory tubercle
Caudate nucleus
Median eminence
Central amygdaloid nucleus
Arcuate nucleus of hypothalamus
Interpenduncular nucleus

Figure 4

of three distinct systems, the nigrostriatal, the tuberoinfundibular and the mesolimbic systems. Each DA system is characterized by the distinct location of the cell bodies and the distinct structure(s) which are innervated.

DA neurons in the zona compacta of the substantia nigra give rise to axons which innervate the striatum. This nigrostriatal DA system has been implicated in the stereotypic behavior observed following agents which cause activation of DA receptors. DA-containing neurons are also found in the arcuate nucleus of the hypothalamus, which project to the median eminence; these neurons release DA into the hypophyseal portal bloodstream; this DA acts on the anterior pituitary to inhibit the release of prolactin. The last DA system is the mesolimbic system; cells from the ventral tegmental region of the brain project to a number of areas including the septum, the amygdala, the olfactory tubercles and the nucleus accumbens. These regions are part of the limbic system and are presumed to be important in the control of emotions. It is obviously this mesolimbic DA system which is of greatest interest in investigations on the effects of hallucinogens.

B. Hallucinogen Interactions with Dopamine Neurons

There is a good deal of evidence to suggest that hallucinogens can act as agonists at DA receptors in the brain. Hallucinogens have been reported to stimulate motor activity and produce stereotyped behavior (Koella et al., 1964; Tilson et al., 1975b; Yamamoto and Ueki, 1975). Both of these functions are presumed to be regulated by DA neuronal activity. Moreover, the use of neuroleptics (DA antagonists)

in the clinical treatment of "bad trips" produced by hallucinogens (Jaffe, 1975) suggests that the effects of hallucinogens may be mediated through DA neurons. In addition, Marrazzi and co-workers (Halasz et al., 1969; Marrazzi and Huang, 1979) have shown that the inhibition of cortical unit firing produced by the intravenous administration of LSD is attenuated by the neuroleptic chlorpromazine.

There is evidence to indicate that hallucinogens can act as both direct and indirect agonists at DA receptors. Evidence that LSD is a direct agonist at DA receptors in the brain comes from studies in which some of the effects of LSD were shown to be pharmacologically similar to apomorphine: 1) the reversal of reserpine-induced depression of motor activity, even after treatment with the putative 5-HT antagonist methysergide (Menon et al., 1977); 2) contralateral turning following unilateral pretreatment with 6-hydroxydopamine (6-OHDA; an agent which selectively destroys DA neurons) into the substantia nigra (Trulson et al., 1977b); 3) autoinhibition of the activity of DA cells in the substantia nigra (Christoph et al., 1977). In addition, Nichols (1976) has noted strong similarities in the chemical structures of LSD and apomorphine.

Evidence regarding other hallucinogens suggests possible indirect agonistic effects at DA neurons. Mescaline and DOM produced ipsilateral turning in animals with unilateral 6-OHDA lesions (Trulson et al., 1977b). Moreover, Vrbanac et al. (1975) have shown that DOM releases catecholamines from the brain. In producing these effects these hallucinogens resemble d-amphetamine, a compound known for its actions as an indirect DA agonist (Rech and Stolk, 1970; Chieuh and

Moore, 1973) and also capable of producing hallucinations in man under chronic conditions of abuse (Innes and Nickerson, 1975; Nielsen et al., 1980).

Thus, there is experimental data to suggest that both 5-HT and DA neurons may be important in mediating the behavioral effects of the indolealkylamine and phenylethylamine hallucinogens. The proposed mechanisms are not mutually exclusive, however, as the effects of these agents may be mediated by influences on both of these neuronal systems.

III. Techniques Used for Disrupting 5-HT or DA Neuronal Activity

Several techniques have been used to disrupt 5-HT or DA neuronal activity. These procedures include electrolytic lesions, surgical procedures such as axotomy or surgical isolation, and administration of short-acting neuroactive drugs (receptor agonists and antagonists; synthesis inhibitors). More recently, the neurotoxins 6-OHDA and 5,7-DHT have been used to produce relatively long-lasting and specific destruction of catecholamine and 5-HT nerve terminals, respectively (Bloom et al., 1969; Breese and Traylor, 1970, 1971; Uretsky and Iversen, 1970; Baumgarten et al., 1973; Björklund et al., 1974, 1975; Daly et al., 1974).

Electrolytic lesions or surgical procedures (axotomy, surgical isolation) can be used to alter neuronal activity, but these techniques generally do not destroy only one neuronal system. The potential for nonspecific effects in the techniques mentioned above precludes their use in studies designed to determine the role(s) of DA

and 5-HT in the manifestation of the behavioral effects of hallucinogens.

A. Neurotoxin Treatments

Recent developments in the use of the neurotoxins 5,7-DHT and 6-OHDA have greatly enhanced their selective toxicity for 5-HT and DA neurons, respectively. The neurotoxins are initially taken up by presynaptic nerve terminals, presumably by the amine reuptake mechanisms in these neurons. Once inside the presynaptic terminal, they produce destruction of the terminal by a mechanism which is still unknown. Since uptake into the presynaptic terminal is a critical step in the neurotoxicity of these agents, selective monoamine reuptake blocking agents would be expected to alter the extent of disruption by preventing the uptake of the neurotoxin into the neurons. Perhaps the best example of this effect is the use of the tricyclic antidepressants desipramine and protriptyline to protect norepinephrine (NE) neurons from the neurotoxicity of both 5,7-DHT and 6-OHDA (Björklund et al., 1975; Gerson and Baldessarini, 1975; Hole et al., 1976; Breese and Howard, 1971). Another manipulation which has been reported to enhance the cytotoxicity of the neurotoxins is treatment with a monoamine oxidase inhibitor. Monoamine oxidase is the intraneuronal enzyme responsible for breakdown of monoamines following their reuptake into the presynaptic terminal. It is thought that this enzyme may also be capable of metabolizing the neurotoxins as well, thus reducing their activity. Pargyline, a monoamine oxidase inhibitor, has been shown to enhance the cytotoxicity of these neurotoxins (Breese et al., 1978) presumably by delaying their metabolism to less active agents.

These agents do not directly affect the post-synaptic neuron; however, compounds which activate post-synaptic receptors (receptor agonists, amino acid precursors) have greater activity in neurotoxin-treated animals as compared to controls (Ungerstedt, 1971; Daly et al., 1974; Stewart et al., 1976; Trulson et al., 1976). In agreement with this "behavioral supersensitivity" is the observation that the number of radiolabelled binding sites (receptor sites) increases following administration of 5,7-DHT (Nelson et al., 1978). Conversely, agents which require a functional (releasable) presynaptic terminal in order to produce their effects (indirect agonists) are considerably less effective following these neurotoxin treatments.

B. Interactions with Neuroactive Drugs

As mentioned above, a number of agents with established mechanism(s) of action used clinically and/or experimentally can be employed as aides in determining the involvement of 5-HT and/or DA neurons in the behavioral effects of hallucinogens. These agents include receptor antagonists, receptor agonists and drugs that inhibit the synthesis of 5-HT or DA.

1. Receptor antagonists. Haloperidol and chlorpromazine are both clinically effective antipsychotic neuroleptics which presumably act as DA receptor antagonists (Byck, 1975). The list in the experimental literature of putative 5-HT antagonists is long, but selecting potent and selective antagonists at central 5-HT receptors is a difficult task. A number of compounds including cinanserin, mianserin, methysergide and cyproheptadine have been reported to

possess peripheral 5-HT antagonist properties, but reports regarding their central 5-HT antagonistic actions are inconsistent (Jacoby et al., 1978; Vargaftig et al., 1971). In addition, these agents produce various non-specific effects, presumably through interactions with other neuronal systems. Methergoline (1-methyl-8-carbobenzyloxyaminomethyl-10α-ergoline) recently has been reported to be a potent and relatively selective central 5-HT antagonist (Samanin et al., 1977, 1979, 1980; Fuxe et al., 1975).

2. Receptor Agonists. Direct activation of DA receptors by the compounds piribedil and apomorphine produces, at low to moderate doses, stereotyped behavior and aphagia (McGeer et al., 1978). Since these two effects greatly interfere with operant responding for food reinforcement, these agents are likely to be ineffective as tools to study the neuronal basis for the effects of hallucinogens in a FR-40 appetitive procedure. The psychomotor stimulant d-amphetamine has been proposed to produce many of its behavioral effects by enhancing the release of DA from nerve terminals (Rech and Stolk, 1970; Chieuh and Moore, 1973). Although this agent also produces aphagia and stereotyped responding, these effects generally require doses greater than those which produce stimulation of motor activity and disruption of operant behaviors (Fibiger et al., 1973).

Quipazine has been proposed to act as a 5-HT agonist (Rodriguez et al., 1973), although a number of additional neurochemical effects have been reported to be produced by this agent. As described above, this agent produces a number of behavioral effects similar to the hallucinogens (Vetulani et al., 1980; White et al.,

1977; Winter, 1978, 1979). m-Chlorophenylpiperazine (MCPP) is a recently developed tool for studying 5-HT neurons. This agent is an extremely effective drug in suppressing feeding behavior, an effect which is presumably related to 5-HT agonistic neuronal activity (Samanin et al., 1979, 1980).

3. Synthesis Inhibitors. Inhibition of catecholamine synthesis by α-methyl-p-tyrosine (αMT) is an extremely selective and potent technique for determining the role of stored catecholamines in mediating the effects of a particular agent (Rech et al., 1966; Spector et al., 1967). Although NE synthesis is also altered by αMT treatment, this effect on NE neurons usually requires larger doses over a longer period and is less dramatic than the effects of αMT on DA synthesis. This differential susceptibility presumably relates to the relatively slow turnover of brain NE compared to that of DA (Demarest et al., 1979).

The phenylalanine analogue PCPA has been used as an effective tool for selectively decreasing stored 5-HT concentrations (Koe and Weissman, 1966) and to examine the role of 5-HT neurons in a number of behaviors (Robichaud and Sledge, 1969; Tenen, 1967; Geller and Blum, 1970; Tilson and Rech, 1974).

STATEMENT OF PURPOSE

The purpose of the present study was to determine if 5-HT or DA neuronal systems play a role in certain aspects of the behavioral effects of hallucinogens in rats. The behavioral effects of both indolealkylamine and phenethylamine hallucinogens were examined in untreated animals and in those in which 5-HT or DA neuronal activity was selectively disrupted by systemic pretreatment with 5-HT or DA synthesis inhibitors, receptor agonists and antagonists and by the intracerebral administration of the neurotoxins 6-OHDA and 5,7-DHT.

MATERIALS AND GENERAL METHODS

I. Behavioral Paradigm

Since the prominent effects of hallucinogens in man are perceptual and subjective in nature, assessment of the effects of these agents in non-human subjects is extremely difficult. Although hallucinogens have been shown to produce a number of behavioral effects in experimental animals, many of these effects are unsuitable as models of the human pharmacology because they are produced only by high doses or are not easily quantifiable. One behavioral test which satisfies these criteria of sensitivity and quantifiability is the fixed ratio (FR) schedule of operant responding in rats, originally described by Ferster and Skinner (1957). Most of the behavioral data presented here are based on the disruptive effects of various drugs on responding in the FR-40 operant paradigm. The question of selectivity of this test in differentiating the effects of hallucinogens from those of a number of non-hallucinogenic psychoactive agents has been a source of contention in previous studies (Appel and Freedman, 1965; Rech et al., 1975) and is also addressed in this study.

II. Subjects

Subjects in all of the FR-40 studies were male Sprague-Dawley rats (Spartan Farms, Haslett, MI). These subjects were housed singly

in a room with a 12-hour day-night light cycle (lights on 0700-1900 hr) and maintained at approximately 70-80 percent of their free-feeding weights.

III. Apparatus

Behavioral testing was conducted between 0700 and 1900 hr in four standard operant chambers (LVE 143-20-215) equipped with food pellet dispensers. These chambers were located in sound-attenuating boxes and contained a single lever which required a force of 10-15 g to activate. All experimental events were controlled by electromechanical programming circuits and responses were recorded on electromagnetic counters and cumulative recorders. Two parameters were monitored in the operant sessions: (1) the number of reinforcements obtained, a reflection of the average response rate, and (2) the periods of non-responding during operant sessions. To accomplish this second measurement, a 10-second "pause" interval counter was incorporated into the program as described below.

Each response by the subject reset a 10-second timer. If the animal responded before 10 seconds elapsed, the timer reset and the program continued. If the animal failed to respond during this 10-second interval, a count was registered and the timer automatically reset. Therefore, the number of counts registered by the pause interval timer was an index of the extent of non-responding in terms of cumulated 10 second pause intervals.

The pause interval in these studies was selected to be 10 seconds for a number of reasons. First, 10 seconds is about the limit of the resolution possible on the cumulative records. Second, with an

interval shorter than 10 seconds, visual double-checking of the pause intervals against a cumulative record would not have been possible. Moreover, in pilot studies utilizing shorter intervals (5-sec, 1-sec), the "turnaround" time of the electromechanical programming equipment became prohibitively slow and introduced error into the measure. This effect on turnaround time often resulted in the development of a true negative; i.e., a situation in which the pausing of an animal is underestimated by the number of pause intervals produced due to the slow turnaround time of the timer. It was also found that longer durations (e.g., 20 seconds) failed to detect many of the "mini-pauses" which occurred during control sessions (usually following the delivery of the food pellet). Since baseline values in this measure are the major advantage of this technique over the "zero baseline" techniques of a number of researchers in this field (Kovacic and Domino, 1976; Kovacic et al., 1978; Ruffing et al., 1979; Ruffing and Domino, 1980) it was felt that the intervals longer than 10 seconds offered a less refined analysis (another true negative).

IV. Behavioral Procedure

The subjects were first trained to respond on a continuous reinforcement (CRF) schedule for food reinforcement (45 mg Noyes Pellets). Daily sessions were 40 minutes in duration. Each animal was run at the same time of day and in the same cage seven days a week. After the subjects were responding on the CRF schedule (approximately 2-4 days) a FR schedule was introduced and gradually (2-3 weeks) increased to FR-40. After an additional 2-3 weeks of control FR-40 sessions, behavioral testing was begun.

In all experiments the order of doses and drugs administered was randomized for each subject. All test drugs were administered intraperitoneally. In these studies drug test days were preceded by at least three non-drug days, unless otherwise specified, to avoid the development of tolerance.

V. Stereotaxic Procedures

In a number of studies the neurotoxins 5,7-DHT and 6-OHDA were administered into various brain regions in order to destroy 5-HT or catecholamine neurons, respectively. These subjects were then trained to perform in the FR-40 paradigm and the effects of various hallucinogens and non-hallucinogens were determined. Subjects in the neurotoxin studies weighed 150-200 gm at the time of neurotoxin administration. Prior to stereotaxic surgery all subjects were anesthetized with Equithesin (3 ml/kg); neurotoxins or vehicle (0.1% ascorbate in saline) were administered through a 30 gauge stainless steel cannula. All neurotoxin administrations were done bilaterally, with the exception of the septal-lesion study (unilateral structure). Table 1 lists the stereotaxic coordinates (König and Klippel, 1967), neurotoxin concentrations, volumes, infusion rates and pretreatments used in all of the neurotoxin studies to be presented.

VI. Neurochemical Analyses

Following completion of behavioral testing in the neurotoxin studies and studies involving PCPA administration the subjects were sacrificed, their brains were removed and the concentrations of 5-HT,

TABLE 1

Administration Parameters for FR-40 Neurotoxin Studies

	Stereotaxic Coordinates from Bregma*			Neurotoxin Concentration	Rate	Pretreatments
	Anterior Posterior	Lateral	Depth			
6-OHDA (HBr)						
intraventricular	0.0	1.5	-3.2	200 µg/10 µl	5 µl/min	----
5,7-DHT (creatinine sulfate)						
intraventricular	0.0	1.5	-3.2	180 µg/10 µl	5 µl/min	Desipramine (25 mg/kg + 45'), Pargyline (40 mg/kg + 40')
n. accumbens	+8.8	0.8	-4.8	8 µg/2 µl	1 µl/min	Desipramine + Pargyline
septum	+9.4	0.0	-4.6	4 µg/1 µl	1 µl/min	
medial forebrain bundle	+2.6	1.0	-7.4	6 µg/2 µl	1 µl/min	Desipramine + Pargyline

*König and Klippel, 1967.

NE, DA and the DA metabolite dihydroxyphenylacetic acid (DOPAC) in various regions were determined. Concentrations in large brain regions (i.e., cortex, hippocampus, hypothalamus and striatum) were analyzed by fluorimetric procedures (Curzon and Green, 1970; Chang, 1964); these analytical procedures were used in the intraventricular neurotoxin studies, in the study employing the administration of 5,7-DHT into the medial forebrain bundle, and in the studies employing PCPA administration. In studies employing the administration of 5,7-DHT into discrete brain regions (where tissue mass limits quantification using fluorimetric procedures; e.g., septum and nucleus accumbens), the concentrations of 5-HT, NE, DA and DOPAC were determined by a modification of the high performance liquid chromatography (HPLC) technique of Felice et al. (1978) using electrochemical detection (Lyness et al., 1980). In the study employing 6-OHDA administration in the nucleus accumbens, DA concentrations in this structure and the substantia nigra were determined using the radioenzymatic procedure of Umezu and Moore (1979); 5-HT concentrations in this instance were analyzed by the HPLC technique cited above for the septum.

VII. Statistical Analyses

Statistical analyses were conducted as described by Klugh (1974). Drug effects were assessed by comparing the data from test days to the average of the three days prior to the test day (baseline). The Student's t-test for paired data was used to evaluate the effects of individual doses of the drugs. Dose-response relationships were compared by analysis of variance (in a block design, when appropriate).

The effects of the neurotoxins and PCPA on regional biogenic amine concentrations were determined by Student's t-test for unpaired data. In all statistical evaluations $p<0.05$ was used as the criterion for statistical significance.

VIII. Drugs

The drugs employed in these studies are listed in Table 2, which indicates the supplier and chemical form used.

TABLE 2

Drugs Used in the FR-40 Operant Studies

Drug	Abbrev.	Dosage Form	Supplier
alpha-methyl-p-tyrosine	αMT	methyl ester	Merck, West Point, PA
d-amphetamine	d-A	sulfate	Sigma Chemical, St. Louis, MO
chlorpromazine	CPZ	hydrochloride	Smith, Kline and French Labs, Philadelphia
cinanserin	CIN	hydrochloride	Squibb
cocaine	COC	hydrochloride	Mallinckrodt, St. Louis, MO
desipramine	DMI	hydrochloride	Merrel Ind., Cincinnati, OH
5,7-dihydroxytryptamine	5,7-DHT	creatinine sulfate	Sigma Chemical
2,5-dimethoxy-4-methylamphetamine	DOM	hydrochloride	NIDA
N,N-dimethyltryptamine	DMT	free base	NIDA
haloperidol	HALO	free base	McNeil Labs, Ft. Washington
6-hydroxydopamine	6-OHDA	hydrobromide	Sigma Chemical
d-lysergic acid diethylamide	LSD	tartrate	NIDA
meta-chlorophenylpiperazine	MCPP	hydrochloride	Farmitalia Labs, Milan, Italy
methaqualone	MQ	free base	W.H. Rorer, Inc. Ft. Washington
methergoline	MTG	free base	Farmitalia Labs

TABLE 2 (Continued)

Drug	Abbrev.	Dosage Form	Supplier
mescaline	MESC	hydrochloride	NIDA
naloxone	NALOX	hydrochloride	Endo Labs, Garden City, NY
para-chlorophenyl-alanine	PCPA	free base	Aldrich Chemical
pargyline	---	hydrochloride	Sigma Chemical
pentobarbital	PB	sodium	Sigma Chemical
phenobarbital	PhB	sodium	Sigma Chemical
quipazine	QUIP	hydrochloride	Miles Labs, Elkhart, IN

RESULTS

Part I. Quantitation of the Disruptive Effects of Hallucinogens and Other Psychoactive Agents on FR-40 Operant Responding with the Pause Interval Timer (Counter)

A. Introduction

Hallucinogens produce a disruption of FR-40 behavior characterized by periods of nonresponding or "pausing" (Freedman et al., 1963; Appel and Freedman, 1964, 1965; Appel et al., 1970; Rech et al., 1975). This pausing occurs at doses which do not necessarily produce sedation or ataxia in these animals. Literature reports regarding the "pause-producing" effects of other psychoactive agents are somewhat less clear. Appel and Freedman (1965) have reported that the psychoactive stimulant d-amphetamine (1.0 mg/kg) produces "pausing" in FR responding. However, Rech et al. (1975) have also reported that the same dose of this agent produces a pattern of disrupted FR-responding characterized by slowed and erratic response rates not characterized by "pausing". Unfortunately, in both of the above studies the conclusions were made on the basis of cumulative records obtained from single subjects. With the use of the 10-second pause interval counter (described above) to quantitative FR-40 "pausing", we now feel that we can indeed resolve this controversy.

B. Methods

In subjects which had achieved stable FR-40 baseline response rates, the effects of a number of psychoactive agents were determined.

These agents included the hallucinogens DMT, DOM, LSD and mescaline, the central nervous system (CNS) stimulants d-amphetamine and cocaine, the CNS depressant phenobarbital, and the neuroleptic chlorpromazine. To further investigate the possible specificity of the "pausing" produced by the hallucinogens versus the disruption produced by d-amphetamine, a series of drug interaction studies were undertaken. In the first phase of this experiment, the effects of various doses of LSD or DOM alone or in combination with 0.25 mg/kg d-amphetamine were determined. This dose of d-amphetamine is at the threshold for the response-rate decreasing effects of this agent. In the second phase of this experiment, the effects of various doses of DOM alone or in combination with 5.0 mg/kg mescaline and 20 µg/kg LSD were determined.

C. Results

Figure 5 illustrates with cumulative recordings the results commonly obtained with saline, LSD (100 µg/kg) or d-amphetamine (1.0 mg/kg) administration immediately prior to the session. Control FR-40 sessions, the response patterns of which were not altered by saline administration, are characterized by a rapid, constant rate of responding with occasional brief pauses throughout the session. These brief pauses often, but not always, follow the delivery of a food pellet. Typically d-amphetamine produces a pattern of disrupted FR-40 responding characterized by slowed and erratic response rates without a significant increase in the extent of "pausing". This pattern of disruption results in a decrease in reinforcements received without any appreciable change in the number of pause intervals produced.

Figure 5. Cumulative recordings illustrating the effects of saline, d-amphetamine (d-A; 1.0 mg/kg) and LSD (100 µg/kg) administration on the pattern of FR-40 responding. Top trace of each panel: Each reponse produced a slight upward deflection of the pen. Approximately 550 responses brought the pen to the top of the record, activating a switch which returned the pen to the start position. Vertical hatch-marks indicate the delivery of a reinforcer. Bottom trace of each panel: Time between vertical hatch-marks equals ten minutes.

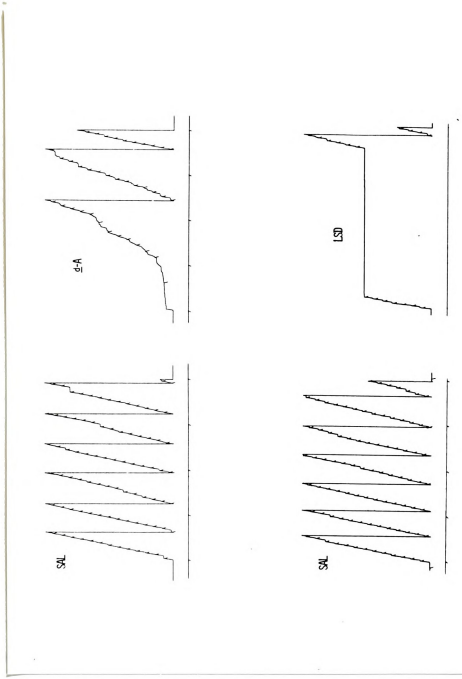


Figure 5

LSD, on the other hand, produces a pattern of disruption characterized by "pausing"; this pattern of disruption results in a decrease in reinforcements received and a concomitant increase in the number of pause intervals produced.

Quantification of these effects of LSD and d-amphetamine in groups of subjects is shown in Figure 6. LSD produces a dose-dependent decrease in reinforcements received and a dose-dependent increase in pause intervals. d-Amphetamine also produces a dose-dependent decrease in reinforcements received; however, unlike LSD, this agent does not produce a dose-dependent increase in pause intervals.

Effects similar to those observed with LSD (dose-dependent decrease in reinforcements paralleled by an increase in pause intervals) have been observed with the other hallucinogens investigated, DMT, DOM and mescaline. Figure 7a illustrates the dose-dependent "pausing" produced by these agents. It should be noted that the relative potency of these agents to produce "pausing" is similar to their potency as hallucinogens in man. It should also be noted that this dose-dependent increase in the duration of total pausing produced by both LSD and DOM is related primarily to a single long pause and not to a series of shorter pauses (Figure 7b). On the other hand, the stimulant cocaine, the depressant phenobarbital and the neuroleptic chlorpromazine have been shown to produce dose-dependent decreases in reinforcements without significantly increasing pausing until gross excitatory, ataxic or sedative doses are administered. In this respect these agents resemble d-amphetamine.

Figure 6. Quantitation of the effects of LSD and d-amphetamine on FR-40 operant responding. The change in pause intervals (circles; left-hand axis) and percent of control reinforcements obtained (squares; right-hand axis) produced by various of LSD (open symbols) or d-amphetamine (filled symbols) during FR-40 operant sessions are illustrated. Change in pause intervals and percent of control reinforcements were determined by comparing data obtained on test days to the average of the three days prior to the test day (baseline). Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects.

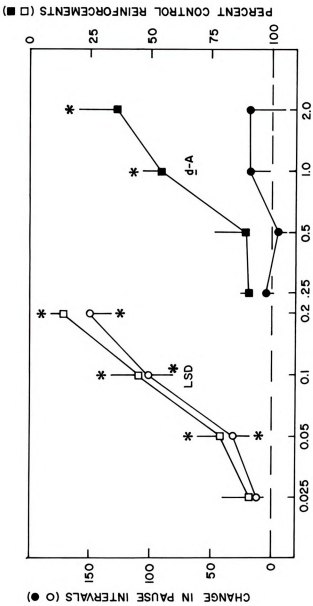

CHANGE IN PAUSE INTERVALS (○ ●)
150
100
50
0
PERCENT CONTROL REINFORCEMENTS (□ ■)
0
25
50
75
100
0.025
0.05
0.1
0.2
.25
0.5
1.0
2.0
DOSE (mg/kg)
LSD
d-A

Figure 6

Figure 7a. The effects of LSD, DOM, DMT and mescaline on FR-40 operant responding. The change in pause intervals is plotted for various doses of LSD (circles), DOM (squares), DMT (hexagons) and mescaline (triangles). Each symbol and vertical bar represents the mean ± S.E.M. for six to eight subjects. See Figure 6 legend for further details.

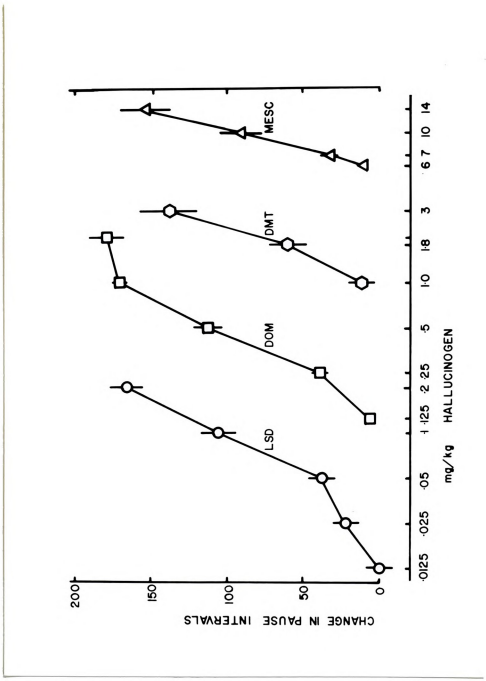

CHANGE IN PAUSE INTERVALS
200
150
100
50
0
LSD
DOM
DMT
MESC
·0125 ·025 ·05 1·125 2·25 5 10 18 3 6·7 10 14
mg/kg HALLUCINOGEN

Figure 7a

Figure 7b. The dose-relationships of LSD and DOM to the longest and second-longest periods of non-responding produced in FR-40 sessions. The length of the longest (open circles) and second-longest (filled circles) pauses produced by various doses of LSD and DOM are plotted. Pause lengths, defined as the distance between single responses, were determined by examination of cumulative records. One mm pause length equals 12 seconds of non-responding. LSD and DOM produced dose-dependent increases in the length of the longest pause. Neither drug had a significant effect on the duration of the second-longest pause. Each symbol and vertical bar represents the mean ± S.E.M. for seven subjects.

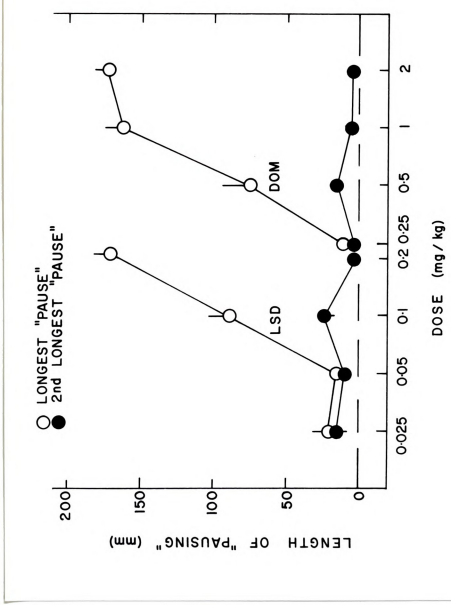

LONGEST "PAUSE"
2nd LONGEST "PAUSE"
LENGTH OF "PAUSING" (m)
200
150
100
50
0
LSD
DOM
0·025
0·05
0·1
0·2
0·25
0·5
1
2
DOSE (mg/kg)

Figure 7b

Since extremely high doses of any of these psychoactive agents can produce complete disruption of FR-40 responding, the best demonstration of this difference between the hallucinogens and non-hallucinogens investigated can be obtained when comparing the extent of pausing at doses which decrease reinforcements received to approximately 50% of control. Table 3 illustrates that the hallucinogens typically produce large (70-100 over baseline) increases in pause intervals at doses which decrease response rates to approximately 50% of control. On the other hand, d-amphetamine, cocaine, phenobarbital and chlorpromazine typically do not produce large increases in pausing at or near ED_{50} doses for decreasing reinforcements. We have occasionally observed "pausing" following administration of lower doses of the latter three agents, but this is clearly the exception and not the rule.

The results of the drug interaction studies, illustrated in Figures 8 and 9, indicated that co-administration of a threshold dose for the response-rate decreasing effects of d-amphetamine did not alter the dose-dependent "pausing" produced by either the indolealkylamine LSD or the phenethylamine DOM. Figure 10 illustrates that the dose-dependent "pausing" produced by DOM is enhanced by the administration of threshold doses of either the indolealkylamine LSD or the phenethylamine mescaline.

D. Discussion

Using the pause interval measurement in conjunction with response rates, the dose-dependent "pausing" produced by hallucinogens, alone or in combination, can be distinguished from the slowed and erratic

TABLE 3

Relationship Between Changes in Reinforcements and Increases in Pausing Induced by Hallucinogens and Non-Hallucinogenic Psychoactive Drugs

Drug and Dose	N	Percent of Control Reinforcements	Increase in Number of Pause Intervals
Hallucinogens			
0.5 mg/kg DOM	8	44± 8*	94±13*
100 µg/kg LSD	8	45±11*	102±21*
1.8 mg/kg DMT	8	52±10*	72±14*
7.1 mg/kg Mescaline	8	59± 6*	69± 6*
Non-Hallucinogens			
1.0 mg/kg d-amphetamine	8	54± 9*	18±16
25 mg/kg phenobarbital	8	63±11*	14±13
0.5 mg/kg chlorpromazine	7	67± 8*	17±10
30 mg/kg cocaine	4	46± 9*	27±21

Each value represents the mean ± S.E.M. Percent of control reinforcements and change in pause intervals were determined as described in Methods. See Methods for details of drug treatments.

*$p<0.05$, Student's t-test for paired values.

Figure 8. The effects of LSD on FR-40 responding alone or in combination with a threshold dose of d-amphetamine. The change in pause intervals produced by various doses of LSD alone (open circles) and in combination with 0.25 mg/kg d-amphetamine (filled circles) are plotted. This dose of d-amphetamine alone, plotted in the far left portion of the figure, had no effect on pausing. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects. d-Amphetamine administration did not alter the pause-producing effects of DOM. See Figure 6 legend for further details.

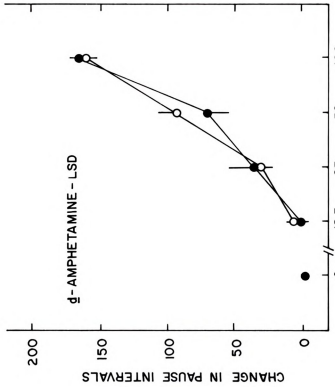

CHANGE IN PAUSE INTERVALS
200
150
100
50
0
d-AMPHETAMINE - LSD
0
12.5
25
50
100
µg / kg d-LSD

Figure 8

Figure 9. The effects of DOM on FR-40 responding alone or in combination with a threshold dose of d-amphetamine. The change in pause intervals produced by various doses of DOM alone (open circles) and in combination with 0.25 mg/kg d-amphetamine (filled circles). This dose of d-amphetamine alone, plotted in the far left portion of the figure, had no effect on pausing. Each symbol and vertical bar represents the mean ± S.E.M. obtained from eight subjects. d-Amphetamine administration did not alter the pause-producing effects of DOM. See Figure 6 legend for further information.

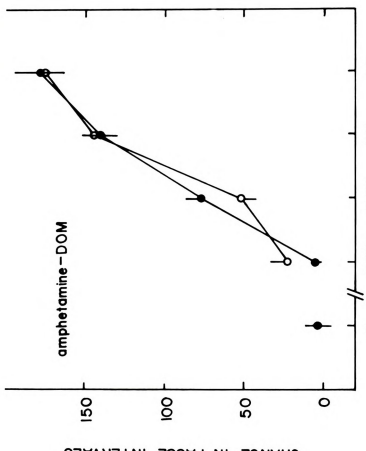

CHANGE IN PAUSE INTERVALS
150
100
50
0
amphetamine-DOM
0
0.125
0.25
0.5
1.0
mg / kg DOM

Figure 9

Figure 10. The effects of DOM on FR-40 responding alone or in combination with threshold doses of LSD or mescaline. The change in pause intervals produced by various doses of DOM alone (open symbols) and in combination with 20 μg/kg LSD (left panel, filled symbols) or 5.0 mg/kg mescaline (right panel, filled symbols) is plotted. The effects of 20 μg/kg LSD and 5.0 mg/kg mescaline alone, plotted in the far left portion of the left and right panels, respectively, were not significant. Each symbol and vertical bar represent the mean ± S.E.M. for 12 (LSD-DOM study) or 8 (mescaline-DOM study) subjects. Combination of either LSD or mescaline with DOM shifted the dose-response curve for DOM to the left ($p<0.05$, analysis of variance in a block design). See Figure 6 legend for further information.

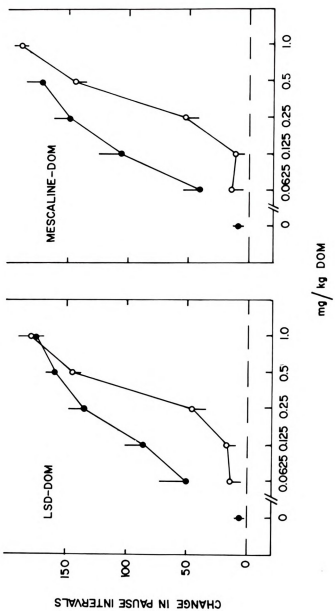

CHANGE IN PAUSE INTERVALS
150
100
50
0
LSD-DOM
MESCALINE-DOM
0
0.0625
0.125
0.25
0.5
1.0
mg/kg DOM

Figure 10

response rates produced by a number of psychoactive non-hallucinogenic drugs. Since co-administration of d-amphetamine failed to alter the "pausing" produced by either LSD or DOM, this agent clearly does not manifest the same spectrum of activity as do the hallucinogens.

Part II. The Role of Catecholamines in the FR-40 Disruptive Effects of Hallucinogens

A. Introduction

As reviewed in the General Introduction, there is considerable evidence to suggest that many of the behavioral effects of hallucinogens are mediated through catecholaminergic neurons in the brain. Members of both the indolealkylamine and phenethylamine classes of hallucinogens stimulate motor activity (Koella et al., 1964; Tilson et al., 1975b; Yamamoto and Ueki, 1975) and, at higher doses, produce stereotyped responding (Koella et al., 1964). Hallucinogens of both classes produce rotational behavior following unilateral destruction of dopaminergic neurons in the substantia nigra produced by 6-OHDA (Pieri et al., 1974; Trulson et al., 1977b). These behavioral effects of hallucinogens, which are also observed following administration of the psychomotor stimulant d-amphetamine (Tilson et al., 1975b; Weston and Overstreet, 1976; Trulson et al., 1977b), are presumed to be mediated by activation of catecholaminergic systems in the brain.

B. Methods

1. Neurotoxin study

The purpose of the first study in this section was to determine the role of catecholaminergic neuronal systems in the effects of

hallucinogens and d-amphetamine on FR-40 operant responding. LSD was chosen as the prototype indolealkylamine hallucinogen. The amphetamine analogue DOM was chosen as a representative member of the phenethylamine class of hallucinogens.

In this study, eight subjects received 6-OHDA and eight subjects received vehicle intraventricularly as detailed in Table 1. The subjects were allowed to recover from surgery, were trained to respond on the FR-40 schedule, and the effects of various doses of LSD, DOM and d-amphetamine were determined. Two of the 6-OHDA-treated subjects failed to reach stable FR-40 response levels and were therefore excluded from all aspects of the study. Following completion of the behavioral testing, the animals were sacrificed, their brains were removed, and the concentrations of DA, 5-HT and NE were determined by fluorimetric procedures as described in Materials and General Methods (Chang, 1964; Curzon and Green, 1970).

2. Drug interaction studies

In addition to the neurotoxin study, a number of drug interaction studies further investigating the role of catecholamines, particularly DA, in the disruptive effects of the hallucinogens were conducted. In these studies, the effects of the indirect DA agonist d-amphetamine, the catecholamine synthesis inhibitor αMT and the neuroleptics chlorpromazine and haloperidol on the dose-dependent "pausing" produced by the hallucinogens LSD and DOM were determined.

C. Results

1. Neurotoxin study

Table 4 summarizes the results of the neurochemical determinations in these subjects. 6-OHDA treatment significantly decreased DA and NE concentrations in all regions tested without altering the concentrations of 5-HT in any of the regions examined.

Table 5 indicates the effects of 6-OHDA-induced destruction of catecholamine neurons on control FR-40 responding. As described above, control FR-40 responding in vehicle-treated subjects was characterized by a rapid, constant rate of responding (approximately 100 responses/min) throughout the session. 6-OHDA treatment decreased the rate of responding, significantly reducing the number of reinforcements obtained, without significantly altering the number of pause intervals produced. Comparison of the effects of 6-OHDA treatment with other agents which disrupt FR-40 responding in regard to these two parameters of FR-40 behavior (Table 3 above) indicates that 6-OHDA is similar to d-amphetamine, cocaine, chlorpromazine and phenobarbital, but different from the hallucinogens.

Cumulative records illustrating the effects of saline, d-amphetamine and DOM in a vehicle- and a 6-OHDA-treated subject are depicted in Figure 11. In the vehicle-treated subject d-amphetamine and DOM produced dose-dependent disruptions of FR-40 operant responding which are both qualitatively and quantitatively different. d-Amphetamine typically produced erratic intrasession response rates, while DOM produced "pausing". Destruction of catecholamine neurons with 6-OHDA decreased the rate of responding relative to control, but

TABLE 4

Effects of Intraventricular 6-OHDA Administration on the Concentrations of 5-HT, DA and NE in Various Brain Regions

	5-HT		DA		NE	
	Vehicle	6-OHDA	Vehicle	6-OHDA	Vehicle	6-OHDA
Cortex	0.43±0.03	0.37±0.02 (86)	0.30±0.02	0.07±0.02* (23)	0.40±0.02	0.03±0.01* (8)
Hippocampus	0.46±0.05	0.32±0.05 (70)	n.d.	n.d.	0.31±0.01	0.04±0.01* (13)
Hypothalamus	1.01±0.04	1.09±0.06 (108)	n.d.	n.d.	1.67±0.10	0.10±0.01* (6)
Striatum	0.77±0.03	0.64±0.11 (83)	5.78±0.17	1.37±0.27* (24)	n.d.	n.d.

Data are expressed in μg amine/g wet tissue weight; each value represents the mean ± S.E.M. obtained from six 6-OHDA-treated (200 μg/10 μl x 2) or eight vehicle-treated animals. Numbers in parentheses represent concentration of amine in 6-OHDA-treated animals expressed as a percentage of vehicle-treated animals.

n.d. = amine concentration not determined.

*$p<0.05$, Student's t-test.

TABLE 5

Effects of Vehicle or 6-OHDA Administration on Control FR-40 Operant Response Parameters

	Reinforcements	Pause Intervals
Vehicle	96±15	56±12
6-OHDA	51± 7* (53)	81±12 (144)

Each value represents the mean ± S.E.M. obtained from six 6-OHDA-treated or eight vehicle-treated animals. Average response parameters for each animal were determined as the mean of the 30-40 control (no injection) FR-40 sessions throughout the study. Numbers in parentheses represent percent of control (vehicle-treated) values.

*$p<0.05$, Student's *t*-test.

Figure 11. Cumulative recordings illustrating the effects of saline, d-amphetamine (d-A) and DOM on FR-40 responding in one vehicle-treated and one 6-OHDA-treated rat. Top two rows: The effects of various doses of d-amphetamine (first row) and DOM (second row) administered to a vehicle-treated subject. Bottom two rows: The effects of various doses of d-amphetamine (third row) and DOM (fourth row) administered to a 6-OHDA-treated subject. Saline-treatment recordings illustrated on far left. See Figure 5 legend for further information.

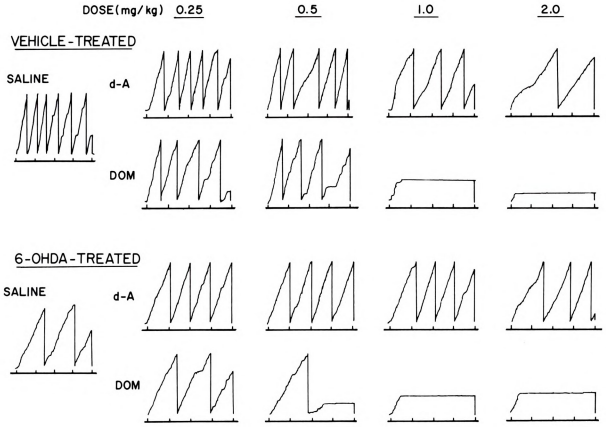

DOSE(mg/kg) 0.25
0.5
1.0
2.0
VEHICLE-TREATED
SALINE
d-A
DOM
6-OHDA-TREATED
SALINE
d-A
DOM

Figure 11

produced neither pausing nor erratic intrasession response rates. In the 6-OHDA-treated subjects the disruptive effects of d-amphetamine were greatly attenuated, while the effects of DOM were not altered.

Quantitative assessment of the effects of DOM on FR-40 responding in vehicle- and 6-OHDA-treated subjects is illustrated in Figure 12. DOM produced a dose-dependent increase in pause intervals and a dose-dependent decrease in reinforcements obtained; these effects were not altered by 6-OHDA treatment. Figure 13 illustrates the effects of LSD on FR-40 responding in vehicle- and 6-OHDA-treated subjects. As with DOM, this hallucinogen produced a dose-dependent increase in pause intervals and a decrease in reinforcements obtained. Again, 6-OHDA treatment failed to alter these effects. Figure 14 quantitates the effects of d-amphetamine on FR-40 operant responding with and without the neurotoxin pretreatment. In vehicle-treated subjects d-amphetamine produced a dose-dependent decrease in reinforcements obtained. Unlike the hallucinogens, this drug did not produce a significant change in pause intervals. Moreover, the effects of d-amphetamine on response rate were significantly attenuated by 6-OHDA treatment.

2. Drug interaction studies

As described above in Part I, co-administration of d-amphetamine at a threshold dose for FR-40 disruptive effects (0.25 mg/kg) did not alter the dose-dependent "pausing" produced by either LSD or DOM (Figures 8 and 9). Similarly, administration of αMT at a dose which did not alter FR-40 responding per se (see Table 6) failed to alter the dose-dependent "pausing" produced by LSD (Figure 15).

Figure 12. The effects of DOM on FR-40 responding in vehicle- and 6-OHDA-treated rats. The change in pause intervals (circles) and percent of control reinforcements (squares) produced by various doses of DOM during FR-40 operant sessions in vehicle- (open symbols, n=8) or 6-OHDA-treated (filled symbols, n=6) subjects are illustrated. Each symbol and vertical bar represents the mean ± S.E.M. There were no significant effects of 6-OHDA pretreatment on the DOM dose-response pattern. See Figure 6 legend for further information.

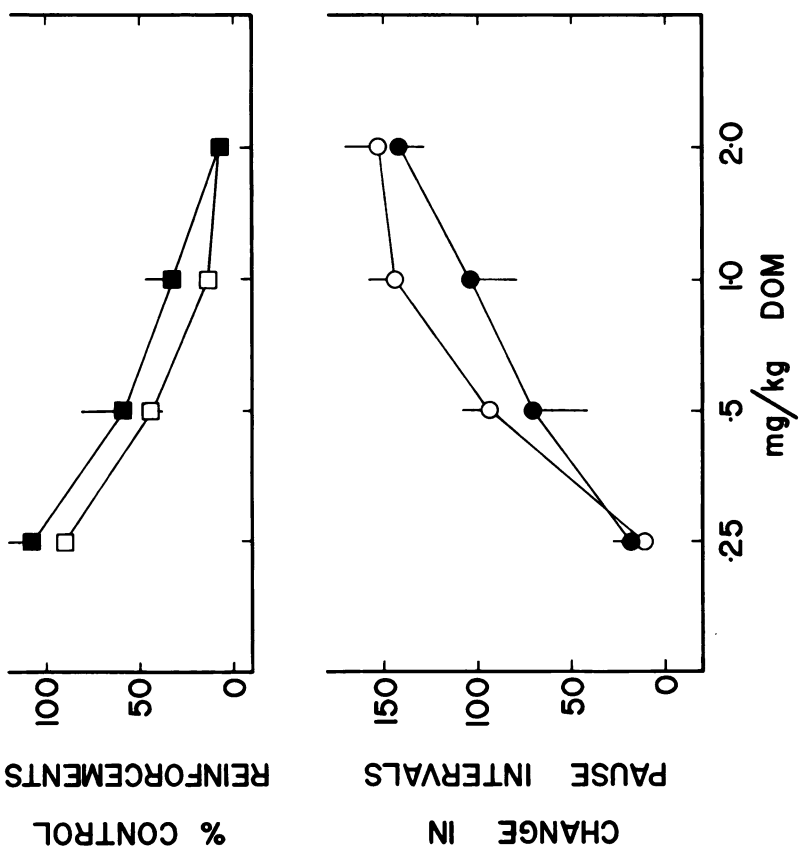

% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
150
100
50
0
.25
.5
1.0
2.0
mg/kg DOM

Figure 12

Figure 13. The effects of LSD on FR-40 responding in vehicle- and 6-OHDA-treated rats. The change in pause intervals (circles) and percent of control reinforcements (squares) produced by various doses of LSD during FR-40 operant sessions in vehicle- (open symbols, n=8) or 6-OHDA-treated (filled symbols, n=6) subjects are illustrated. There were no significant effects of 6-OHDA on the LSD dose-response pattern. See Figure 6 legend for further information.

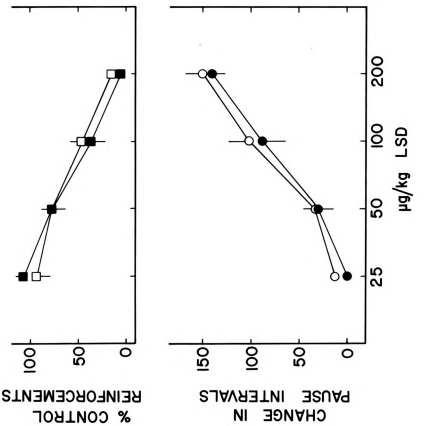

% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
150
100
50
0
25
50
100
200
µg/kg LSD

Figure 13

Figure 14. The effects of d-amphetamine on FR-40 operant responding in vehicle- and 6-OHDA-treated rats. The change in pause intervals (circles) and percent of control reinforcements (squares) produced by various doses of d-amphetamine during FR-40 operant sessions in vehicle- (open symbols, n=8) or 6-OHDA-treated (filled symbols, n=6) subjects are illustrated. 6-OHDA treatment significantly attenuated the effects of d-amphetamine on the number of reinforcements obtained. See Figure 6 legend for further information.

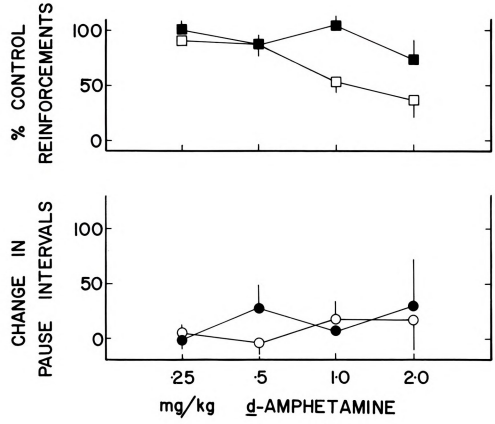

% CONTROL REINFORCEMENTS
100
50
0
CHANGE IN PAUSE INTERVALS
100
50
0
·25
·5
1·0
2·0
mg/kg d-AMPHETAMINE

Figure 14

TABLE 6

The Effects of α-Methyl-p-Tyrosine (αMT) on the Characteristics of FR-40 Operant Responding

Treatment	N	Percent of Control Reinforcements	Change in Pause Intervals
50 mg/kg αMT + 40'	7	95±3	6±4
100 mg/kg αMT + 40'	8	99±4	4±4

Figure 15. The effects of LSD on FR-40 responding alone or in combination with a threshold dose of α-methyl-p-tyrosine (αMT). The change in pause intervals produced by various doses of LSD alone (open symbols) or in combination with 100 mg/kg d,l-αMT is plotted. This dose of αMT alone, plotted in the far left portion of the figure, had no effect on pausing. Each symbol and vertical bar represents the mean ± S.E.M. for six subjects. αMT pretreatment did not alter the FR-40 disrupting effects of LSD. See Figure 6 legend for further details.

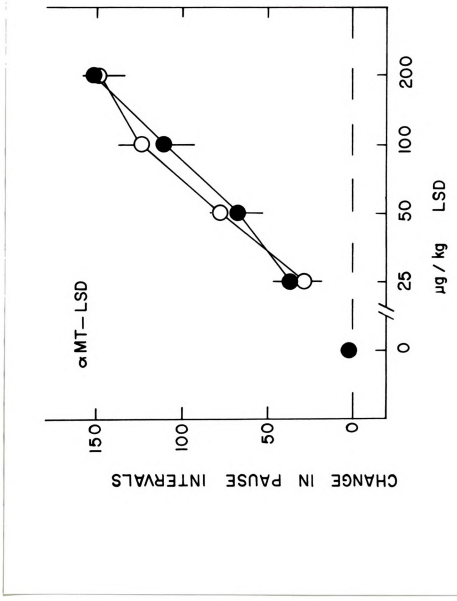

CHANGE IN PAUSE INTERVALS
150
100
50
0
αMT–LSD
0
25
50
100
200
µg / kg LSD

Figure 15

Surprisingly, this αMT treatment did not alter the FR-40 disruptive effects of d-amphetamine (Figure 16).

Lastly, administration of chlorpromazine (for the effects of chlorpromazine alone on FR-40 responding, see Table 7) clearly did not attenuate the FR-40 pause-producing effects of DOM or LSD (Figures 17 and 18). Again, however, this treatment regimen with chlorpromazine failed to alter the disruptive effects of d-amphetamine (Figures 19 and 20). Similarly, haloperidol was found to have no effect on the dose-dependent pausing produced by DOM (Figure 17).

D. Discussion

These data suggest that the catecholamines are not involved in FR-40 disruptive effects of the hallucinogens LSD or DOM. The results of the 6-OHDA study suggest that normal catecholamine neuronal activity is required for the disruptive effects of d-amphetamine. In contrast to these findings are those of Petersen and Sparber (1974), who reported that 6-OHDA treatment failed to alter the FR disruptive effects of d-amphetamine. However, these investigators found that 22 weeks after 6-OHDA (200 μg; intraventricular administration) NE concentrations were reduced to approximately 50 percent in all brain areas and DA concentrations in the striatum (the only area examined for DA) were not altered. These authors therefore concluded that NE neurons were not involved in the FR-disruptive effects of d-amphetamine. It would appear, then, that the attenuation by 6-OHDA of the d-amphetamine-induced disruption of FR-40 operant behavior is due to the destruction of DA and not NE neurons in the brain. This finding

Figure 16. The effects of d-amphetamine on FR-40 responding alone or in combination with a threshold dose of αMT. The change in pause intervals (circles) and percent of control reinforcements received (squares) after various doses of d-amphetamine alone (open symbols) or in combination with 100 mg/kg d,l-αMT are plotted. This dose of αMT alone, plotted in the far left portion of the figure, had no effect on pausing. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects. αMT pretreatment did not alter the FR-40 disrupting effects of d-amphetamine. See Figure 6 legend for further details.

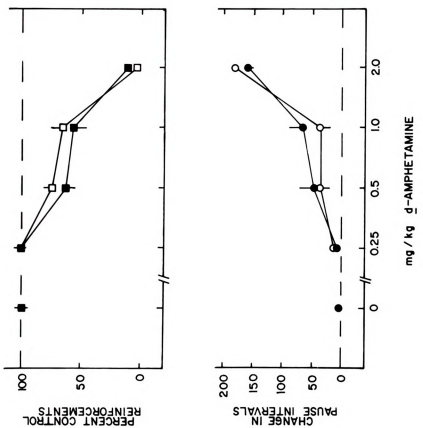

PERCENT CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
150
100
50
0
0
0.25
0.5
1.0
2.0
mg/kg d-AMPHETAMINE

Figure 16

TABLE 7

The Effects of Chlorpromazine on FR-40 Operant Responding

Dose (mg/kg)	PERCENT CONTROL REINFORCEMENTS	CHANGE IN PAUSE INTERVALS
0.25	104± 5	5± 8
0.5	104± 5	-21± 7*
1.0	67± 8*	17±10
2.0	34±11*	83±17*

*$p<0.05$, Student's t-test.

Figure 17. The effects of DOM on FR-40 responding alone or in combination with a threshold dose of haloperidol or chlorpromazine. The change in pause intervals produced by various doses of DOM alone (open symbols) and in combination with 0.025 mg/kg haloperidol (left panel; filled symbols) or 0.5 mg/kg chlorpromazine (right panel; filled symbols) is plotted. The effects of these doses of haloperidol and chlorpromazine alone are shown in the far left of the left and right panels, respectively. Haloperidol or chlorpromazine administration did not alter the pausing produced by DOM. See Figure 6 legend for further details.

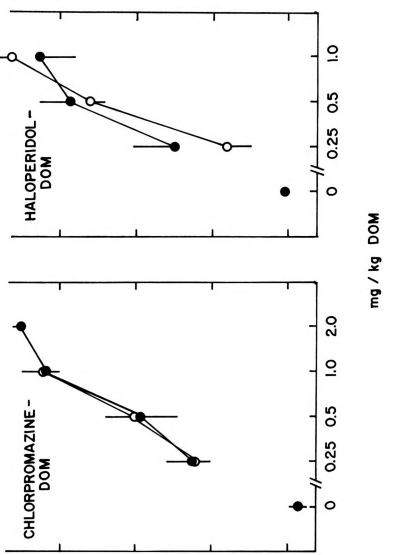

CHANGE IN PAUSE INTERVALS
150
100
50
0
CHLORPROMAZINE - DOM
HALOPERIDOL - DOM
0
0.25
0.5
1.0
2.0
mg / kg DOM

Figure 17

Figure 18. The effects of LSD on FR-40 responding alone or in combination with a threshold dose chlorpromazine. The change in pause intervals (circles) and percent of control reinforcements received (squares) are plotted for various doses of LSD alone (open symbols) and after pretreatment with 0.5 mg/kg chlorpromazine (filled symbols). Chlorpromazine alone, plotted in the far left portion of the figure, had no effect on pausing. Each symbol and vertical bar represents the mean ± S.E.M. for six subjects. Chlorpromazine pretreatment did not alter the FR-40 disrupting effects of LSD. See Figure 6 legend for further information.

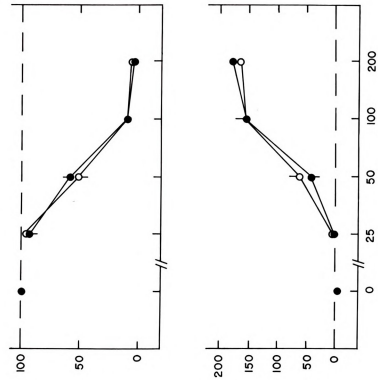

CHLORPROMAZINE-LSD
% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
150
100
50
0
0
25
50
100
200
µg/kg LSD

Figure 18

Figure 19. The effects of d-amphetamine on FR-40 responding alone or in combination with a threshold dose of chlorpromazine. The change in pause intervals (circles) and percent of control reinforcements received (squares) after various doses of d-amphetamine alone (open symbols) or in combination with 0.5 mg/kg chlorpromazine (filled symbols) are plotted. This dose of chlorpromazine alone, plotted in the far left portion of the figure, did not increase the extent of pausing. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects. Chlorpromazine pretreatment did not alter the FR-40 disrupting effects of d-amphetamine. See Figure 6 legend for further details.

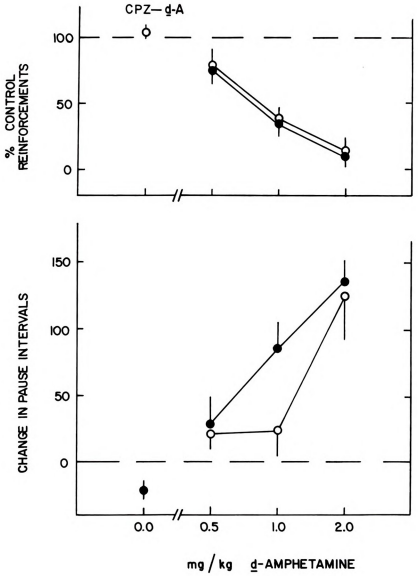

CPZ—d-A
100
50
0
% CONTROL REINFORCEMENTS
150
100
50
0
CHANGE IN PAUSE INTERVALS
0.0
0.5
1.0
2.0
mg / kg d-AMPHETAMINE

Figure 19

Figure 20. The effects of d-amphetamine on FR-40 responding alone or in combination with 1.0 mg/kg chlorpromazine. The change in pause intervals (circles) and percent of control reinforcements (squares) after various doses of d-amphetamine alone (open symbols) or in combination with 1.0 mg/kg chlorpromazine (filled symbols) are plotted. The effects of this dose of chlorpromazine alone are plotted on the far left portion of each panel. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects. Chlorpromazine pretreatment did not alter the FR-40 disrupting effects of d-amphetamine. See Figure 6 legend for further details.

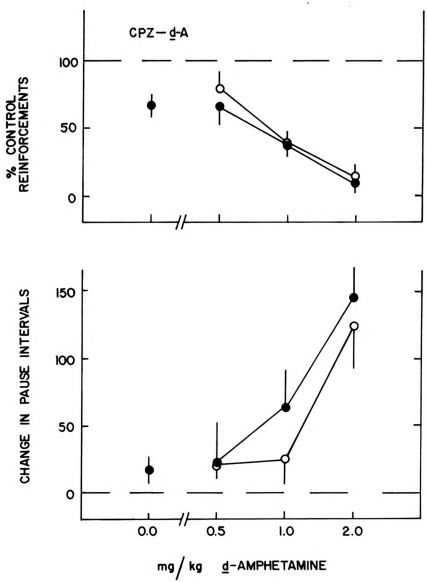

CPZ— d-A
% CONTROL REINFORCEMENTS
100
50
0
CHANGE IN PAUSE INTERVALS
150
100
50
0
0.0
0.5
1.0
2.0
mg / kg d-AMPHETAMINE

Figure 20

is consistent with the established mechanism of action of d-amphetamine (i.e., release of catecholamines, in particular DA, in the brain; Chiueh and Moore, 1973; Rech and Stolk, 1970). These data are also in agreement with those of Fibiger et al. (1973) and Roberts and Fibiger (1975) who reported that the stimulant, taste aversion- and stereotypie-inducing properties of d-amphetamine are markedly attenuated by 6-OHDA treatment.

The αMT and chlorpromazine studies, however, do not support the contention that the FR-40 disruptive effects of d-amphetamine are mediated via alterations in DA neuronal activity. These data are perplexing in that they do not agree with effects observed in 6-OHDA-treated rats. However, the time allowed for the αMT effect to develop may not have been optimal (Rech et al., 1966). Clearly, further experimentation in this area would be of great help in solving this enigma.

Part III. The Role of 5-HT Neurons in the FR-40 Disruptive Effects of Hallucinogens

A number of studies were conducted to investigate the role of 5-HT neurons in the FR-40 disruptive effects of the hallucinogens. These approaches included neurotoxin experiments, studies involving the use of the 5-HT synthesis inhibitor PCPA, and interactions employing putative 5-HT agonists and antagonists.

A. Neurotoxin Studies

1. Introduction

As discussed in the General Introduction, the destruction of 5-HT neurons induced by intraventricular administration of the 5-HT neurotoxin 5,7-DHT enhances the effects of single doses of LSD and mescaline on FR operant responding in rats (Appel et al., 1977; Joseph and Appel, 1977). However, dose-response analyses for the effects of these agents in 5,7-DHT-treated rats were not conducted in these previous studies. Therefore, a number of experiments were conducted to investigate the effects of 5,7-DHT administration on the disruption of FR-40 operant behavior produced by a full range of doses for a number of agents. These studies utilized the intraventricular administration of 5,7-DHT to destroy 5-HT neurons throughout the brain and more localized administration of 5,7-DHT in an effort to determine the site or sites of action of the hallucinogens.

2. Methods

a. Intraventricular 5,7-DHT. In the first of a series of studies utilizing the 5-HT neurotoxin 5,7-DHT, subjects received either 5,7-DHT or its vehicle intraventricularly as detailed in Table 1. Desipramine and pargyline pretreatments were employed in this study to enhance the selectivity and efficacy, respectively, of the neurotoxic effect (Breese et al., 1978; Björklund et al., 1975). Following recovery from surgery, the subjects were trained to respond on the FR-40 schedule and the effects of various doses of LSD, DOM, mescaline and phenobarbital were determined. The hallucinogens were administered immediately prior to the start of the FR-40 session;

phenobarbital was administered thirty minutes prior to the start of the session.

Twenty-four hours after the last test dose, the subjects were sacrificed, their brains were removed, and the concentrations of 5-HT and NE in selected brain regions (cortex, hippocampus, hypothalamus and striatum) were determined by fluorimetric procedures (Chang, 1964; Curzon and Green, 1970). In addition, the concentrations of 5-HT and the dopamine metabolite DOPAC were determined in the septum using high performance liquid chromatography with electrochemical detection (Lyness et al., 1980).

b. Administration of 5,7-DHT into specific brain nuclei. Additional studies were conducted utilizing local injections of 5,7-DHT in an attempt to define the site(s) of action of the hallucinogens. These studies concentrated on the septum and the nucleus accumbens because these structures are a part of the limbic system and receive a relatively dense 5-HT input from the raphé neurons.

1) 5,7-DHT administration into the septum. In this study the subjects were randomly assigned to one of two groups and received either 5,7-DHT (4 µg/µl, 2 µl; n=8) or its vehicle (n=8) into the septum. See Table 1 for parameters of neurotoxin administration. Following recovery from surgery, the subjects were trained to respond on the FR-40 schedule and the effects of LSD and DOM were determined. The effects of 5,7-DHT administration into the septum on the concentrations of NE and 5-HT in the septum, hippocampus and occipital cortex were determined in comparably-treated animals. These latter subjects were sacrificed two weeks after administration of the neurotoxin.

2) 5,7-DHT administration into the nucleus accumbens. In this study the subjects were first trained to respond on the FR-40 schedule. They were then randomly assigned to one of two groups and were administered either 5,7-DHT (8 µg/2 µl; n=6) or its vehicle (n=5) bilaterally into the nucleus accumbens. See Table 1 for the parameters of the neurotoxin treatment. Following recovery of the subjects from surgery, FR-40 sessions were reinstated. After approximately one week responding stabilized and the effects of LSD, DOM, mescaline and d-amphetamine were determined. The subjects were then sacrificed and the concentrations of 5-HT and DA in the nucleus accumbens and striatum were determined as detailed in Materials and General Methods.

c. Administration of 5,7-DHT into the medial forebrain bundle (MFB). As described in the General Introduction, the processes of the raphé nuclei extend forward to various forebrain structures via the MFB. 5,7-DHT administration into the MFB has been shown to damage selectively 5-HT neurons in these fiber tracts (Lorden et al., 1978). Therefore, a study was conducted to determine the effects of neurotoxin administration into this site on the behavioral disruption produced by a number of hallucinogens.

Neurochemical lesions were placed bilaterally in the MFB as detailed in Table 1. Following recovery from surgery, the subjects were trained to respond on the FR-40 schedule and the disruptive effects of various doses of LSD, DOM and mescaline were determined. Five days after completion of the behavioral testing the animals were sacrificed by decapitation, their brains removed, and the

hypothalamus, hippocampus, striatum, and cortex dissected out and weighed. Fluorimetric procedures were utilized to analyze 5-HT in all four regions as described by Curzon and Green (1970). Concentrations of NE and DA were likewise analyzed fluorimetrically as described by Chang (1964).

3. Results

a. Intraventricular 5,7-DHT. Intraventricular administration of 5,7-DHT significantly decreased 5-HT concentrations in the cortex, hippocampus, hypothalamus, and striatum, while NE concentrations in those regions examined were unaltered relative to vehicle-treated controls (Table 8). Concentrations of 5-HT in the septum of 5,7-DHT-treated subjects were not different from blank measurements at the level of sensitivity of the HPLC method (<3 ng/mg protein). These values were significantly different from vehicle-treated values of 54±3 ng/mg protein ($p<0.05$, 95% confidence limits). Septal DOPAC concentrations in the 5,7-DHT treated subjects, 4.0±0.9 ng/mg protein, were not significantly different from vehicle-treated subjects, 4.3±0.4 ng/mg protein.

As described above, control FR-40 responding is characterized by a rapid, constant rate of responding throughout the session, with brief pauses following the delivery of the food pellet reinforcements. In the present study vehicle-treated subjects received 104±6 reinforcements and produced 70±13 pause intervals in control FR-40 sessions. 5,7-DHT treatment did not significantly alter these characteristics of FR-40 responding (Table 9). As described previously, administration of the hallucinogens invariably resulted in a cessation

TABLE 8

Effects of Intraventricular 5,7-DHT Administration on the Concentrations of 5-HT and NE in Various Brain Regions

	5-HT		NE	
	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Cortex	340±29	53±18* (16)	311±12	389±47 (125)
Hippocampus	279±15	42± 8* (15)	364±21	361±32 (99)
Hypothalamus	836±40	282±61* (34)	1796±79	1751±189 (97)
Striatum	408±27	53±18* (13)	n.d.	n.d.

Data are expressed as ng/gm wet tissue weight as determined fluorimetrically. Each value represents the mean ± S.E.M. obtained from four 5,7-DHT-treated (180 µg/10 µl) or six vehicle-treated animals. Numbers in parentheses represent concentration of amine in 5,7-DHT-treated rats expressed as a percentage of vehicle-treated controls.

n.d. - amine concentration not determined.

*$p<0.05$ Student's t-test.

TABLE 9

The Effects of Vehicle or 5,7-DHT Administration on Control FR-40 Operant Response Parameters

TREATMENT	REINFORCEMENTS	PAUSE INTERVALS
Vehicle	104±6	70±13
5,7-DHT	81±6	73±19
Percent of vehicle value	77%	105%

Each value represents the mean ± S.E.M. obtained from four 5,7-DHT-treated (180 µg 5,7-DHT/10 µl) or six vehicle-treated subjects. Average response parameters for each subject were determined as the mean of the control (no injection) FR-40 sessions throughout the study. No significant differences between vehicle- and 5,7-DHT-treated animals were found.

of FR-40 responding for some portion of the test session, followed by reinstatement of responding at or very near the control response rate ("pausing"). This pattern of disruption resulted in a decrease in reinforcements received and a correlated increase in the number of pause intervals produced. Occasional viewing of the performing subjects through a wide angle lens in the door of the sound-attenuated boxes revealed no overt signs of altered behavior. Administration of these agents to other animals not trained in FR-40 did not result in ataxia or a loss of motor function. Therefore, this pausing is presumably not due to motor deficits. On the other hand, phenobarbital most often produced slowed, erratic response rates throughout the session, with no clear-cut "pausing". This pattern of disruption also resulted in a decrease in reinforcements received, but with little or no effect on the number of pause intervals produced. Higher doses (35, 50 mg/kg) would produce "pausing" in some animals, but this was associated with ataxia and motor deficits. Cumulative recordings illustrating the effects of saline, LSD and phenobarbital in vehicle- and 5,7-DHT-treated subjects are shown in Figure 21.

In vehicle-treated subjects, all four agents produced effects ranging from little or no change in reinforcements received at the lowest doses to nearly complete disruption of FR-40 behavior at the highest doses. The hallucinogens also produced a concomitant increase in pause intervals which was well correlated with the decrease in reinforcements received, as observed earlier in control subjects receiving these doses of the hallucinogens. This increase in pause intervals was between 70-100 counts over baseline values following

Figure 21. Cumulative recordings from one vehicle-treated subject (row) and one intraventricular 5,7-DHT-treated subject (bottom row) illustrating the effects of saline, LSD (100 µg/kg) and phenobarbital (PHB; 25 mg/kg) administration on FR-40 responding. See Figure 5 legend for further information.

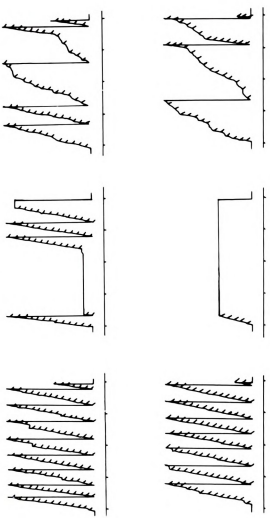


Figure 21

doses of the hallucinogens which decreased reinforcements by nearly 50 percent (50 µg/kg LSD, 0.5 mg/kg DOM and 10.0 mg/kg mescaline). In contrast, the 25.0 mg/kg dose of phenobarbital, which decreased reinforcements received to less than 70 percent of control, had no effect on the number of pause intervals produced. Moreover, the 50.0 mg/kg dose of this latter agent, while decreasing reinforcements to less than 20 percent of control, still produced a mean increase of less than 60 pause intervals.

In the 5,7-DHT treated subjects, the effects of the hallucinogens on both reinforcements received and pause intervals produced were potentiated (Figure 22 illustrates the effects on pause intervals). Moreover, these two measures were still well correlated for the hallucinogens in the 5,7-DHT-treated subjects. The effects of phenobarbital on the change in pause intervals and percent of control reinforcements received were not altered by 5,7-DHT treatment (Figure 23). In the 5,7-DHT-treated subjects injected with this agent, as with the vehicle-treated subjects, there was a dissociation between the decrease in reinforcements received and the change in pause intervals produced. For example, the 25.0 mg/kg dose decreased reinforcements received by almost 25 percent but actually produced a tendency to decrease, not increase, the number of pause intervals.

b. Administration of 5,7-DHT into specific brain nuclei

1) 5,7-DHT administration into the septum. The effects of 5,7-DHT administration into the septum on regional brain amine concentrations are shown in Table 10. Concentrations of 5-HT

Figure 22. The effects of LSD, DOM and mescaline on FR-40 operant responding in vehicle- or intraventricular 5,7-DHT-treated rats. The change in pause intervals produced by various doses of LSD (circles), DOM (squares) and mescaline (triangles), during FR-40 operant sessions in vehicle (open symbols) or 5,7-DHT-treated (filled symbols) subjects is shown. Each symbol and vertical bar represents the mean ± S.E.M. for four (5,7-DHT) or six (vehicle) subjects. Potentiation of the effects of all three agents after 5,7-DHT is indicated by the significant shifts to the left in the dose-response curves, $p<0.05$ by factorial analysis of variance. See Figure 6 legend for further information.

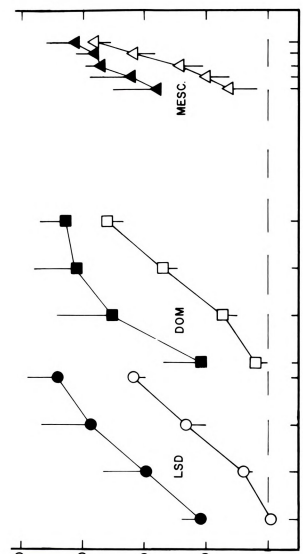

CHANGE IN PAUSE INTERVALS
200
150
100
50
0
LSD
DOM
MESC.
0·0125
0·025
0·05
0·1
0·25
0·5
1
2
7·1
10
14·2
DOSE (mg/kg)

Figure 22

Figure 23. The effects of phenobarbital on FR-40 operant responding in vehicle- and intraventricular 5,7-DHT-treated rats. The change in pause intervals (bottom panel) and percent of control reinforcements obtained (top panel) after various doses of phenobarbital in vehicle (open squares) or 5,7-DHT-treated (filled squares) subjects are plotted. *Significantly different from baseline values, $p<0.05$, Student's t-test for paired values. There was no significant difference between the dose-response curves in vehicle- and 5,7-DHT-treated rats. See Figure 6 legend for further information.

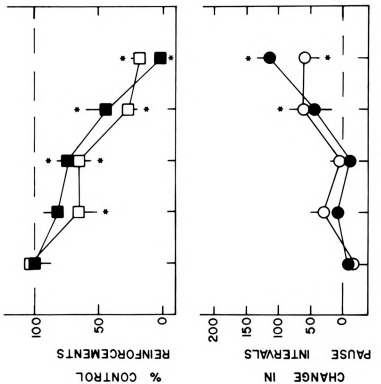

% CONTROL REINFORCEMENTS
100
50
0
CHANGE IN PAUSE INTERVALS
200
150
100
50
0
12·5
17·5
25
35
50
mg/kg PHENOBARBITAL

Figure 23

TABLE 10

The Effects of 5,7-DHT Administration into the Septum on the Concentrations of 5-HT and NE in Various Brain Regions

	5-HT		NE	
	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Hippocampus	256±15	82± 2* (32)	235± 9	253±19 (108)
Septum	55± 4	14± 3* (25)	----	----
Occipital Cortex	243±17	99±15* (41)	299±28	224± 6 (75)

Data are expressed in ng amine/gm tissue wet weight for hippocampus and occipital cortex, as determined fluorimetrically. Septal values represent ng amine/mg protein, as determined by HPLC. Each value represents the mean ± S.E.M. for four subjects. Numbers in parentheses represent concentration of amine in 5,7-DHT-treated animals expressed as a percentage of vehicle-treated animals.

*$p<0.05$, Student's t-test.

in the septum, hippocampus and occipital cortex were significantly decreased; NE concentrations in the hippocampus and occipital cortex were not altered by administration of 5,7-DHT into the septum. Table 11 illustrates that 5,7-DHT administration into the septum did not alter the characteristics of FR-40 responding. The effects of LSD and DOM in these two groups are shown in Figure 24. Both agents produced dose-dependent pausing in vehicle-treated subjects; these effects were not altered by 5,7-DHT administration into the septum.

2) 5,7-DHT administration into the nucleus accumbens. The effects of 5,7-DHT administration into the nucleus accumbens on regional brain amine concentrations are shown in Table 12. 5-HT concentrations were significantly decreased in the nucleus accumbens, while DA concentrations in this region were not changed. Administration of 5,7-DHT into this nucleus had no effect on the concentrations of 5-HT or DA in the nearby striatum. Thus, it appears that local administration produced a relatively selective destruction of 5-HT nerve terminals in the nucleus accumbens alone. Table 13 illustrates that this disruption of 5-HT input into the nucleus accumbens did not alter the characteristics of control FR-40 responding. The effects of the hallucinogens LSD, DOM and mescaline in these two groups of subjects are shown in Figure 25. All three agents produced dose-dependent pausing in vehicle-treated subjects. These effects were not altered by 5,7-DHT administration into the nucleus accumbens.

c. Administration of 5,7-DHT into the MFB. 5,7-DHT injection into the MFB significantly decreased the concentrations of

TABLE 11

Effects of 5,7-DHT Administration into the Septum on the Characteristics of Control FR-40 Operant Responding

	Reinforcements	Pause Intervals
Vehicle	85±10	66±10
5,7-DHT	70± 5	78± 7
Percent of Control	82	109

Each value represents the mean ± S.E.M. obtained from seven 5,7-DHT treated or eight vehicle-treated subjects. Average response parameters for each subject were determined as the mean of the control (no injection) FR-40 sessions throughout the study. No significant differences between vehicle- and 5,7-DHT-treated subjects were found.

Figure 24. The effects of LSD and DOM on FR-40 operant responding in rats treated with 5,7-DHT or its vehicle into the septum. The change in pause intervals produced by various doses of LSD (circles) and DOM (squares) is plotted for vehicle- (open symbols) or 5,7-DHT-treated (filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for seven 5,7-DHT-treated or eight vehicle-treated subjects. No significant effect of 5,7-DHT was observed. See Figure 6 legend for further information.

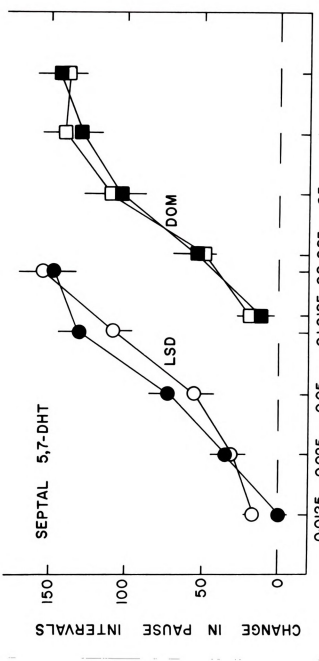

SEPTAL 5,7-DHT
LSD
DOM
CHANGE IN PAUSE INTERVALS
150
100
50
0
0·0125
0·025
0·05
0·1
0·125
0·2
0·25
0·5
1
2
DOSE (mg / kg)

Figure 24

TABLE 12

The effects of 5,7-DHT administration into the nucleus accumbens on the concentration of 5-HT and DA in the nucleus accumbens and striatum

	5-HT		DA	
	Vehicle	5,7-DHT	Vehicle	5,7-DHT
N. Accumbens	11.5±0.9	2.7±0.3* (23)	84±4	98±6 (117)
Striatum	6.6±0.4	5.6±0.5 (85)	102±5	101±6 (99)

Data are expressed in ng amine/mg protein, as determined by HPLC. Each value represents the mean ± S.E.M. for six (5,7-DHT-treated) or 5 (vehicle-treated) subjects. Numbers in parentheses represent concentration of amine in 5,7-DHT-treated animals expressed as a percentage of vehicle-treated animals.

*$p<0.05$, Student's *t*-test.

TABLE 13

The Effects of 5,7-DHT Administration into the Nucleus Accumbens on the Characteristics of FR-40 Operant Responding

	Reinforcements	Pause Intervals
Vehicle	104± 8	39±8
5,7-DHT	117±11 (113)	31±7 (79)

Each value represents the mean ± S.E.M. obtained from six (5,7-DHT-treated) or five (vehicle-treated) subjects. Average response parameters for each subject were determined as the mean of the control (no injection) FR-40 sessions throughout the study. No significant differences between vehicle- and 5,7-DHT-treated subjects were found.

Figure 25. The effects of LSD, DOM and mescaline on FR-40 operant responding in rats treated with 5,7-DHT or its vehicle into the nucleus accumbens. The change in pause intervals produced by various doses of LSD (circles), DOM (squares), and mescaline (triangles) is plotted for vehicle- (open symbols) or 5,7-DHT-treated (filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for six (5,7-DHT) or five (vehicle) subjects. No significant effect of 5,7-DHT was observed. See Figure 6 legend for further details.

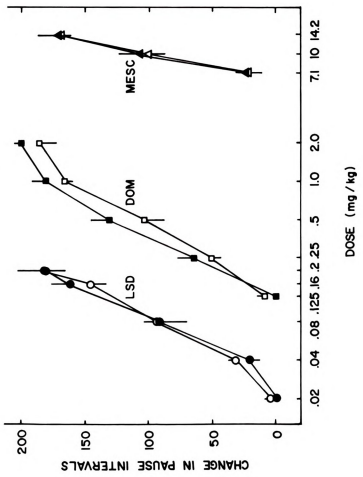

CHANGE IN PAUSE INTERVALS
200
150
100
50
0
LSD
DOM
MESC
.02 .04 .08 .125 .16 .2 .25 .5 1.0 2.0 7.1 10 14.2
DOSE (mg/kg)

Figure 25

5-HT in the cortex, hippocampus, and hypothalamus (Table 14). Concentrations of 5-HT in the cortex and hippocampus were reduced to fifty percent, while the concentration of 5-HT in the hypothalamus was reduced to only 70 percent; striatal 5-HT concentrations were not significantly altered by 5,7-DHT administration into the MFB. Similarly, DA and NE concentrations in all areas examined were not altered by this treatment.

Administration of 5,7-DHT into the MFB did not change control FR-40 operant responding as measured by either total reinforcements obtained or pause intervals produced in control sessions (Table 15). The hallucinogens, as before, produced a dose-dependent increase in pause intervals (Figure 26). The effects of LSD were potentiated by administration of 5,7-DHT into the MFB, while the effects of DOM were attenuated in these animals and the disruptive effects of mescaline were not altered by this 5,7-DHT treatment.

4. Discussion

a. Intraventricular 5,7-DHT. As described above, the hallucinogens produced a disruption of FR-40 behavior characterized by periods of non-responding. This dose-related effect was dramatically potentiated by 5,7-DHT pretreatment to about the same extent for LSD, DOM and mescaline. The effects of phenobarbital were considerably different, however. In vehicle-treated animals this agent produced disruptions of behavior characterized by periods of erratic responding, a finding which is consistent with the data shown in Table 3. This pattern of disruption produced by phenobarbital results in a decrease in reinforcements obtained similar to that observed with the

TABLE 14

The Effects of 5,7-DHT Administration into the MFB on Regional Brain Amine Concentrations

	5-HT		DA		NE	
	Vehicle	5,7-DHT	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Cortex	0.42±0.02	0.22±0.01* (52)	n.d.	n.d.	0.22±0.02	0.25±0.01 (111)
Hippocampus	0.41±0.02	0.20±0.03* (48)	n.d.	n.d.	0.37±0.03	0.34±0.04 (92)
Hypothalamus	1.09±0.05	0.78±0.04* (71)	n.d.	n.d.	2.01±0.16	1.82±0.27 (90)
Striatum	0.43±0.04	0.35±0.10 (81)	5.12±0.41	6.12±0.58 (120)	n.d.	n.d.

Data are expressed as µg amine/gm wet tissue weight as determined fluorimetrically. Each value represents the mean ± S.E.M. from four (5,7-DHT-treated) or eight (vehicle-treated) subjects. Numbers in parentheses represent concentrations of amine in 5,7-DHT-treated subjects expressed as a percentage of controls.

n.d. = amine concentration not determined.

*$p<0.05$ Student's *t*-test.

TABLE 15

The Effects of 5,7-DHT Administration into the MFB on the Characteristics of Control FR-40 Operant Responding

	Reinforcements	Pause Intervals
Vehicle	95± 5	36± 6
5,7-DHT	96±18 (101)	48±15 (133)

Each value represents the mean ± S.E.M. obtained from four (5,7-DHT-treated) or eight (vehicle-treated) subjects. Average response parameters for each subject were determined as the mean of the control (no injection) FR-40 sessions throughout the study. No significant differences between vehicle- and 5,7-DHT-treated subjects were found.

Figure 26. The effects of LSD, DOM and mescaline on FR-40 operant responding in rats treated with 5,7-DHT or its vehicle into the MFB. The change in pause intervals produced by various doses of LSD (circles), DOM (squares) and mescaline (triangles) is plotted for vehicle- (open symbols) or 5,7-DHT-treated (filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for four (5,7-DHT) or eight (vehicle) subjects. 5,7-DHT treatment significantly potentiated the effects of LSD, while the effects of DOM were significantly attenuated by 5,7-DHT treatment. There was no change in the response to mescaline. See Figure 6 legend for further information.

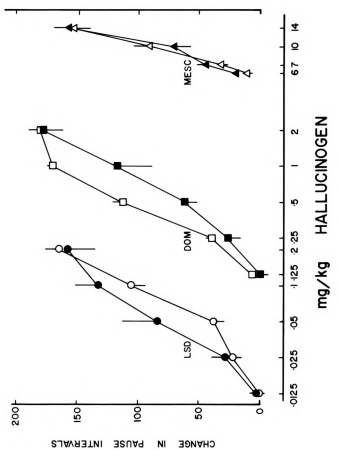

CHANGE IN PAUSE INTERVALS
200
150
100
50
0
LSD
DOM
MESC
-0125
-025
-05
-1
-125
2
25
5
1
2
67
10
14
mg/kg HALLUCINOGEN

Figure 26

hallucinogens, but without "pausing". Destruction of 5-HT neurons by 5,7-DHT treatment did not alter the effects of phenobarbital.

These data indicate that central 5-HT systems are important in the disruptive effects of the hallucinogens LSD, DOM and mescaline. Moreover, these effects seem to be somewhat specific, since 5,7-DHT treatment failed to alter the disruptive effects of phenobarbital. These findings extend the earlier studies by Appel et al. (1977) and Joseph and Appel (1977) in which it was reported that 5,7-DHT treatment enhanced the FR disruptive effects of single doses of LSD and mescaline.

b. Administration of 5,7-DHT into specific brain nuclei. On the basis of the above results, it appears that the 5-HT neurons innervating either the septum or nucleus accumbens are not critically important for the manifestation of the behavioral effects of hallucinogens. It is quite possible, moreover, that there is no single brain region which, upon destruction of the 5-HT input, will result in an alteration of the behavioral effects of the hallucinogens. Before this statement can be strongly supported a number of additional brain regions should also be investigated utilizing the local administration of 5,7-DHT. Unfortunately, pilot studies have indicated that a number of brain regions, for unknown reasons, appear to be relatively resistant to the selective 5-HT-depleting effects of local 5,7-DHT administration. These areas include the dorsal and median raphé nuclei and the amygdala.

c. Administration of 5,7-DHT into the MFB. As anticipated, 5,7-DHT administration injected into the MFB produced different neurochemical effects when compared to intraventricular injection. Injection of 5,7-DHT into the MFB produced moderate decreases in the concentrations of 5-HT in the cortex and hippocampus, with only a slight decrease in the hypothalamus. There was no significant change in striatal 5-HT concentration in these animals. Intraventricular 5,7-DHT produced large (80-90%) decreases in 5-HT concentrations in all of these areas (see Table 8 above). These differences in the pattern of 5-HT depletion produced by either intraventricular or MFB 5,7-DHT administration presumably relate to the differences in the response to the various drugs. Indeed, treatment probably also depends on the route of administration and the quantity administered. Intraventricular injection of the neurotoxin potentiated the disruptive effects of LSD, DOM and mescaline to a similar extent (see Figure 22 above). Injection of 5,7-DHT into the MFB potentiated the effects of LSD while the effects of DOM were attenuated, the effects of mescaline being unchanged. Both neurotoxin treatments suggest a role of 5-HT neuronal systems in the behavioral effects of hallucinogens. The more specific treatment with 5,7-DHT injection into the MFB yields evidence that the brain sites and/or mechanisms of action of these hallucinogens may differ slightly.

B. PCPA Studies

1. Introduction

As mentioned above, pretreatment with the 5-HT synthesis inhibitor PCPA has been reported to potentiate the behavioral effects of the hallucinogens LSD and mescaline, but not those of d-amphetamine (Appel and Freedman, 1964; Appel et al., 1968, 1970, 1977; Joseph and Appel, 1977). The present study replicates the findings reported with LSD and extends them to the hallucinogenic amphetamine analogue DOM.

2. Methods

Seven subjects were used in the PCPA-DOM study and five subjects were used in the PCPA-LSD study. The test doses of DOM and LSD were 0.5 mg/kg and 50 μg/kg, respectively; these doses were chosen because they produce a reliable increase in "pausing" which is clearly sub-maximal. In these studies, days 1-3 were control FR-40 sessions. On day 4 the subjects were given the test dose of either LSD or DOM immediately prior to the start of the session. Days 5-7 were control sessions. Again on day 8, the appropriate dose of the hallucinogen was administered before the start of the FR-40 sessions. No drugs were given before operant sessions on days 9-11. However, shortly (approximately, 30 minutes) after each of these sessions all subjects were administered 100 mg/kg PCPA intraperitoneally. On day 12, after 3 days of 100 mg/kg PCPA per day, the hallucinogens were again administered prior to the start of the session. For subjects in the PCPA-DOM study, the animals were sacrificed approximately six hours after the last session (day 12) and the concentrations of DA, 5-HT and NE in various brain regions were determined by fluorimetric methods as

described in Materials and General Methods. Subjects in the PCPA-LSD study were run in control FR-40 sessions on day 13; six hours later these subjects were sacrificed and the brain amine analyses described above were conducted.

3. Results

The behavioral results of the PCPA-DOM study are shown in Figure 27. The number of pause intervals produced during control FR-40 sessions (days 1-3, 5-7 and 9) did not change over the course of the experiment. Daily administration of 100 mg/kg PCPA failed to alter the number of pause intervals produced in subsequent control sessions (days 10-11). Administration of a low dose (0.5 mg/kg) of DOM produced a slight but significant increase in the number of pause intervals (days 4, 8). After 3 days of PCPA administration, however, this same dose of DOM (day 12) resulted in a significantly greater "pause" than produced by DOM previously. Cumulative recordings obtained from four subjects illustrating the above-mentioned results are shown in Figure 28. Table 16 summarizes the biochemical data obtained from these animals. 5-HT concentrations were significantly decreased in all brain regions following PCPA treatment. A modest, though significant, decrease in the concentration of NE in the hypothalamus was observed following this treatment. Hippocampal NE and striatal DA concentrations were not significantly different from control values.

The behavioral results of the PCPA-LSD study are shown in Figure 29. Again, the number of pause intervals produced during control FR-40 sessions (days 1-3, 5-7 and 9) did not change over the course of the experiment. Daily administration of 100 mg/kg PCPA

Figure 27. The effects of PCPA pretreatment on the FR-40 response to DOM. Ordinate: The number of reinforcements received (top panel) and pause intervals produced (bottom panel) by various treatments. Each point represents the mean ± S.E.M. for seven rats. Abscissa: Days of the study and various treatments. Days 1-3 and 5-7 and 9-11 were control days (no injection before session). DOM, 0.5 mg/kg, was administered IP on days 4, 8 and 12 immediately before the start of the 40 min FR-40 session. PCPA, 100 mg/kg, was administered IP 30 min after the FR-40 session on days 9-11. On day 12 the effects of 0.5 mg/kg DOM were determined 24 hr following the last PCPA injection. *Significantly different ($p<0.05$) from control. The DOM response on Day 12 (after PCPA) was significantly different from DOM responses on Day 4 and 8.

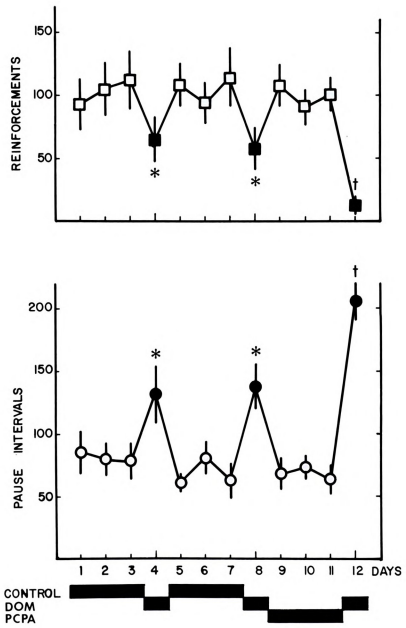

REINFORCEMENTS
150
100
50
*
*
†
PAUSE INTERVALS
200
150
100
50
*
*
†
1 2 3 4 5 6 7 8 9 10 11 12 DAYS
CONTROL
DOM
PCPA

Figure 27

Figure 28. Cumulative recordings illustrating the response patterns of four rats (B-1, B-2, A-1, A-2) receiving the schedule of drug treatments as indicated in Figure 27. Treatment and day are indicated by column headings. See Figure 5 legend for further information.

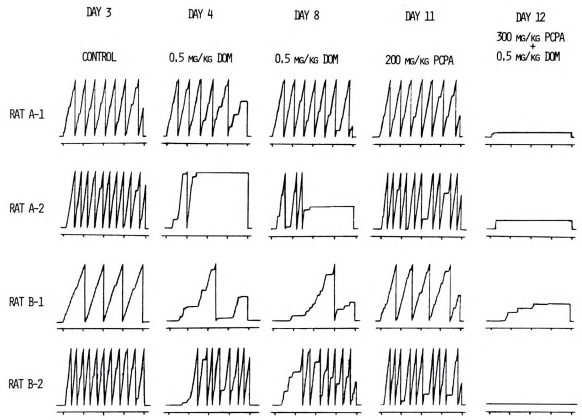

DAY 3
CONTROL
DAY 4
0.5 MG/KG DOM
DAY 8
0.5 MG/KG DOM
DAY 11
200 MG/KG PCPA
DAY 12
300 MG/KG PCPA
+
0.5 MG/KG DOM
RAT A-1
RAT A-2
RAT B-1
RAT B-2

Figure 28

TABLE 16

The Concentrations of 5-HT, NE and DA in Various Brain Regions Following Administration of PCPA (100 mg/kg/day) for 3 days (PCPA-DOM study)

	Cortex		Hippocampus		Hypothalamus		Striatum	
	Control	PCPA	Control	PCPA	Control	PCPA	Control	PCPA
5-HT	417±25	110±18* (26%)	468±41	97±27* (21%)	1270± 75	187± 60* (15%)	472± 30	117± 32* (25%)
NE	N.D.	N.D.	442±24	385±35 (87%)	2542±161	1859±197* (73%)	N.D.	N.D.
DA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	9470±850	8210±1240 (87%)

Values represent concentration of amines (ng/g wet weight); each value represents mean ± SEM for 7 rats, as determined fluorimetrically. Animals were sacrificed approximately 30 hours after the last PCPA injection. Numbers in parentheses indicate percent of control.

*$p<0.05$ compared to control values.

N.D. = Not Determined.

Figure 29. The effects of PCPA pretreatment on the FR-40 response to LSD. Ordinate: The number of pause intervals produced by various treatments. Each symbol and vertical bar represents the mean ± S.E.M. for five subjects. Abscissa: days of the study and various treatments. Days 1-3, 5-7, 9-11 and 13 were control days (no injection before the session). LSD, 50 µg/kg, was administered IP on days 4, 8 and 12 immediately before the start of the 40-min FR-40 session. PCPA, 100 mg/kg, was administered IP 30 minutes after the FR-40 sessions on days 9-11. On day 12 the effects of 50 µg/kg LSD were determined 24 hr following the last PCPA injection. The LSD response on day 12 (after PCPA) was significantly different from those responses on days 4 and 8.

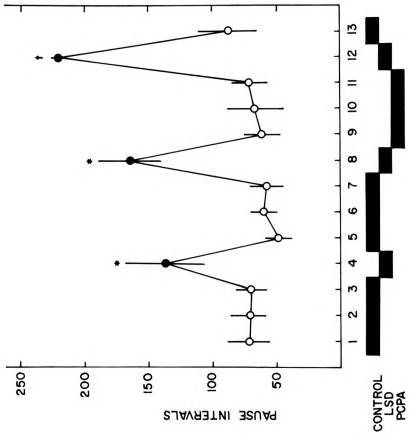

250
200
150
100
50
PAUSE INTERVALS
1 2 3 4 5 6 7 8 9 10 11 12 13
CONTROL
LSD
PCPA

Figure 29

failed to alter the number of pause intervals produced in subsequent control sessions (days 10, 11 and 13). Administration of a low dose (50 μg/kg) of LSD produced a slight but significant increase in the number of pause intervals (days 4, 8). After 3 days of PCPA administration, however, this same dose of LSD (day 12) resulted in a significantly greater "pause" than produced by LSD previously. Table 17 summarizes the biochemical data obtained from these animals. 5-HT concentrations were significantly decreased in all brain regions examined following PCPA treatment; DA and NE concentrations were unaltered in this study.

4. Discussion

In summary, decreasing whole brain 5-HT concentrations with PCPA, while not affecting FR-40 operant responding by itself, potentiated the pause-producing effects of both LSD and DOM. These data correlate well with those provided earlier using intraventricular administration of 5,7-DHT.

C. Interactions between Hallucinogens and Putative 5-HT Agonists

1. Introduction

Hallucinogens have been shown to produce effects similar to those observed following administration of the putative 5-HT agonists quipazine and MCPP. LSD and quipazine both produce head twitching in mice, although there seem to be some quantitative differences in the effects of these drugs on this behavior (Vetulani et al., 1980). Both quipazine and MCPP decrease free-feeding presumably through their 5-HT agonistic properties (Samanin et al., 1977, 1979); LSD has also been reported to decrease free-feeding behavior in rats (Hamilton and

TABLE 17

Concentrations of 5-HT, NE and DA in Various Brain Regions Following Administration of PCPA (100 mg/kg/day) for 3 Days (PCPA-LSD Study)

	Cortex		Hippocampus		Hypothalamus		Striatum	
	Control	PCPA	Control	PCPA	Control	PCPA	Control	PCPA
5-HT	413±18	107±39 (26)	557±69	113±71 (20)	1561±39	224±94 (14)	612± 78	107± 45 (17)
NE	N.D.	N.D.	370±27	383±33 (104)	2104±220	2349±169 (112)	N.D.	N.D.
DA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	7818±976	7234±657 (93)

Values represent concentrations of amines (ng/g wet weight) as determined fluorimetrically. Each value represents the mean ± S.E.M. for 5 (PCPA) or 4 (control) rats. Animals were sacrificed approximately 30 hours after the last PCPA injection. Numbers in parentheses indicate percent of control.

*$p<0.05$ compared to control values.

N.D. = not determined.

Wilpizeski, 1961). Moreover, stimulus control experiments by Winter (1978, 1979) and White et al. (1977) have shown that hallucinogens substitute for quipazine as a discriminative cue and these effects are blocked by the putative 5-HT antagonist BC-105. In light of these similarities a number of studies were undertaken to evaluate the FR-40 disruptive effects of these putative 5-HT agonists alone and in combination with various agents.

2. Methods

Various doses of the putative 5-HT agonists quipazine and MCPP were administered to subjects responding on the FR-40 schedule. MCPP solutions were prepared fresh shortly (within an hour) before injection and were administered ten minutes prior to the start of the session. In another study involving the putative 5-HT agonists, a group of subjects were administered various doses of DOM alone or in combination with 0.25 mg/kg MCPP or 0.25 mg/kg quipazine.

3. Results

As seen in Figures 30 and 31, both agents produced dose-dependent increases in pausing and decreases in reinforcements received. Table 18 illustrates the relationship between these two parameters at near ED_{50} doses (1.0 mg/kg quipazine; 0.5 mg/kg MCPP) for the response-rate decreasing effects. As can be seen in this table, these agents produce large increases in pause intervals at doses which decrease responding to near 50% of control. In this respect, these agents resemble the hallucinogens and are different from d-amphetamine, cocaine, phenobarbital and chlorpromazine as

Figure 30. The effects of quipazine on the characteristics of FR-40 responding. The change in pause intervals (circles) and percent of control reinforcements (squares) after various doses of quipazine are plotted. Each value represents the mean ± S.E.M. for six subjects. Filled symbols represent values significantly different from control values. See Figure 6 legend for further details.

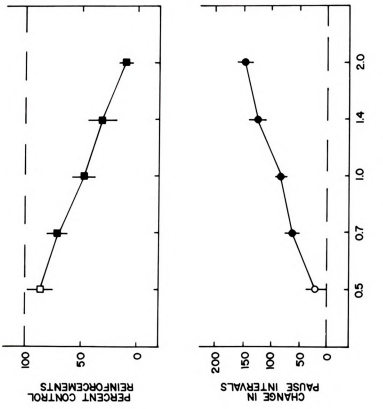

PERCENT CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
150
100
50
0
0.5
0.7
1.0
1.4
2.0
mg/kg QUIPAZINE

Figure 30

Figure 31. The effects of MCPP on the characteristics of FR-40 responding. The change in pause intervals (circles) and percent of control reinforcements (squares) after various doses of MCPP are plotted. Each value represents the mean ± S.E.M. for eight subjects. Filled symbols represent values significantly different from control values. See Figure 6 legend for further details.

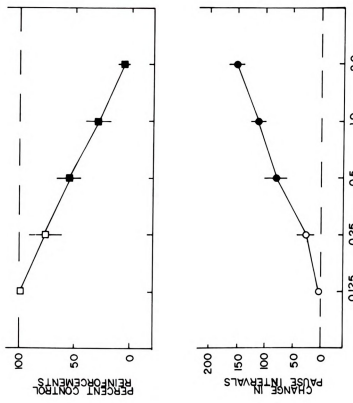

PERCENT CONTROL REINFORCEMENTS
100
50
0
CHANGE IN PAUSE INTERVALS
200
150
100
50
0
0.125
0.25
0.5
1.0
2.0
mg/kg MCPP

Figure 31

TABLE 18

Relationship of Changes in Reinforcements to Changes in Pausing Induced by the Putative 5-HT Agonists Quipazine and MCPP

Drug and Dose	N	Percent of Control Reinforcements	Change in Pause Intervals
1.0 mg/kg Quipazine	6	84± 9*	47± 9*
0.5 mg/kg MCPP	8	80±20*	55±11*

*$p<0.05$ compared to control values.

discussed earlier (see Table 3). Figure 32 illustrates that the pause-producing effects of DOM were enhanced by pretreatment with these agents.

4. Discussion

The two putative 5-HT agonists examined both produced dose-dependent disruptions of FR-40 responding similar to that seen with the hallucinogens. Comparison of pausing produced by these agents with those induced by the hallucinogens and non-hallucinogens previously examined (Table 3), at near-ED_{50} doses for response-rate decrease, indicates that these agents resemble the hallucinogens in producing pausing rather than slowed and erratic intrasession response rates. In addition, the dose-dependent pausing produced by DOM was enhanced by a threshold dose of either MCPP or quipazine. These data suggest that the hallucinogens may be producing "pausing" through the activation of 5-HT receptors in the brain. These findings are in agreement with other reports (Samanin et al., 1977, 1979; Hamilton and Wilpizelski, 1961; Vetulani et al., 1980; Winter, 1978, 1979; White et al., 1977) in which the effects of hallucinogens and putative 5-HT agonists were similar.

D. Interactions between Hallucinogens and Putative 5-HT Antagonists

1. Introduction

A number of studies have examined interactions of putative 5-HT antagonists on the behavioral effects of hallucinogens and putative 5-HT agonists. Stimulus control experiments have shown that the stimulus cues produced by quipazine and both phenethylamine and

Figure 32. The effects of DOM on FR-40 responding alone or in combination with threshold doses of MCPP or quipazine. The change in pause intervals produced by various doses of DOM alone (open symbols) and in combination with 0.25 mg/kg MCPP (left panel, filled symbols) or 0.25 mg/kg quipazine (right panel, filled symbols) is plotted. These doses of MCPP and quipazine alone, plotted in the far left portion of the left and right panels, respectively, did not alter pausing. Each symbol and vertical bar represents the mean ± S.E.M. for six (MCPP-DOM study) or seven (quipazine-DOM study) subjects. Combination of either MCPP or quipazine with DOM shifted the DOM dose-response curve to the left ($p<0.05$, analysis of variance in a block design). See Figure 6 legend for further information.

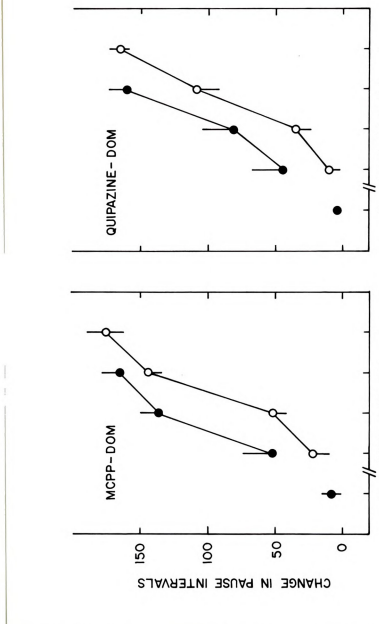

CHANGE IN PAUSE INTERVALS
150
100
50
0
MCPP-DOM
QUIPAZINE-DOM
0
0.125
0.25
0.5
1
mg / kg DOM

Figure 32

indolealkylamine hallucinogens are blocked by the 5-HT antagonists BC-105, cinanserin, methysergide, methergoline and methiothepin (Järbe, 1980; White et al., 1977; Winter, 1978, 1979, 1980; Glennon et al., 1979; Silverman and Ho, 1980). Preliminary reports by Rech et al. (1975) have shown that the 5-HT antagonist cinanserin blocks the FR-40 pause-producing effects of single doses of a number of hallucinogens in both the phenethylamine and indolealkylamine classes. Sloviter et al. (1980) have recently shown that the "5-HT syndrome" characterized by side-to-side headweaving or head tremor, forepaw padding and splayed hindlimbs produced by both indolealkylamine and phenethylamine hallucinogens is blocked by the 5-HT antagonists methergoline, methysergide and mianserin. In addition, Samanin et al. (1977, 1979) have reported that the food-intake suppressing effects of both quipazine and MCPP are attenuated by the putative 5-HT antagonist methergoline. Surprisingly, these 5-HT antagonists have never been reported to produce any effects per se on FR operant response rates. We, therefore, initiated a series of studies investigating the FR-40 effects of a number of putative 5-HT antagonists alone and in combination with hallucinogens.

2. Methods

Various doses of the putative 5-HT antagonists cinanserin and methergoline were administered to subjects responding in the FR-40 paradigm. Cinanserin was injected 80 minutes and methergoline 180 minutes prior to the start of the session. In another series of studies involving the putative 5-HT antagonists, the FR-40 disruptive effects of a number of hallucinogens and non-hallucinogens were

examined in control subjects or in subjects following pretreatment with cinanserin or methergoline.

Initial studies utilized 1.0 mg/kg of the methergoline dose. In these studies 16 subjects were used. Half of the subjects received various doses of LSD (12.5-400 µg/kg), mescaline (5.0-28.4 mg/kg) and d-amphetamine (0.25-2.0 mg/kg) alone and after pretreatment with methergoline (180 minutes prior to testing). The other half received various doses of DOM (0.125-4.0 mg/kg), DMT (1.0-8.0 mg/kg) and phenobarbital (12.5-50.0 mg/kg) alone and after methergoline pretreatment. All subjects received methergoline alone at some point in the study. The order of drugs and doses administered was completely randomized for each subject. The hallucinogens and d-amphetamine were administered immediately prior to the start of the FR-40 session; phenobarbital was administered thirty minutes prior to the start of the session.

Subsequent studies investigated the interaction between 0.1 mg/kg methergoline and DOM, as well as the putative 5-HT agonist quipazine. In addition, an experiment was conducted in which a 20 mg/kg dose of cinanserin was paired with various doses of LSD and DOM.

3. Results

As can be seen in Tables 19 and 20, these putative 5-HT antagonists used did not produce "pausing"; in fact, the 20 mg/kg cinanserin and 0.1 and 1.0 mg/kg methergoline doses actually produced a modest, yet significant, decrease in "pausing". Figure 33 illustrates that pretreatment with 20 mg/kg cinanserin antagonized the

TABLE 19

Effects of Cinanserin on FR-40 Operant Responding

	Reinforcements	Pause Intervals
Control	108±8	55±10
Cinanserin	97±6 (90)	39±13* (71)

Values represent mean ± S.E.M. for 7 subjects. Cinanserin (20 mg/kg) administered i.p. 80 minutes prior to the operant session. Numbers in parentheses indicate percent of control for the values in treated subjects.

*$p<0.05$, Student's t-test for paired values.

TABLE 20

The Effects of Methergoline on FR-40 Operant Responding

	Reinforcements	Pause Intervals
Control	116±7	47±6
0.1 mg/kg Methergoline	129±7* (111)	32±6* (68)
1.0 mg/kg Methergoline	126±7* (108)	28±5* (60)

Values represent mean ± S.E.M. for 8 subjects. Methergoline was administered 180 minutes prior to the start of the operant session. Numbers in parentheses indicate percent of control for the values from treated subjects.

*$p<0.05$, Student's t-test for paired values. Reinforcement data normalized by square root transformation prior to statistical analysis.

Figure 33. Cinanserin antagonism of the effects of LSD and DOM. The change in pause intervals produced by various doses of LSD (squares) and DOM (circles) in control (open symbols) and cinanserin-pretreated (20 mg/kg, 80 minute pretreatment; filled symbols) subjects is plotted. Each symbol and vertical bar represents the mean ± S.E.M. for six to eight subjects. Cinanserin pretreatment produced a significant ($p<0.05$) shift to the right in the dose-response curves for both LSD and DOM. See Figure 6 legend for further information.

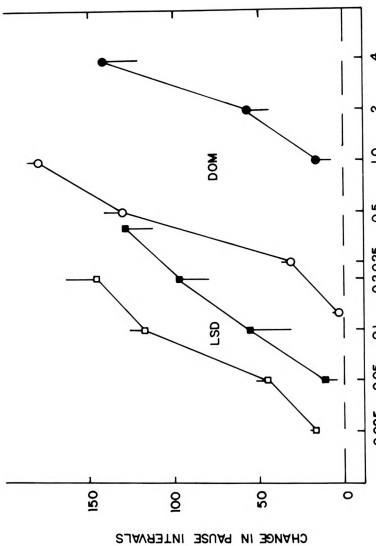

CHANGE IN PAUSE INTERVALS
150
100
50
0
0.025
0.05
0.1
0.2
0.25
0.5
1.0
2
4
mg/kg HALLUCINOGEN
LSD
DOM

Figure 33

pause-producing effects of both LSD and DOM, with about a 2-fold shift in the LSD dose-response curve and about a 4-fold shift in the DOM dose-response curve for the production of "pausing".

As described above, control FR-40 responding is characterized by a rapid, constant rate of responding with a number of brief "minipauses" throughout the session. These "minipauses" often but not always follow the delivery of a food pellet. The response pattern of an individual animal is depicted in Figure 34, panel A, and the characteristics of control responding in all animals are summarized in Table 20. Methergoline treatment alone slightly increased the number of reinforcements obtained and reduced the number of pause intervals produced (Table 20; Figure 34, panel E).

The pattern and extent of disruption produced by LSD, DOM and d-amphetamine alone or after methergoline pretreatment are illustrated in Figure 34. LSD alone (200 µg/kg; panel B) produced the pattern of disrupted FR-40 responding characterized by a fairly long period of non-responding, as described previously. Methergoline pretreatment greatly reduced, but did not eliminate, the pause-producing effect of this dose of LSD (panel F). DOM alone (2.0 mg/kg; panel C) produced a prolonged period of non-responding; methergoline pretreatment completely antagonized this effect (panel G). Administration of d-amphetamine (1.0 mg/kg; panel D) produced a pattern of disruption characterized by slowed, erratic intrasession response rates without any clear-cut "pausing". Methergoline pretreatment did not alter this pattern of disruption produced by d-amphetamine (panel H).

Figure 34. Cumulative recordings illustrating the effects of various treatments on FR-40 responding. A: Saline; B: 200 μg/kg LSD; C: 2.0 mg/kg DOM; D: 1.0 mg/kg d-amphetamine (d-A). Treatments A-D were administered by i.p. injection immediately prior to the start of the FR-40 session. Treatments E, F, G and H denote methergoline pretreatment (1.0 mg/kg, 180 min prior to the start of the session) and administration of saline, LSD, DOM, or d-amphetamine, respectively, as in A, B, C and D. See Figure 6 legend for further information.

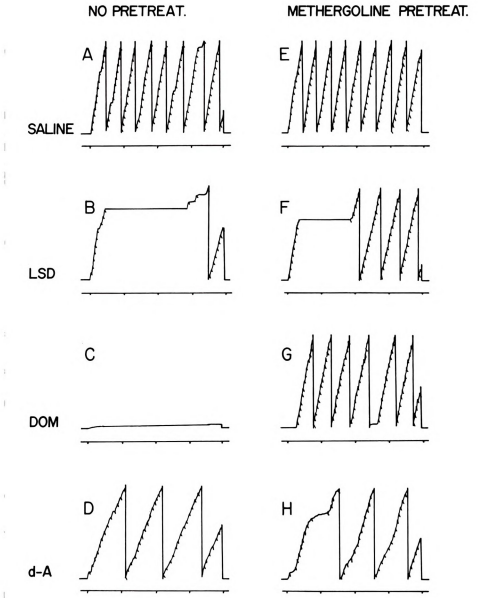

NO PRETREAT.
METHERGOLINE PRETREAT.
A
E
SALINE
B
F
LSD
C
G
DOM
D
H
d-A

Figure 34

Quantification of the effects of methergoline pretreatment on the disruption of FR-40 performance produced by the indolealkylamine hallucinogens LSD and DMT can be seen in Figure 35. In control animals these agents produced dose-dependent increases in pause intervals and correlated dose-dependent decreases in reinforcements, as indicated earlier. These hallucinogen-induced effects were significantly attenuated but not blocked by methergoline pretreatment, as evidenced by the shift to the right in the dose-response curves ($p<0.05$, by factorial analysis of variance).

The effects of methergoline pretreatment on the FR-40 disruptive effects of the phenethylamines DOM and mescaline are shown in Figure 36. These agents administered alone also produced dramatic dose-dependent increases in the number of pause intervals and dose-dependent decreases in the number of reinforcements. Methergoline pretreatment completely blocked the effects of these agents, even when they were administered at supramaximal doses (4.0 mg/kg DOM; 28.4 mg/kg mescaline).

In control animals d-amphetamine produced a dose-dependent decrease in the number of reinforcements similar to that observed following administration of the hallucinogens, but did not produce an increase in pause intervals, except at the highest dose (Figure 37). Examination of the cumulative records from these animals indicated that (as shown in Figure 34, panel D) administration of d-amphetamine generally produces slowed, erratic intrasession response rates not characterized by pausing. The pattern of disruption produced by this

Figure 35. Methergoline antagonism of the effects of indolealkylamine hallucinogens. The change in pause intervals and percent of control reinforcements produced by various doses of LSD (circles) and DMT (hexagons) during FR-40 operant sessions are plotted for control (open symbols) or methergoline-pretreated (1 mg/kg, 180 min prior to session; filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects; where vertical lines are not shown, the S.E.M. is less than the radius of the symbol. See Figure 6 legend for further information.

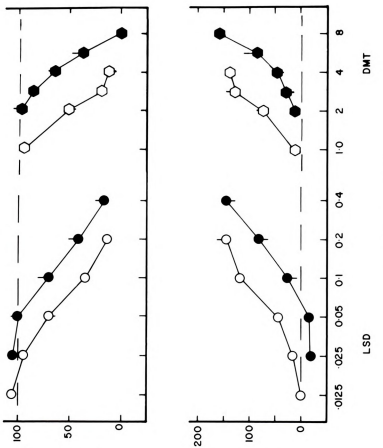

% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
100
0
.025 .025 .05 .1 .2 .4 1.0 2 4 8
LSD
DMT
DOSE (mg/kg)

Figure 35

Figure 36. Methergoline antagonism of the effects of the phenethylamine hallucinogens. The change in pause intervals and percent of control reinforcements produced by various doses of DOM (circles) and mescaline (triangles) are plotted for control (open symbols) or methergoline-pretreated (filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects; where vertical lines are not shown, the S.E.M. is less than the radius of the symbol. See Figure 6 legend for further information.

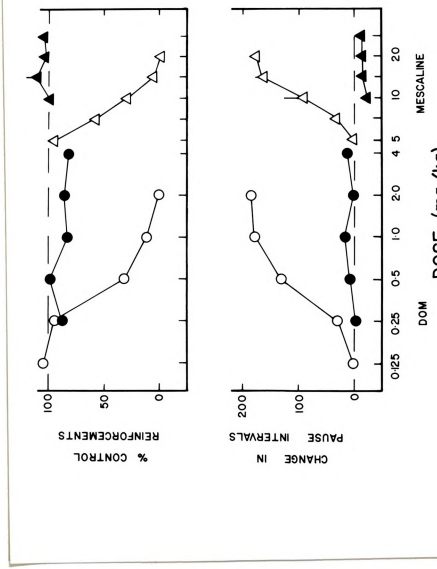

% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
100
0
0125 0.25 0.5 1.0 2.0 4 5 10 20
DOM
MESCALINE
DOSE (mg/kg)

Figure 36

Figure 37. The effects of methergoline on the disruption of FR-40 operant responding produced by d-amphetamine and phenobarbital. The change in pause intervals and percent of control reinforcements produced by various doses of d-amphetamine and phenobarbital are plotted for control (open symbols) or methergoline-pretreated (filled symbols) subjects. Each symbol and vertical bar represents the mean ± S.E.M. for eight subjects; where vertical bars are not shown, the S.E.M. is less than the radius of the symbol. See Figure 6 legend for further information.

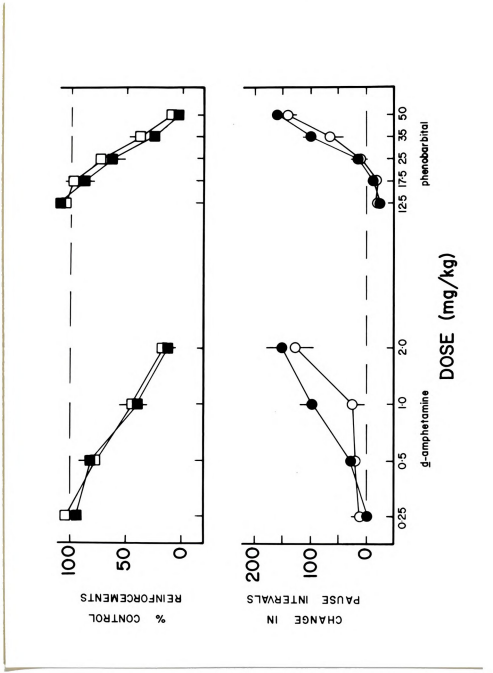

% CONTROL
REINFORCEMENTS
100
50
0
CHANGE IN
PAUSE INTERVALS
200
100
0
0.25
0.5
1.0
2.0
d-amphetamine
1.25
1.75
2.5
3.5
5.0
phenobarbital
DOSE (mg/kg)

Figure 37

agent results in a decrease in reinforcements with little or no change in pause intervals except at higher doses. Methergoline pretreatment did not significantly alter the effects of d-amphetamine on reinforcements and pause intervals.

Phenobarbital, like d-amphetamine, produced a dose-dependent decrease in reinforcements without altering the number of pause intervals until relatively high doses (35, 50 mg/kg) were administered (Figure 37). Examination of the cumulative records indicated that, as with d-amphetamine, administration of this agent also produced slow and erratic response rates within the FR-40 session. Methergoline pretreatment did not alter the disruptive effects of phenobarbital.

Figure 38 indicates that, if the doses of DOM are increased sufficiently, the blockade produced by 1.0 mg/kg methergoline can indeed be overcome. Moreover, the 0.1 mg/kg dose of methergoline is also quite effective in antagonizing the pause-producing effects of DOM, although this dose is significantly less effective than the 1.0 mg/kg methergoline dose. Similar studies with quipazine showed that the effects of this drug are also antagonized by methergoline and that this antagonism is similar to that observed with DOM, but different from LSD (Figure 39). That is, 0.1 mg/kg methergoline, and to a greater extent 1.0 mg/kg, produced a very dramatic shift to the right in the dose-response curve for the pausing produced by quipazine.

4. Discussion

The data with cinanserin and methergoline alone illustrating the decrease in pausing relative to control levels further support the hypothesis that this pausing is produced by 5-HT agonistic actions and

Figure 38. Antagonism of the effects of DOM by methergoline. The change in pause intervals produced by various doses of DOM alone (open symbols) or in combination with 1.0 mg/kg (filled symbols) or 0.1 mg/kg (half-filled symbols) methergoline (180 minute pretreatment) is shown. Each symbol and vertical bar represent the mean ± S.E.M. obtained from 5-9 animals. One mg/kg methergoline, and to a lesser extent 0.1 mg/kg, produced a significant shift to the right in the dose-response curve for DOM ($p<0.05$, factorial analysis of variance). See Figure 6 legend for further information.

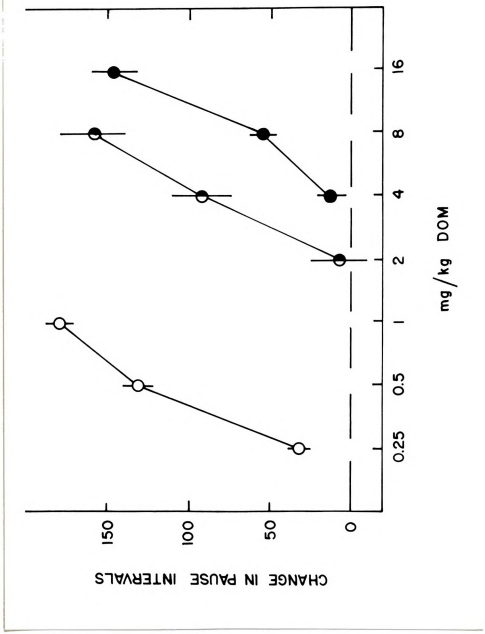


Figure 38

Figure 39. Antagonism of the effects of quipazine by methergoline. The change in pause intervals produced by various doses of quipazine alone (open symbols) or in combination with 1.0 mg/kg (filled symbols) or 0.1 mg/kg (half-filled symbols) methergoline (180 minute pre-treatment) is shown. Each symbol and vertical bar indicate the mean ± S.E.M. obtained from 5-9 animals. One mg/kg methergoline, and to a lesser extent 0.1 mg/kg, produced a significant shift to the right in the dose-response curve for quipazine. See Figure 6 legend for further information.

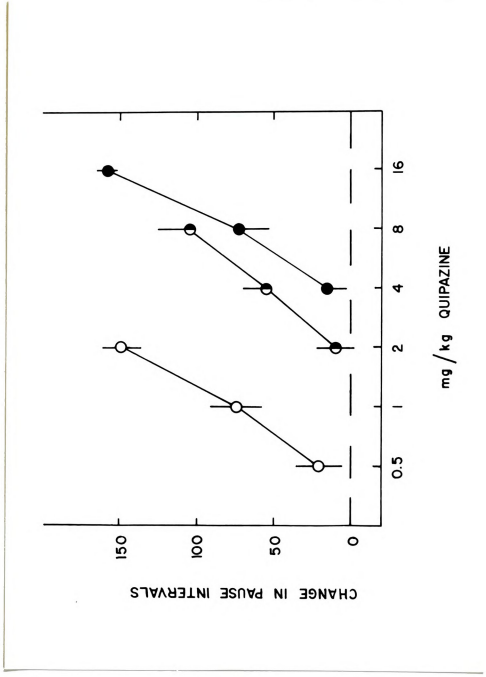

CHANGE IN PAUSE INTERVALS
150
100
50
0
0.5
1
2
4
8
16
mg / kg QUIPAZINE

Figure 39

imply that the endogenous neurotransmitter exerts a tonic influence. Moreover, the antagonism of the pause-producing effects of both LSD and DOM by cinanserin further supports this 5-HT agonistic hypothesis. The difference in degree in the extent of antagonism of LSD and DOM by cinanserin could be explained by either a difference in the affinities of these agents for the receptor (presumably 5-HT) occupied by cinanserin or a difference in the receptor populations upon which the two hallucinogens act.

The findings with methergoline are in agreement with the cinanserin data, indicating again that 5-HT agonistic actions are involved in the pause-producing effects of the hallucinogens. Moreover, the slight difference in the antagonism of LSD vs. DOM observed with cinanserin is considerably more apparent in the methergoline studies and has been extended (with the DMT and mescaline interactions) to suggest that these differences in the blockade by the putative 5-HT antagonists are indeed class differences.

These studies also demonstrate that the methergoline antagonism of the pausing produced by the 5-HT agonist quipazine is similar in pattern to that seen when the antagonist was combined with the phenethylamine DOM and dissimilar to that with the indolealkylamines LSD and DMT (see above). Moreover, these studies also indicate that this methergoline antagonism of the effects of DOM and quipazine is indeed dependent on the dose of methergoline employed. These studies suggest that members of the two classes of hallucinogens interact with two receptor (presumably 5-HT) populations differentiated by the

methergoline sensitivity. Alternatively, they may affect the same receptor populations, but by very different mechanisms.

Part IV. Additional Behavioral Studies

A. Introduction

In addition to their disruption of FR-40 operant behavior, the hallucinogens produce a number of other behavioral effects. One of the reported effects involves the release of responding that is suppressed by punishment. This release of punished responding has been suggested to be related to alterations in the activity of brain 5-HT neurons (Schoenfeld, 1976).

Two studies were undertaken to explore the possible anti-conflict effects of the hallucinogens. One study investigated the effects of the hallucinogens in animals treated intraventricularly with 5,7-DHT to destroy 5-HT neurons; the other study investigated the effects of methergoline pretreatment on the behavioral effects of hallucinogens in punishment-suppressed responding. In the first study, several non-hallucinogenic agents with established anti-conflict activity were also examined.

B. Methods

1. Intraventricular 5,7-DHT and the effects of various agents on punished and unpunished responding. The purpose of the first study was to determine the effects of pentobarbital, methaqualone, LSD and DOM on punished and unpunished responding. The drugs were tested first in control animals and later in the same animals following destruction of 5-HT neurons with the neurotoxin 5,7-DHT.

The subjects were six female Sprague-Dawley rats (200-225 g). These subjects were housed three per cage in a room with a 12-hour day-night cycle (lights on 0700-1900 hr). Although the subjects had received previous drug treatments, a three-week drug-free period was observed before the start of the present experiment.

Behavioral training and testing was conducted between 1400-1600 hr in the conditioned suppression chamber described by Ford et al. (1979). The testing chamber was a rectangular box with plexiglass sides and a metal floor and top. Protruding from one wall was a metal drinking tube. Seven-second tone periods were produced intermittently (variable interval - 21 sec) throughout the 10-minute session. During the latter 5 seconds of these 7-second periods contact between the floor and the drinking tube closed a circuit which resulted in the delivery of a 0.03 mA shock to the subject. All experimental events were controlled by electromechanical programming circuits and shocks were recorded on electromagnetic counters. Attached to the metal drinking tube was a calibrated (0.5 ml units) polyethylene drinking tube used to measure the volume of fluid drunk.

During training water-deprived subjects were first placed in the experimental chamber and allowed to drink freely without the shock contingency. After approximately one week of nonshock sessions, the shock contingency was incorporated into the session. Initially the shock inhibited all drinking. After several days, however, all subjects came to drink stable volumes of water and received a relatively constant number of shocks from day to day. After responding had become stable for each rat, the effects of

various doses of LSD, DOM, pentobarbital and methaqualone were determined in each rat. All drugs were administered i.p. 10 minutes prior to the start of the test session. The order of drugs and doses administered was completely randomized for each subject. Drug test days were separated by at least three nondrug sessions to avoid the possibility of tolerance development.

After the effects of these agents were determined, the subjects were anesthetized with equithesin (3 ml/kg) and placed in a stereotaxic apparatus. All subjects received 5,7-DHT (200 µg/10 µl) intraventricularly. All subjects also received 25 mg/kg desipramine HCl 45 minutes prior to administration of the neurotoxin to protect against the destruction of norepinephrine neurons (Björklund et al., 1975). All subjects were allowed 3-5 days to recover from surgery before behavioral testing was reinstated. After an additional week of control sessions, water intake and the number of shocks received had stabilized for each rat and the effects of LSD, DOM, pentobarbital and methaqualone were again determined as described above.

Following completion of the post-lesion behavioral analyses, the subjects and six untreated animals were sacrificed, their brains were removed, and the concentrations of 5-HT, NE and DA in a number of brain regions were determined by fluorimetric procedures (Chang, 1964; Curzon and Green, 1970) as described in Materials and General Methods.

Drug effects were assessed by comparing the data from test days to the average of the three days prior to and three days

after the test day (baseline). Student's t-test for paired data was used to evaluate the effects of individual doses of the agents used and to evaluate the effects of 5,7-DHT treatment on baseline conditioned suppression performance. Dose-response relationships were examined by analysis of variance in a block design. In all statistical evaluations $p<0.05$ was used as the criterion for statistical significance.

2. 5-HT agonists and antagonists and the effects of hallucinogens on punished and unpunished responding. The purpose of the second study was to examine the effects of LSD, DOM and quipazine in the conditioned suppression paradigm alone and after methergoline pretreatment.

The subjects in this study were twelve drug-naive female Sprague-Dawley (Spartan Farms, Haslett, MI) rats weighing between 200-225 g at the start of the experiment. All subjects were housed singly in a room with a 12-hr light-dark cycle (lights on 0700-1900 hr) and food-deprived to approximately 80% of their free-feeding body weights.

Behavioral training and testing was conducted between 1500-1700 hr in the conditioned suppression chamber described above. In these studies a chocolate-flavored liquid diet ("very" chocolate Sego; Pet, St. Louis, MO) served as the liquid reinforcer, as opposed to the water used in the previous study (see above).

During training the food-deprived subjects were placed in the experimental chamber and allowed to consume liquid diet freely

without the shock contingency. After approximately one week of non-shock sessions, the shock contingency was incorporated into the session and training progressed as detailed above. After responding had become stable for each rat, the effects of various doses of LSD, DOM and quipazine were determined in each rat either alone or after methergoline pretreatment. LSD, DOM and quipazine were administered i.p. 10 minutes prior to the test session; methergoline was administered 180 minutes prior to the session. Drug effects were assessed as described above. Student's t-test for paired data were used to evaluate the effects of individual doses of the agents used. Dose-response relationships were examined by analysis of variance in a block design. In all statistical evaluations $p<0.05$ was used as the criterion for statistical significance.

C. Results

1. Intraventricular 5,7-DHT and the effects of various agents on punished and unpunished responding. Pretreatment with 5,7-DHT significantly decreased the concentration of 5-HT in all brain areas examined, when these subjects were compared with untreated control rats (Table 21). The concentrations of DA and/or NE in these areas were not significantly altered. The disruption of 5-HT neuronal activity did not significantly alter the number of shocks received in post-lesion control sessions (Table 22). Water intake was significantly greater after the 5,7-DHT treatment. However, this effect was probably not due to the 5,7-DHT treatment per se since we have observed that a similar group of six untreated subjects showed an

TABLE 21

Effects of Intraventricular 5,7-DHT Administration on the Concentrations of 5-HT, DA and NE in Various Brain Regions (Conflict Procedure)

	5-HT		DA		NE	
	Control	5,7-DHT	Control	5,7-DHT	Control	5,7-DHT
Cortex	0.27±0.04	0.04±0.01* (15)	0.30±0.02	0.30±0.01 (100)	0.32±0.02	0.36±0.01 (113)
Hippocampus	0.46±0.03	0.08±0.01* (18)	n.d.	n.d.	0.31±0.01	0.32±0.02 (103)
Hypothalamus	1.14±0.03	0.47±0.09* (39)	n.d.	n.d.	1.61±0.13	1.82±0.11 (113)
Striatum	0.64±0.05	0.06±0.01* (9)	6.29±0.32	6.40±0.36 (102)	n.d.	n.d.

Data are expressed in μg amine/gm wet tissue weights; each value represents the means ± S.E.M. obtained from six 5,7-DHT-treated (200 μg/10 ml) or six control (no treatment) animals. Numbers in parentheses represent concentrations of amine in 5,7-DHT-treated animals expressed as a percentage of control animal values.

n.d. = amine concentration not determined.

*$p<0.05$, Student's *t*-test.

TABLE 22

The Effects of 5,7-DHT Treatment on Control Conditioned Suppression Performance

	SHOCKS RECEIVED	WATER INTAKE (ml)
PRE-5,7-DHT	20±1	13.5±0.5
POST-5,7-DHT	25±4 (123)	15.0±0.5* (113)

Each value represents the mean ± S.E.M. obtained from six subjects before (PRE-5,7-DHT) or after (POST-5,7-DHT) administration of 5,7-DHT. Average response parameters for each animal were determined as the mean of the control (no injection) sessions throughout the study. Numbers in parentheses represent percent of control (PRE-5,7-DHT) values.

*$p<0.05$, Student's t-test.

increase from 13.3±0.3 to 14.9±0.7 ml water consumed over the course of two consecutive 3-month intervals (the time elapsed during the present study).

Before 5,7-DHT treatment, pentobarbital produced a dose-dependent increase in the number of shocks received (relative to baseline) between 2.5 and 10 mg/kg, and a dose-dependent decrease in water intake between 10 and 20 mg/kg (Figure 40). A decrease in punished responding at 20 mg/kg was correlated with the highly sedative effects of this dose. Maximal anti-conflict activity for pentobarbital was observed at the 10.0 mg/kg dose, at which dose punished responding was increased to greater than 600 percent of control. Water intake was slightly depressed by this dose. Treatment with 5,7-DHT did not significantly alter the effects of pentobarbital on either punished or unpunished responding.

In control animals, methaqualone produced a dose-dependent increase in punished responding between 2.5 and 10.0 mg/kg (Figure 41). Maximal anti-conflict activity of this agent was also obtained at the 10.0 mg/kg dose, which increased punished responding to almost 400 percent of control. Water intake was not depressed by this dose of methaqualone. A significant decrease in water intake was observed at the 20.0 mg/kg dose in control animals, however. Treatment with 5,7-DHT did not significantly alter the effects of methaqualone on either punished or unpunished responding, although there was a tendency for methaqualone to produce a greater increase in punished responding after 5,7-DHT treatment.

Figure 40. The effects of pentobarbital on shocked drinking tube behavior in rats before and after intraventricular 5,7-DHT treatment. Top panel: percentage of control shocks received (punished responding); bottom panel: control water intake (unpunished responding). Doses of pentobarbital, dissolved in saline, were administered 10 minutes prior to testing. Open symbols and vertical bars represent mean ± S.E.M. from six subjects prior to administering the neurotoxin; filled symbols represent the data obtained after intracerebroventricular 5,7-DHT (200 µg/10 µl) treatment. Percent of control for each subject was determined by comparing the data on test days to the average of the three days prior to and the three days after the test day (baseline). *Significantly different from baseline values; $p<0.05$ by Student's *t*-test for paired values. 5,7-DHT administration did not change the response to various doses of pentobarbital.

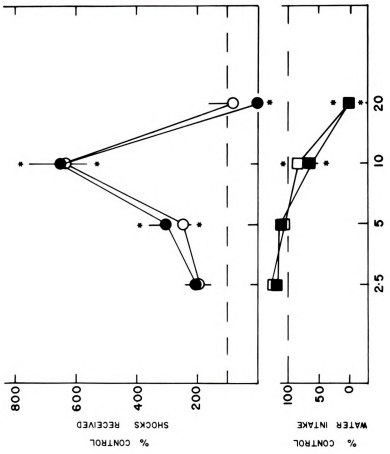

% CONTROL
SHOCKS RECEIVED
800
600
400
200
% CONTROL
WATER INTAKE
100
50
0
2·5
5
10
20
mg/kg PENTOBARBITAL

Figure 40

Figure 41. The effects of methaqualone on shocked drinking tube behavior in rats before (open symbols) and after (filled symbols) 5,7-DHT treatment. Doses of methaqualone were administered 10 minutes prior to testing. *Significantly different from baseline values; $p<0.05$ by Student's *t*-test. 5,7-DHT administration did not significantly change the response to various doses of methaqualone. See Figure 40 legend for further information.

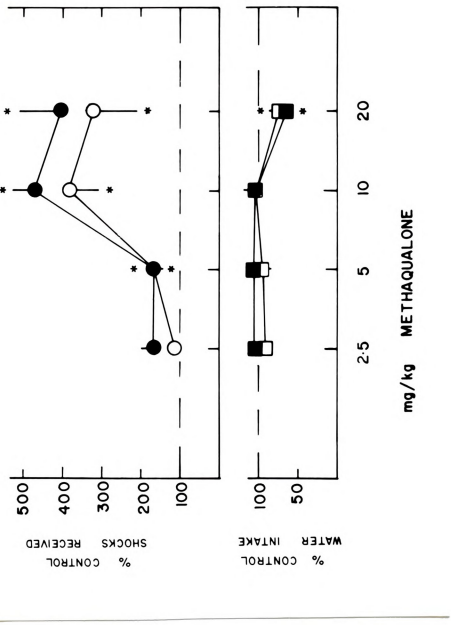

% CONTROL SHOCKS RECEIVED
500
400
300
200
100
% CONTROL WATER INTAKE
100
50
2·5
5
10
20
mg/kg METHAQUALONE

Figure 41

In control animals both LSD and DOM produced a dose-dependent decrease in water intake (Figure 42). Many doses of these agents produced a tendency for a modest increase in punished responding, but this effect was found to be significant only with the 25 µg/kg LSD and 0.35 mg/kg DOM doses. A trend for maximal anticonflict activity was observed with the 35 µg/kg LSD and 0.5 mg/kg DOM doses, at which doses mean values for punished responding were increased to 142 and 175 percent of control, respectively. Pretreatment with 5,7-DHT attenuated the effects of both LSD and DOM to increase punished responding over the entire range of doses. However, 5,7-DHT treatment potentiated the dose-dependent depression of water intake observed after both hallucinogens.

2. 5-HT agonists and antagonists and the effects of hallucinogens on punished responding. Subjects in the conditioned suppression paradigm utilizing liquid diet reinforcement received an average of 15-25 shocks and consumed 15-20 ml fluid per session. These control values, and the effects of various doses of methergoline alone on punished and unpunished responding are shown in Table 23. As can be seen, methergoline treatment alone did not alter punished responding, but did reduce unpunished responses at the highest dose tested (2.0 mg/kg). LSD (Figure 43) and DOM (Figure 44) in these subjects produced a modest increase in punished responding at the 50 µg/kg and 0.5 mg/kg doses, respectively. Quipazine (Figure 45) produced a tendency for release of this behavior at the 1.0 mg/kg dose, but this effect was not significant. All three agents produced dose-dependent decreases in fluid consumption (Figures 43-45). Pretreatment

Figure 42. The effects of LSD and DOM on shocked drinking tube behavior in rats before (open symbols) and after (filled symbols) 5,7-DHT treatment. The effects of various doses of LSD (12.5-100 µg/kg) are represented by the symbols on the left side of the figure; the effects of DOM (0.125-1.0 mg/kg) are represented by symbols on the right side of the figure. Both agents were dissolved in saline and administered 10 minutes prior to the start of the session. *Significantly different from baseline values, $p<0.05$ by Student's t-test for paired values. 5,7-DHT significantly attenuated the effects of both agents to increase number of shocks received (top panel); 5,7-DHT treatment significantly potentiated the capacity of both agents to decrease water intake (bottom panel). See Figure 40 legend for further information.

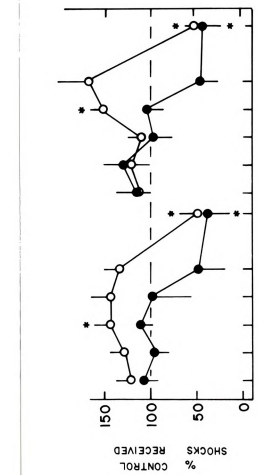

% CONTROL SHOCKS RECEIVED
150
100
50
0
% CONTROL WATER INTAKE
100
50
0
0·0125
LSD
0·025
0·05
0·1
0·125
0·25
0·5
1·0
DOM
mg/kg

Figure 42

TABLE 23

Effects of Methergoline on Conditioned Suppression Responding

Methergoline Dose (mg/kg)	Punished Responding	Unpunished Responding
0.1	113±32	98± 3
0.25	75±19	110± 5
0.5	157±50	115±13
1.0	147±30	100± 6
2.0	69±21	61± 6*

Values represent the mean ± S.E.M. (percent of control) obtained from six subjects for the number of shocks received (punished responding) and the volume (in ml) of fluid drunk (unpunished responding). Control values were 16±4 shocks/session and 16.0±1.0 ml liquid diet/session.

*$p<0.05$, Student's t-test for paired values.

Figure 43. Antagonism of the effects of LSD on punished and unpunished responding by methergoline. The effects of LSD alone (open symbols) or after 1.0 mg/kg methergoline pretreatment (filled symbols) are plotted on the basis of the percent of control shocks received (top panel) and the percent of control fluid intake (bottom panel) in conditioned suppression testing. Each symbol and vertical bar represent the mean ± S.E.M. obtained from six subjects. Methergoline pretreatment significantly attenuated the fluid intake-decreasing effects of LSD, $p<0.05$, Student's _t_-test for paired values. See Figure 40 legend for further information.

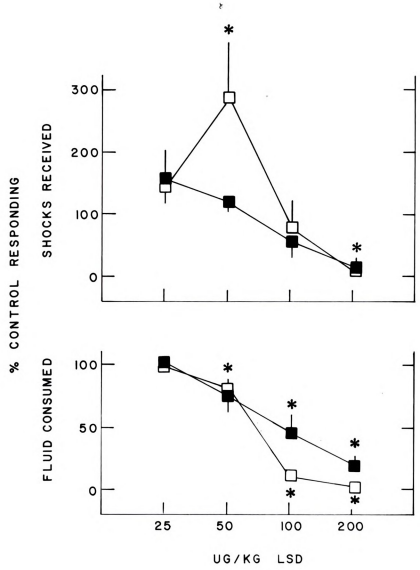

% CONTROL RESPONDING
SHOCKS RECEIVED
300
200
100
0
FLUID CONSUMED
100
50
0
25
50
100
200
UG/KG LSD

Figure 43

Figure 44. Antagonism of the effects of DOM on punished and unpunished responding by methergoline. The effects of DOM alone (open symbols) or after 0.1 mg/kg (half-filled symbols) or 1.0 mg/kg (filled symbols) methergoline are plotted on the basis of the percent of control shocks received (top panel) and the percent of control fluid intake (bottom panel) in conditioned suppression testing. One mg/kg methergoline, and to a lesser extent 0.1 mg/kg, significantly shifted the DOM dose-response curves to the right, $p<0.05$ by analysis of variance. *$p<0.05$, Student's t-test for paired values. See Figure 40 legend for further information.

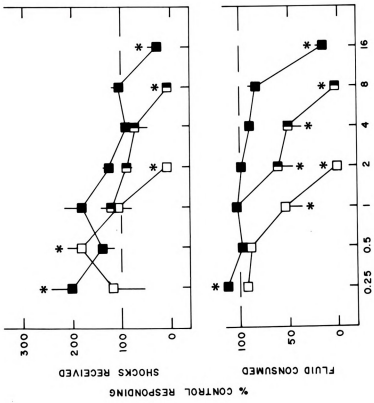

% CONTROL RESPONDING
SHOCKS RECEIVED
FLUID CONSUMED
mg/kg DOM
300
200
100
0
100
50
0
0.25
0.5
1
2
4
8
16

Figure 44

Figure 45. Antagonism of the effects of quipazine on punished and unpunished responding by methergoline. The effects of quipazine alone (open symbols) or after 0.1 mg/kg (half-filled symbols) or 1.0 mg/kg (filled symbols) methergoline are plotted on the percent of control shocks received (top panel) and the percent of control fluid intake (bottom panel) in conditioned suppression testing. One mg/kg methergoline, and to a lesser extent 0.1 mg/kg, significantly shifted the dose-response curves to the right, $p<0.05$ by analysis of variance. *$p<0.05$, Student's t-test for paired values. See Figure 40 legend for further information.

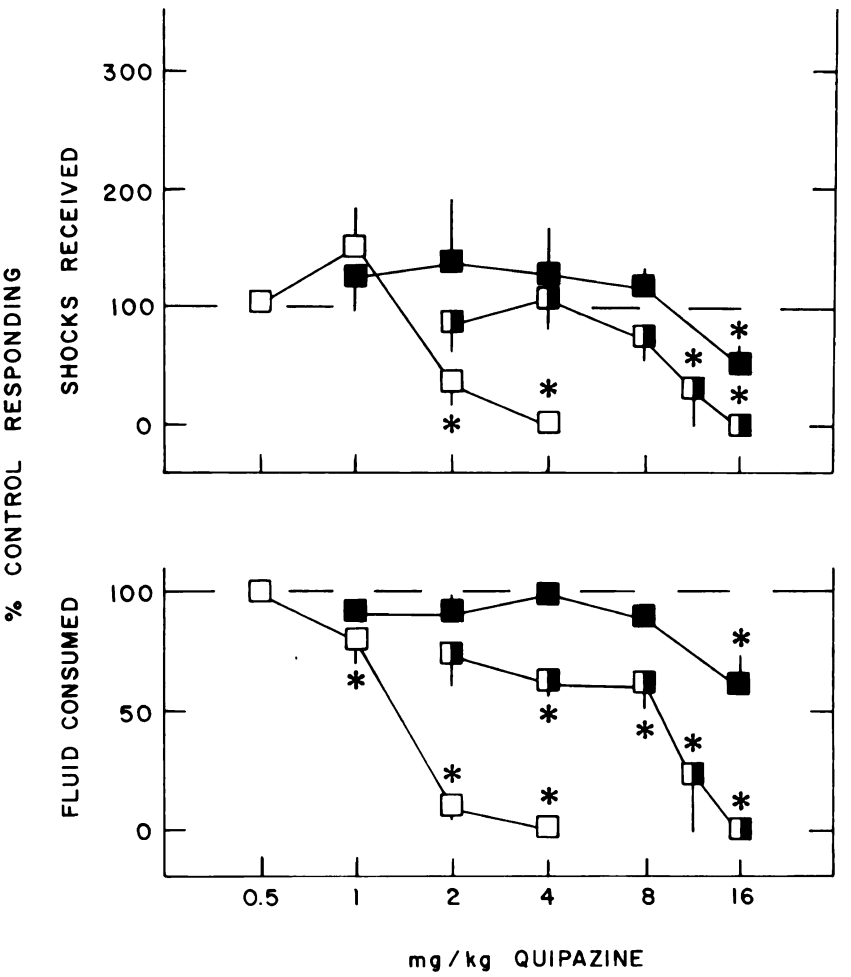

% CONTROL RESPONDING
SHOCKS RECEIVED
300
200
100
0
FLUID CONSUMED
100
50
0
0.5
1
2
4
8
16
mg/kg QUIPAZINE

Figure 45

with 1.0 mg/kg methergoline significantly attenuated the fluid-intake decreasing effects of LSD (Figure 43). This same dose of methergoline blocked the capacity of DOM and quipazine to decrease fluid intake up to doses of 8.0 mg/kg of the agonists, but not for higher doses (Figures 44 and 45). The lower dose of methergoline (0.1 mg/kg) antagonized these effects of both DOM and quipazine to a lesser extent than the 1.0 mg/kg dose. However, this antagonism was relatively much greater than the antagonism of the LSD effects produced by 1.0 mg/kg methergoline.

D. Discussion

In both the water reinforced and food-reinforced conflict procedures, the hallucinogens (LSD and DOM) produced a mild increase in punished responding. Quipazine also produced a tendency for an increase in punished responding in the food-reinforced conflict procedure. These effects were shown to be extremely weak in comparison to the anxiolytics pentobarbital and diazepam (Kilts, 1979; Ford et al., 1979). In addition to their varying effects on punished responding, all the agents examined produced a dose-dependent decrease in unpunished responding (fluid intake).

Neither the 5,7-DHT nor methergoline treatments (except at the highest dose of methergoline) had any effects on punished or unpunished responding. Also, neither the punishment-releasing nor the depressant effects of pentobarbital or methaqualone were altered by 5,7-DHT treatment. These data are in agreement with earlier results with phenobarbital in FR-40 (Figure 23). The weak punishment-releasing

effects of both LSD and DOM were blocked by 5,7-DHT treatment; moreover, the dose-dependent decreases in unpunished responding were potentiated by 5,7-DHT treatment. These data are also in agreement with earlier studies on FR-40 responding (Figure 22). Methergoline pretreatment alone produced no discernible change in unpunished responding. The effects of the hallucinogens and quipazine on unpunished responding, however, were antagonized by methergoline pretreatment. Again, as observed in the FR-40 studies, the effects of LSD were weakly (at best 2-fold) antagonized while those of DOM and quipazine were antagonized considerably more effectively (8- to 16-fold; see Figures 35, 36, 38 and 39). Also, as in the FR-40 studies (Figures 38 and 39), the methergoline antagonism of DOM and quipazine was found to be dependent on the dose of methergoline used. These results in the conditioned suppression paradigm provide no really new information; rather, they corroborate the critical results of the earlier studies conducted in the FR-40 paradigm.

SUMMARY AND GENERAL DISCUSSION

As had been reported by other investigators, the hallucinogens produced a disruption of FR-40 operant behavior characterized by periods of non-responding or "pausing" (Freedman et al., 1963; Appel and Freedman, 1964, 1965; Appel et al., 1970; Rech et al., 1975). Estimation of the pausing can be obtained by either the increase in the number of pause intervals or the decrease in reinforcements produced. In contrast, the non-hallucinogenic psychoactive agents d-amphetamine, phenobarbital, cocaine and chlorpromazine typically produced disruptions of FR-40 operant behavior characterized by slowed and erratic response rates within the operant session and not by pausing. Although an occasional subject would be observed to "pause" following administration of one of these agents (see also Appel and Freedman, 1965), quantitation of the pausing in a group of these subjects indicated that there was no increase in pause intervals apparent, although a dose-dependent decrease in reinforcements received (response rate) was observed. These findings are in agreement with an earlier report by Rech et al. (1975).

The capacity of the pause interval counter to differentiate the hallucinogens from the four non-hallucinogens was most apparent at near ED_{50} doses for the response-rate decreasing effects of all agents.

All four hallucinogens produced large increases in pausing at ED_{50} doses for a decrease in reinforcements, while the non-hallucinogens were typically without significant effect on pause intervals at dose levels reducing reinforcements to a 50 percent level.

Co-administration of a threshold dose of the stimulant d-amphetamine (for the response-rate decreasing effects) with various doses of LSD or DOM failed to alter the dose-response curves for the pausing produced by the hallucinogens. On the other hand, threshold doses of either the indolealkylamine LSD or the phenethylamine mescaline shifted the DOM dose-response curve to the right. These data further illustrate the capacity of the pause interval counter to differentiate the hallucinogens from another class of psychoactive agents.

As discussed in the General Introduction, the hallucinogens produce a number of behavioral effects that are presumably mediated through interactions with catecholamines, particularly DA neurons (Christoph et al., 1977; Koella et al., 1964; Meltzer et al., 1978; Menon et al., 1977; Trulson et al., 1977b; Tilson et al., 1975b; Yamamoto and Ueki, 1975). The data presented demonstrate that the FR-40 pausing produced by hallucinogens is not mediated through DA neurons. First, destruction of catecholamine neurons in the brain with intraventricular 6-OHDA failed to alter the dose-dependent pausing produced by either LSD or DOM. The FR-40 disrupting effects of d-amphetamine, in addition to being different in pattern from those produced by the hallucinogens, were attenuated by 6-OHDA treatment. This is in agreement with the presumed mechanism of action for this

agent (i.e., release of catecholamines from the brain; Rech and Stolk, 1970; Chiueh and Moore, 1973).

The lack of catecholamine involvement in the pause-producing effects of the hallucinogens was further substantiated by the results of the drug interaction studies using the neuroleptic agents chlorpromazine and haloperidol and the catecholamine synthesis inhibitor αMT. It has been reported previously that chlorpromazine actually enhances some of the effects of LSD (Marrazzi and Huang, 1979; Halasz et al., 1969). These data again suggest that, although hallucinogens may produce a number of effects mediated by catecholamine systems, the FR-40 pausing effect appears not to involve catecholamine neurons.

The results with αMT extend data from previous reports examining one dose of LSD (Appel and Freedman, 1964; Appel et al., 1970) to an entire dose-response analysis. However, a note of caution must be added in evaluating the results from the chlorpromazine and αMT interaction studies, since these treatments also failed to alter the dose-dependent disruption produced by d-amphetamine in the way that 6-OHDA did. Since the 6-OHDA-induced attenuation of the d-amphetamine disruption of FR-40 operant responding was earlier used as positive evidence for disruption of normal catecholamine activity, it may be suggested that these other treatments were without effect on catecholamine neurons. For example, it is possible that the pretreatment time for αMT was not long enough to allow for optimal antagonism of the effects of d-amphetamine, especially since they relate to disruptive actions rather than response enhancing properties (Rech et al., 1966; 1968; Rech and Moore, 1968; Stolk and Rech, 1970.

Destruction of 5-HT neurons with the intraventricular administration of 5,7-DHT selectively potentiated the FR-40 disruptive effects of both indolealkylamine and phenethylamine hallucinogens equally, as evidenced by similar shifts in the dose-response curves. These results extend earlier single-dose studies by investigators in Appel's laboratory (Appel et al., 1977; Joseph and Appel, 1977). Intraventricular 5,7-DHT administration also selectively altered the effects of hallucinogens on punished and unpunished responding, since the dose-dependent decreases in fluid intake produced by LSD and DOM were also potentiated by this 5,7-DHT treatment. These data strongly imply that hallucinogens interact with 5-HT neurons and/or receptors in the brain. Depletion of whole brain 5-HT by treatment with PCPA potentiated the FR-40 disruptive effects of LSD and mescaline, but not d-amphetamine (Appel et al., 1977); the present studies replicate the findings with LSD and extend them to the amphetamine analog, DOM.

Two approaches to the depletion of brain 5-HT concentrations have yielded similar results; i.e., depletion of central 5-HT enhances the effects of both phenethylamine and indolealkylamine hallucinogens equally. Several hypotheses can be advanced to explain these effects.

One possible explanation for these data is that, following 5,7-DHT treatment, the hallucinogens are acting as agonists at supersensitive 5-HT receptors. A number of investigators have suggested that hallucinogens are agonists at postsynaptic central 5-HT receptors (Andén et al., 1968, 1971, 1974; Appel et al., 1977; Browne and Ho, 1975; Glennon et al., 1979; Joseph and Appel, 1977; Kuhn et al., 1978; Silverman and Ho, 1980; White et al., 1980; Winter, 1969, 1978,

1980). As indicated above, the putative 5-HT antagonists methergoline and cinanserin block the decrease in reinforcements and "pause-producing" effects of hallucinogens without attenuating the effects of d-amphetamine or phenobarbital. Moreover, Nelson et al. (1978) have reported an increase in the number of hippocampal 5-HT binding sites (receptor supersensitivity) following 5,7-DHT treatment. Therefore, it may be postulated that (assuming the hallucinogens are acting as postsynaptic agonists in control animals) the 5,7-DHT-treated subjects exhibit a greater effect in response to these agonists as they interact with an increased number of receptors. However, a number of other drugs (e.g., fenfluramine, p-chloroamphetamine) activate post-synaptic 5-HT receptors, but they are not hallucinogenic in man (Trulson and Jacobs, 1976a). Perhaps the hallucinogens produce a particular pattern of regional post-synaptic receptor activation which differs from that produced by fenfluramine and p-chloroamphetamine, and this difference accounts for the hallucinogenic effects.

Another possible explanation for these data relates to the hypothesis for hallucinogenic drug actions originally proposed by Aghajanian et al. (1975). According to this hypothesis, hallucinogens activate autoreceptors on the cell bodies of 5-HT neurons in the raphé nuclei (Aghajanian, 1976; Aghajanian et al., 1970, 1972, 1975; Aghajanian and Haigler, 1974, 1975; Aghajanian and Wang, 1978; DeMontigny and Aghajanian, 1977; Haigler and Aghajanian, 1973, 1977) which in turn results in a cessation of discharge of these neurons. Since many central 5-HT neurons appear to be tonically inhibitory in nature (Aghajanian and Wang, 1978; Bloom et al., 1972; Haigler and Aghajanian,

1977), attenuation of this discharge would result in "disinhibition" in many forebrain areas, and presumably hallucinations, as higher centers received excessive and inappropriate input. According to this hypothesis, the threshold for the effects of the hallucinogens would be determined by the concentration of drug required to inhibit raphé cell firing. In 5,7-DHT treated animals, in which a large portion of the 5-HT pathways to the forebrain have been destroyed with a reduction in the reserve of the system, this threshold may be lowered relative to control subjects. Therefore, the effects of the hallucinogens would be potentiated as described here. One major problem with the hypothesis involving raphé neuron inhibition, however, is that a number of non-hallucinogenic agents are also potent inhibitors of raphé cell activity. This list includes the LSD analogue lisuride (Rogawski and Aghajanian, 1979), the 5-HT precursor 5-hydroxytryptophan (Trulson and Jacobs, 1976b) and the 5-HT releasing agents fenfluramine and p-chloroamphetamine (Sheard, 1974). Moreover, Trulson et al. (1977a) have demonstrated that hallucinogen-induced inhibition of raphé cell activity persists despite the demonstration of tolerance to the behaviorally disruptive effects of these agents in the same animals. These discrepancies must be resolved through further experimentation in order to assess the feasibility of this latter hypothesis for explaining the central nervous actions of hallucinogenic drugs.

Localized neurotoxin administration was used in an attempt to determine whether actions of these agents at post-synaptic 5-HT receptors in the forebrain or autoreceptors in the raphé nuclei were critical for the production of this pausing. Unfortunately, these studies

proved to be inconclusive because a number of brain regions were, for one reason or another, resistant to the selective neurotoxicity of 5,7-DHT. The nucleus accumbens and the septum, regions which had demonstrated effective destruction of 5-HT neurons in pilot studies, both proved to be negative in testing for a critical site of action for the effects of the hallucinogens. If the parameters of neurotoxin administration can be manipulated to provide adequate 5-HT neuronal destruction for the resistant brain regions, perhaps a number of additional brain sites can be examined with similar designs in future studies. Such possible sites include the amygdala and the median or dorsal raphé nuclei, to name a few.

Administration of 5,7-DHT into the MFB produced a pattern of central depletion that was different from that observed with intraventricular administration of the neurotoxin. This infusion of 5,7-DHT into the MFB also produced effects on the disruptive influences of the hallucinogens that differed from those of intraventricular 5,7-DHT. Instead of potentiating the effects of the hallucinogens equally, 5,7-DHT into the MFB potentiated the effects of LSD and attenuated the effects of DOM. The effects of mescaline, however, were unchanged by this treatment. The LSD potentiation was not as great in magnitude as that observed in the animals treated intraventricularly with 5,7-DHT. Similarly, the attenuation of the effects of DOM was not outstanding. Yet both were statistically significant. These data are the first that clearly suggest that the various hallucinogens may disrupt behavior by somewhat different mechanisms at various sites in the brain.

The putative 5-HT agonists quipazine and MCPP both produced a disruption of FR-40 operant responding characterized by pausing. This effect is most apparent at the near-ED_{50} dose to reduce reinforcements for each of these agents; at these doses pausing was significantly increased (80 or more counts over baseline) to an extent similar to that observed with the hallucinogens (see Table 3). These findings with quipazine are in agreement with those of White et al. (1977) and Winter (1979), in which the discriminative stimulus cues of quipazine were found to generalize to the hallucinogens. In addition to their pause-producing effects, threshold doses of both quipazine and MCPP shift the dose-response curve for the pause induced by DOM to the left, much as did the hallucinogens LSD and mescaline (see Figures 10 and 32). Since quipazine and MCPP are presumed to be post-synaptic 5-HT agonists, these data suggest more strongly that the pause-producing effects of the hallucinogens may be the result of post-synaptic 5-HT receptor activation.

The putative 5-HT antagonists cinanserin and methergoline, administered alone, produced a surprising decrease in the duration of pausing relative to control sessions. Methergoline actually increased FR-40 response rates over control levels, while cinanserin had no effect on response rates. These data suggest that the pausing produced during control FR-40 sessions may also be due to activation of 5-HT receptors by endogenous 5-HT activity. When administered in combination with the hallucinogens, both cinanserin and methergoline antagonized the pause-producing effects of all the hallucinogens examined. However, there were class differences in the nature of this

antagonism. The dose-response curves for the indolealkylamines were shifted at best 2-fold with either 5-HT antagonist. The DOM dose-response curve was shifted 3- to 4-fold to the right by the 20 mg/kg cinanserin dose. Methergoline pretreatment blocked the effects of DOM and mescaline to a greater extent, as the 1.0 mg/kg dose of the antagonist produced at least a 16-fold shift in the DOM dose-response curve. Thus, the effects of a normally supramaximal dose (28.4 mg/kg) of mescaline were completely blocked by the 1.0 mg/kg methergoline dose. This antagonism of the phenethylamine effects was found to be dependent on the methergoline dose, as 0.1 mg/kg produced only about an 8-fold shift in the dose-response curve for DOM. Quipazine, the putative 5-HT agonist, was found to be similar to the phenethylamines regarding the nature of its interaction with methergoline. That is, 1.0 mg/kg methergoline produced a greater than 8-fold shift in the dose-response curve for this agent to produce pausing, while the 0.1 mg/kg dose was somewhat less effective, producing a 4- to 6-fold shift.

Similar differences in the interaction of methergoline with LSD vs. DOM have been shown in the conditioned suppression paradigm. Methergoline by itself had no significant effect on punished or unpunished responding in the paradigm. The dose-dependent decrease in fluid intake produced by LSD was shifted to the right approximately 2-fold following pretreatment with the 1.0 mg/kg methergoline dose. As in the FR-40 studies (above) this same dose of methergoline shifted the dose-response curve for the fluid-intake decreasing effects of DOM to the right greater than 8-fold; 0.1 mg/kg methergoline produced a

somewhat smaller (still 4-fold) shift in this dose-response curve. Again, quipazine was found to be similar to DOM, and dissimilar to LSD, in the pattern of antagonism of its effects by methergoline.

The majority of the literature reports regarding 5-HT neurons and the behavioral effects of hallucinogens and 5-HT agonists has supported a common site of action and level of sensitivity (Andén et al., 1968, 1971, 1974; Appel et al., 1977; Browne and Ho, 1975; Glennon et al., 1979; Kuhn et al., 1978; Silverman and Ho, 1980; Schechter and Rosecrans, 1972; Winter, 1969, 1978, 1980). However, differences in the hallucinogenic drug classes and quipazine regarding their interactions with 5-HT antagonists have been suggested by investigators using other behavioral measures. For example, although head-twitching is produced by both LSD and quipazine, the capacity of the putative 5-HT antagonist methysergide to block this effect is much greater for quipazine than LSD (Vetulani et al., 1980). Moreover, although there have been no reports of class differences in hallucinogens regarding their discriminative stimulus properties in rats, a recent study by Järbe (1980) has suggested that pigeons trained to discriminate LSD will generalize readily to other indolealkylamine hallucinogens but do not generalize a strongly to the phenethylamine mescaline. Furthermore, the LSD discriminative cue is not antagonized by methergoline. Unfortunately, this paper did not address the capacity of methergoline to antagonize the mescaline cue.

The results of the present experiments strongly implicate 5-HT neurons in the behavioral effects of hallucinogens. However, there are now clear-cut differences between the phenethylamine and indolealkylamine classes as shown by the methergoline studies. Much of the data may be explained by postulating the presence of two sites of action for the effects of hallucinogens on FR-40 responding. The first type (type-I; Indolealkylamine) would be activated strongly by the indolealkylamines LSD and DMT and may not interact with the phenethylamines DOM and mescaline. The second type (type-P; phenethylamine) would be activated strongly by DOM, mescaline and quipazine and possibly more weakly by LSD and DMT. Activation of either or both receptor sites is presumed to disrupt the balance of brain functions to favor hallucinatory-type drug effects. Type-I receptors are apparently non-responsive or only weakly responsive to methergoline blockade; type-P receptors would be more effectively blocked by this agent. It is further proposed that the type-P receptor represents a postsynaptic 5-HT receptor, since the results of electrophysiological (Aghajanian et al., 1970, 1972, 1975; Aghajanian and Haigler, 1974, 1975; DeMontigny and Aghajanian, 1977; Haigler and Aghajanian, 1973, 1977) and behavioral (Andén et al., 1968, 1971, 1974; Browne and Ho, 1975; Glennon et al., 1979; Kuhn et al., 1978; Silverman and Ho, 1980; Winter,1969, 1978, 1980) studies have suggested that the hallucinogens of both classes activate post-synaptic receptors in the brain. Depletion of whole brain 5-HT by intraventricular 5,7-DHT or systemic PCPA potentiates the effects of LSD, DOM and mescaline equally. This potentiation

is most likely related to type-P receptor denervation supersensitivity. Indeed, 5,7-DHT treatment has been shown to increase the number of hippocampal (post-synaptic) 5-HT binding sites (Nelson et al., 1978). Working from this proposed model, one may explain the weak antagonism of LSD and DMT by methergoline on the basis of actions of the indolealkylamines on the methergoline-insensitive type-I receptors. The question remains of course, what is the type-I receptor and what is its normal function? Electrophysiological studies have shown that the indolealkylamine hallucinogens have the capacity to directly activate autoreceptors on the cell bodies of raphé neurons (Aghajanian et al., 1970, 1972, 1975; Aghajanian and Haigler, 1974, 1975; DeMontigny and Aghajanian, 1977; Haigler and Aghajanian, 1973, 1977). The phenethylamine mescaline and DOM are without such an effect directly (Aghajanian et al., 1970; Haigler and Aghajanian, 1973). These data can be interpreted to suggest that the type-I receptor site is the autoreceptor on the raphé cells. Conversely, the type-P receptor would involve neuronal inputs into the raphé neurons or would relate to post-synaptic 5-HT receptors in the forebrain.

Alternative proposals are not difficult to imagine. Both classes may exert their effects on postsynaptic 5-HT receptors in various regions of the forebrain, but by different mechanisms. The indole hallucinogens may bind at a receptor site that very effectively activates the effector changes, i.e., resulting in altered neuronal activity of the postsynaptic neurons. Methergoline may compete for this site with some degree of affinity, but little intrinsic activity (i.e.,

a competitive antagonist). Phenethylamine-type hallucinogenic agents may act at an allosteric site on the receptor to alter reactivity to the normal transmitter or even to activate the receptor but in a less direct manner. Methergoline, by blocking "downstream" from this site, would be much more effective as an antagonist against this class, since there would not be direct agonist-antagonist competition for the same site. Analyses of these proposals will require careful, appropriately designed experimental approaches beyond the scope of the current effort.

In summary, the present studies demonstrate that the FR-40 pause-producing effects of phenethylamine and indolealkylamine hallucinogens can be differentiated from the pattern of slowed and erratic intra-session response rates produced by a number of non-hallucinogenic psychoactive agents. The analyses combined the assessment of pausing, through the use of the pause interval timer, and the number of reinforcements obtained. Studies employing the neurotoxin 6-OHDA, the catecholamine synthesis inhibitor αMT, and receptor antagonists haloperidol and chlorpromazine have indicated that catecholamine neurons appear not to be involved in this pausing manifested by the hallucinogens. Studies employing agents to produce comparable manipulations of 5-HT neurons indicated that the hallucinogens appear to produce pausing through their agonistic actions at 5-HT receptors. Lastly, studies with the 5-HT antagonist methergoline, in which it was shown that this agent was more effective in antagonizing the effects of phenethylamines than indolealkylamine hallucinogens, have indicated

that there appear to be at least slight differences in the mechanisms by which agents of these two classes produce their effects.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aghajanian, G.K.: LSD and 2-bromo-LSD: Comparison of effects on serotonergic neurons and neurons in two serotonergic projection areas, the ventral lateral geniculate and amygdala. Neuropharmacol. 15: 521-528, 1976.

Aghajanian, G.K. and Haigler, H.J.: Mode of action of LSD on serotonergic neurons. Adv. Biochem. Psychopharmacol. 10: 167-177, 1974.

Aghajanian, G.K. and Haigler, H.J.: Hallucinogenic indoleamines: Preferential action upon presynaptic serotonin neurons. Psychopharmacol. Communs. 1: 619-629, 1975.

Aghajanian, G. and Wang, R.: The physiology and pharmacology of central serotonergic neurons. In: Psychopharmacology, A Review of Progress, 1967-1977, ed. by M. Lipton, A. DiMascio and K. Killam, Raven Press, 1978.

Aghajanian, G.K., Foote, W.E. and Sheard, M.E.: Action of psychotogenic drugs on single midbrain raphe neurons. J. Pharmacol. Exp. Ther. 171: 178-187, 1970.

Aghajanian, G., Haigler, H. and Bennett, J.: Amine receptors in the CNS. III. 5-Hydroxytryptamine in brain. In, Handbook of Psychopharmacology, Vol. 6, ed. by L.L. Iversen, S.D. Iversen and S.H. Snyder, pp. 63-96, Plenum Publishing Co., New York, 1975.

Aghajanian, G.K., Haigler, H.J. and Bloom, F.E.: Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons in rat. brain. Life Sci. 11: 615-622, 1972.

Andén, N.E., Corrodi, H. and Fuxe, K.: Hallucinogenic drugs of the indolealkylamine type and central monoamine neurons. J. Pharmacol. Exp. Ther. 179: 236249, 1971.

Andén, N.E., Corrodi, H., Fuxe, K. and Hökfelt, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Brit. J. Pharmacol. 34: 1-7, 1968.

Andén, N.E., Corrodi, H., Fuxe, K. and Meek, J.L.: Hallucinogenic phenethylamines: Interactions with serotonin turnover and receptors. Eur. J. Pharmacol. 25: 176-184, 1974.

Appel, J. and Freedman, D.: Chemically-induced alterations in the behavioral effects of LSD-25. Biochem. Pharmacol. 13: 861-869, 1964.

Appel, J. and Freedman, D.: The relative potencies of psychotomimetic drugs. Life Sci. 4: 2181-2186, 1965.

Appel, J. and Freedman, D.: Tolerance and cross-tolerance among psychotomimetic drugs. Psychopharmacologia (Berl.) 13: 267-274, 1968.

Appel, J., Joseph, J., Utsey, E., Hernandez, L. and Boggan, W.: Sensitivity to psychoactive drugs and the serotonergic neuronal system. Commun. Psychopharmacol. 1: 541-551, 1977.

Appel, J., Lovell, R. and Freedman, D.: Alterations in the behavioral effects of LSD by pretreatment with p-chlorophenylalanine and α-methyl-p-tyrosine. Psychopharmacologia (Berl.) 48: 387-406, 1970.

Baumgarten, H.G., Victor, S.J. and Lovenberg, N.: Effect of intraventricular injection of 5,7-dihydroxytryptamine on regional tryptophan hydroxylase of rat brain. J. Neurochem. 21: 251-253, 1973.

Björklund, A., Baumgarten, H.G. and Nobin, A.: Chemical lesioning of central monoamine axons by means of 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine. Adv. Biochem. Psychopharmacol. 10: 13-33, 1974.

Björklund, A., Baumgarten, H.G. and Rensch, A.: 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. J. Neurochem. 24: 833-835, 1975.

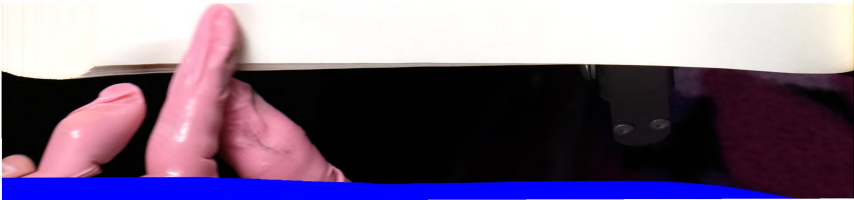
Bloom, F.E., Algeri, S., Groppetti, A., Reveutta, A. and Costa, E.: Lesions of central norepinephrine terminals with 6-OH-dopamine: Biochemistry and fine structure. Science 166: 1284-1286, 1969.

Bloom, F.E., Hoffer, B.J., Siggins, G.R., Barker, J.L. and Nicoll, R.A.: Effects of serotonin on central neurons: Microiontophoretic administration. Fed. Proc. 31: 97-106, 1972.

Brawley, P. and Duffield, J.: The pharmacology of hallucinogens. Pharmacol. Rev. 24: 31-67, 1972.

Breese, G.R. and Howard, J.L.: Effect of central catecholamine alterations on the hypothermic response to 6-hydroxydopamine in desipramine-treated rats. Brit. J. Pharmacol. 43: 671-674, 1971.

Breese, G.R. and Traylor, T.D.: Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. J. Pharmacol. Exp. Ther. 174: 413-420, 1970.



Breese, G.R. and Traylor, T.D.: Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. Brit. J. Pharmacol. 42: 88-99, 1971.

Breese, G.R., Vogel, R.A. and Mueller, R.A.: Biochemical and behavioral alterations in developing rats treated with 5,7-dihydroxytryptamine. J. Pharmacol. Exp. Ther. 205: 587-595, 1978.

Browne, R.G. and Ho, B.T.: Role of serotonin in the discriminative stimulus properties of mescaline. Pharmacol. Biochem. Behav. 3: 429-435, 1975.

Byck, R.: Drugs and the treatment of psychiatric disorders. In, The pharmacological Basis of Therapeutics, ed. by L.S. Goodman and A. Gilman, pp. 152-200, Macmillan Publishing Co., New York, 1975.

Cameron, O.G. and Appel, J.B.: A behavioral and pharmacological analysis of some discriminable properties of d-LSD in rats. Psychopharmacologia (Berl.) 33: 117-134, 1973.

Chang, C.: A sensitive method for spectrofluorimetric assay of catecholamines. Intl. J. Neuropharmacol. 3: 643-649, 1964.

Chiueh, C. and Moore, K.: Release of endogenously synthesized catechols from the caudate nucleus by stimulation of the nigrostriatal pathway and by the administration of d-amphetamine. Brain Research 50: 221-225, 1973.

Christoph, G., Kuhn, D. and Jacobs, B.: Electrophysiological evidence for a dopaminergic action of LSD: Depression of unit activity in the substantia nigra of the rat. Life Sci. 21: 1585-1596, 1977.

Cooper, J.R., Bloom, F.E. and Roth, R.H.: The Biochemical Basis of NeuroPharmacology. Oxford University Press, New York, 272 pp., 1974.

Curzon, G. and Green, A.: Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol. 39: 653-655, 1970.

Daly, J., Fuxe, K. and Johnsson, G.: 5,7-Dihydroxytryptamine as a tool for the morphological and functional analysis of central 5-hydroxytryptamine neurons. Res. Comm. Chem. Path. Pharmacol. 7: 175-187, 1974.

Demarest, K.T., Alper, R.H. and Moore, K.E.: Dopa accumulation is a measure of dopamine synthesis in the median eminence and posterior pituitary. J. Neural Transmission 46: 183-193, 1979.

DeMontigny, C. and Aghajanian, G.: Preferential action of 5-methoxytryptamine and 5-methoxydimethyltryptamine on presynaptic serotonin receptors: A comparative iontophoretic study with LSD and serotonin. Neuropharmacol. 16: 811-818, 1977.

Felice, L., Felice, J. and Kissinger, P.: Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. J. Neurochem. 31: 1461-1465, 1978.

Ferster, C. and Skinner, B.: Schedules of Reinforcement, Appleton-Century-Crofts, New York, 1957.

Fibiger, H., Fibiger, H. and Zis, A.: Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. Brit. J. Pharmacol. 47: 683-692, 1973.

Ford, R.D., Rech, R.H., Commissaris, R.L. and Meyer, L.Y.: Effects of acute and chronic interactions of diazepam and d-amphetamine on punished behavior of rats. Psychopharmacology 65: 197-204, 1979.

Freedman, D., Appel, J., Hartman, F. and Molliver, M.: Tolerance to behavioral effects of LSD-25 in the rat. J. Pharmacol. Exp. Ther. 143: 309-313, 1963.

Fuxe, K., Agnati, L. and Everitt, B.: Effects of methergoline on central monoamine neurons. Evidence for a selective blockade of central 5-HT receptors. Neuroscience Letters 1: 283-290, 1975.

Gaddum, J.H.: Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. J. Physiol. (London) 121: 15P, 1953.

Geller, I. and Blum, K.: The effects of 5-HTP on p-chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. Eur. J. Pharmacol. 9: 319-324, 1970.

Gerson, S. and Baldessarini, R.J.: Selective destruction of serotonin terminals in rat forebrain by high doses of 5,7-dihydroxytryptamine. Brain Res. 85: 140-145, 1975.

Glennon, R., Rosecrans, J., Young, R. and Gaines, J.: Hallucinogens as a discriminative stimuli: Generalization of DOM to a 5-methoxy-N,N-dimethyltryptamine stimulus. Life Sci. 24: 993-998, 1979.

Glennon, R., Young, R., Rosecrans, J. and Kallman, M.: Hallucinogenic agents as discriminative stimuli: A correlation with serotonin receptor affinities. Psychopharmacology 68: 155-158, 1980.

Haigler, H.J. and Aghajanian, G.K.: Mescaline and LSD: Direct and indirect effects on serotonin-containing neurons in brain. Eur. J. Pharmacol. 21: 53-60, 1973.

Haigler, H.J. and Aghajanian, G.K.: Serotonin receptors in the brain. Fed. Proc. 36: 2159-2164, 1977.

Halasz, M., Formanek, J. and Marrazzi, A.: Hallucinogen-tranquilizer interaction: Its nature. Science 164: 569-571, 1969.

Hamilton, C.L. and Wilpizeski, C.: Effect of LSD-25 on food intake in the rat. Proc. Soc. Exp. Biol. Med. 108: 319-321, 1961.

Hole, K., Fuxe, K. and Jonsson, G.: Behavioral effects of 5,7-dihydroxytryptamine lesions of ascending 5-hydroxytryptamine pathways. Brain Res. 107: 385-399, 1976.

Innes, I.R. and Nickerson, M.: Norepinephrine, epinephrine and the sympathomimetic amines. In, The Pharmacological Basis of Therapeutics, ed. by L.S. Goodman and A. Gilman, pp. 477-513, Macmillan Publishing Co., New York, 1975.

Jacobs, B.: Dreams and hallucinations: A common neurochemical mechanism mediating their phenomenological similarities. Neurosci. Biobehav. Rev. 2: 59-69, 1978.

Jacoby, J., Poulakos, J. and Bryce, G.: On the central anti-serotonergic actions of cyproheptadine and methysergide. Neuropharmacol. 17: 299-306, 1978.

Jaffe, J.: Drug addiction and drug abuse. In: The Pharmacological Basis of Therapeutics, ed. by L.S. Goodman and A. Gilman, pp. 284-324, Macmillan Publishing Co., New York, 1975.

Järbe, T.U.C.: LSD-25 as a discriminative stimulus for response selection by pigeons. Pharmacol. Biochem. Behav. 13: 549-554, 1980.

Joseph, J.A. and Appel, J.B.: Behavioral insensitivity to LSD: Depending upon the pattern of central 5-HT depletion. Pharmacol. Biochem. Behav. 6: 499-504, 1977.

Kilts, C.D.: Relationship of amygdaloid 5-hydroxytryptamine-containing neurons to anticonflict effects of benzodiazepines in the rat. Doctoral Dissertation, Michigan State University, 1979.

Klugh, H.E.: Statistics: The Essentials for Research, John Wiley and Sons, New York, 1974.

Koe, K.B. and Weissman, A.: p-Chlorophenylalanine: A specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154: 499-516, 1966.

Koella, W., Beaulieu, R. and Bergen, J.: Stereotyped behavior and cyclic changes in response produced by LSD in goats. Intl. J. Neuropharmacol. 3: 397-403, 1964.

König, J. and Klippel, R.: The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins Co., New York, 1967.

Kovacic, B. and Domino, E.: Tolerance and limited cross-tolerance to the effects of N,N-dimethyltryptamine (DMT) and lysergic acid diethylamide-25 (LSD) on food-rewarded bar pressing in the rat. J. Pharmacol. Exp. Ther. 197: 495-502, 1976.

Kovacic, B., Lu, L.W., Ruffing, D. and Domino, E.F.: Interactions of partial LSD analogs with behavioral disrupting effects of LSD and DMT in the rat. Eur. J. Pharmacol. 47: 37-44, 1978.

Krynock, G. and Rosecrans, J.: Morphine as a discriminative stimulus: Role of periaqueductal gray neurons. Res. Comm. Chem. Pathol. Pharmacol. 23: 49-60, 1979.

Kuhn, D., White, F. and Appel, J.: The discriminative stimulus properties of LSD: Mechanisms of action. Neuropharmacology 17: 257-263, 1978.

Lorden, J.F., Oltmans, G.A., Dawson, R., Jr. and Callahan, M.: Evaluation of the non-specific effects of catecholamine and serotonin neurotoxins by injection into the medial forebrain bundle of the rat. Pharmac. Biochem. Behav. 10: 79-86, 1979.

Lyness, W., Friedle, N. and Moore, K.: Measurement of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in discrete brain nuclei using reverse phase liquid chromatography with electrochemical detection. Life Sci. 26: 1109-1114, 1980.

Marrazzi, A. and Huang, C.: Qualitative identity of cerebral neuronal membrane actions of 5-HT, LSD and CPZ. Biol. Psychiat. 14: 637-644, 1979.

McGeer, P., Eccles, J. and McGeer, E.: Molecular Neurobiology of the Mammalian Brain. Plenum Press, New York, 1974.

Meltzer, H.Y., Fessler, R.G., Siminovic, M. and Fang, V.S.: Stimulation of rat prolactin secretion by indolealkylamine hallucinogens. Psychopharmacology 56: 255-259, 1978.

Menon, M., Clark, W. and Masuoka, D.: Possible involvement of the central dopaminergic system in the antireserpine effect of LSD. Psychopharmacology 52: 291-297, 1977.

Minnema, D., Krynock, G., Young, R., Glennon, R. and Rosecrans, J.: LSD as a discriminative stimulus: Role of dorsal raphe nucleus. Substance and Alcohol Actions/Misuse 1: 29-34, 1980.

Nelson, D.L., Herbert, A., Burgoin, S., Glowinski, J. and Hamon, M.: Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat. Mol. Pharmacol. 14: 983-995, 1978.

Nichols, D.: Structural correlation between apomorphine and LSD: Involvement of dopamine as well as serotonin in the actions of hallucinogens. J. Ther. Biol. 59: 167-177, 1976.

Nielsen, E.B., Lee, T.H. and Ellison, G.: Following several days of continuous administration d-amphetamine acquires hallucinogen-like properties. Psychopharmacology 68: 197-200, 1980.

Peterson, D.W. and Sparber, S.B.: Increased fixed ratio performance and differential d- and l-amphetamine action following norepinephrine depletion by intraventricular 6-hydroxydopamine. J. Pharmacol. Exp. Ther. 191: 349-357, 1974.

Pieri, L., Pieri, M. and Haefely, W.: LSD as an agonist of dopamine receptors in the striatum. Nature (Lond.) 252: 586-588, 1974.

Poling, A. and Appel, J.B.: Effects of quipazine on behavior under a multiple schedule of reinforcement. Pharmacol. Biochem. Behav. 8: 491-492, 1978.

Rech, R.H. and Moore, K.E.: Interactions between d-amphetamine and α-methyltyrosine in rat shuttle-box behavior. Brain Res. 8: 398-400, 1968.

Rech, R.H. and Stolk, J.: Amphetamine-drug interactions that relate catecholamines to behavior. In: Amphetamines and Related Compounds, ed. by E. Costa and S. Garatini, Raven Press, New York, pp. 385-413, 1970.

Rech, R.H., Borys, H.K. and Moore, K.E.: Alterations in behavior and brain catecholamine levels in rats treated with α-methyltyrosine. J. Pharmacol. Exp. Ther. 153: 412-419, 1966.

Rech, R.H., Carr, L.A. and Moore, K.E.: Behavioral effects of α-methyltyrosine following prior depletion of brain catecholamines. J. Pharmacol. Exp. Ther. 160: 326-335, 1968.

Rech, R.H., Tilson, H.A. and Marquis, W.J.: Adaptive changes in behavior after repeated administration of various psychoactive drugs. In: Neurobiological Mechanisms of Adaptation and Behavior, ed. A.J. Mandell, Raven Press, New York, pp. 263-286, 1975.

Roberts, D. and Fibiger, H.: Attenuation of amphetamine-induced conditioned taste aversion following intraventricular 6-hydroxydopamine. Neuroscience Letters 1: 343-347, 1975.

Robichaud, R. and Sledge, K.: The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. Life Sci. 8: 965-969, 1969.

Rodriguez, R., Rojas-Ramirez, J. and Drucker-Colin, R.: Serotonin-like actions of quipazine on the central nervous system. Eur. J. Pharmacol. 24: 164-171, 1973.

Rogawski, M. and Aghajanian, G.: Response of central monoaminergic neurons to lisuride: Comparison with LSD. Life Sci. 24: 1289-1298, 1979.

Ruffing, D., Kovacic, B., Demetriou, S. and Domino, E.F.: Naloxone enhancement of DMT and LSD-25-induced suppression of food-rewarded bar pressing behavior in the rat. Psychopharmacology 62: 207-210, 1979.

Ruffing, D.M. and Domino, E.F.: First dose behavioral tolerance to phencyclidine on food-rewarded bar pressing behavior in the rat. Psychopharmacology 69: 1-4, 1980.

Samanin, R., Bendotti, C., Candelarssi, G. and Garattini, S.: Specificity of serotonergic involvement in the decrease of food intake induced by quipazine in the rat. Life Sci. 21: 1259-1266, 1977.

Samanin, R., Mennini, T., Ferraris, A., Bendotti, C., Borsini, F. and Garattini, S.: m-Chlorophenylpiperazine: A central serotonin agonist causing powerful anoxia in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 159-163, 1979.

Samanin, R., Mennini, T., Ferraris, A., Bendotti, C. and Borsini, F.: Hyper-and hyposensitivity of central serotonin receptors: [^{3}H]Serotonin binding and functional studies in the rat. Brain Res. 189: 449-457, 1980.

Schechter, M.D. and Rosecrans, J.A.: Lysergic acid diethylamide (LSD) as a discriminative cue: Drugs with similar stimulus properties. Psychopharmacologia (Berl.) 26: 313-316, 1972.

Schoenfeld, R.: Lysergic acid diethylamide- and mescaline-induced attenuation of the effect of punishment in the rat. Science 192: 801-803, 1976.

Shannon, H.E.: MDA and DOM: Substituted amphetamines that do not produce amphetamine-like discriminative stimuli in the rat. Psychopharmacology 67: 311-312, 1980.

Sheard, M.: The effect of p-chloroamphetamine on single raphe neurons. Adv. Biochem. Psychopharmacol. 10: 179-184, 1974.

Silverman, P.B. and Ho, B.T.: The discriminative stimulus properties of 2,5-dimethoxy-4-methylamphetamine (DOM): Differentiation from amphetamine. Psychopharmacology 68: 209-215, 1980.

Sloviter, R.S., Drust, E.G., Damiano, B.P. and Connor, J.D.: A common mechanism for lysergic acid, indolealkylamine and phenethylamine hallucinogens: Serotonergic mediation of behavioral effects in rats. J. Pharmacol. Exp. Ther. 214: 231-238, 1980.

Spector, S., Sjoerdsma, A. and Udenfriend, S.: Blockade of endogenous norepinephrine synthesis by α-methyltyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 158: 140-149, 1967.

Stewart, R.M., Growdon, J.H., Cancian, D. and Baldessarini, R.J.: 5-Hydroxytryptophan-induced myoclonus: Increased sensitivity to serotonin after intracranial 5,7-dihydroxytryptamine in the adult rat. Neuropharmacol. 15: 449-455, 1976.

Stolk, J.M. and Rech, R.H.: Antagonism of d-amphetamine alpha-methyl-L-tyrosine: Behavioral evidence for the participation of catecholamine stores and synthesis in the amphetamine stimulant response. Neuropharmacology 9: 249-263, 1970.

Tenen, S.: The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. Psychopharmacologia (Berl.) 10: 204-210, 1967.

Tilson, H.A. and Rech, R.H.: The effects of p-chlorophenylalanine on morphine analgesia, tolerance and dependence development in two strains of rats. Psychopharmacologia (Berl.) 35: 45-60, 1974.

Tilson, H.A., Baker, T.G. and Gylys, J.A.: A comparison of the discriminative stimulus properties of R-2,5-dimethoxy-4-methylamphetamine (R-DOM) and S-amphetamine in the rat. Psychopharmacologia (Berl.) 44: 225-228, 1975.

Tilson, H., Baker, T., Chamberlain, J., Marquis, W. and Rech, R.: Behavioral and neuropharmacological analysis of amphetamine and 2,5-dimethoxy-4-methylamphetamine in rats. Psychopharmacologia (Berl.) 44: 229-239, 1975.

Trulson, M.E., Eubanks, E.E. and Jacobs, B.L.: Behavioral evidence for supersensitivity following destruction of central serotonergic nerve terminals by 5,7-dihydroxytryptamine. J. Pharmacol. Exp. Ther. 198: 23-32, 1976.

Trulson, M.E. and Jacobs, B.L.: Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. Eur. J. Pharmacol. 36: 149-154, 1976a.

Trulson, M. and Jacobs, B.: Dose-response relationships between systemically administered l-tryptophan or l-5-hydroxytryptophan and raphe unit activity in the rat. Neuropharmacol. 15: 339-344, 1976b.

Trulson, M.E. and Jacobs, B.L.: Effects of LSD on behavior and raphe unit activity in freely moving rats. Fed. Proc. 37: 346, 1978.

Trulson, M.E., Ross, C.A. and Jacobs, B.L.: Lack of tolerance to the depression of raphe unit activity by lysergic acid diethylamide. Neuropharmacol. 16: 771-774, 1977a.

Trulson, M., Stark, A. and Jacobs, B.: Comparative effects of hallucinogenic drugs on rotational behavior in rats with unilateral 6-hydroxydopamine lesions. Eur. J. Pharmacol. 44: 113-119, 1977b.

Umezu, K. and Moore, K.E.: Effects of drugs on regional brain concentrations of dopamine and dihydroxyphenylacetic acid. J. Pharmacol. Exp. Ther. 208: 49-56, 1979.

Ungerstedt, U.: Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. 367: 69-93, 1971.

Uretsky, N.J. and Iversen, L.L.: Effects of 6-hydroxydopamine on catecholamine neurons in the rat brain. J. Neurochem. 17: 269-278, 1970.

Vargaftig, B.B., Coignet, J.L., DeVos, C.J., Grijsen, H. and Bonta, I.L.: Mianserin hydrochloride: Peripheral and central effects in relation to antagonism against 5-hydroxytryptamine and tryptamine. Eur. J. Pharmacol. 16: 336-346, 1971.

Vetulani, J., Bednarczyk, B., Reichenberg, K. and Rokosz, A.: Head twitches induced by LSD and quipazine: Similarities and differences. Neuropharmacol. 19: 155-158, 1980.

Vrbanac, J., Tilson, H., Moore, K. and Rech, R.: Comparison of 2,5-dimethoxy-4-methylamphetamine (DOM) and d-amphetamine for in vivo efflux of catecholamines from rat brain. Pharmacol. Biochem. Behav. 3: 57-64, 1975.

Weston, P. and Overstreet, D.: Does tolerance develop to low doses of d- and l-amphetamine on locomotor activity in rats? Pharmacol. Biochem. Behav. 5: 645-649, 1976.

White, F., Kuhn, D. and Appel, J.: Discriminative stimulus properties of quipazine. Neuropharmacol. 16: 827-832, 1977.

White, F., Simmons, M., West, K., Holohean, A. and Appel, J.: The effect of serotonin depletion on the discriminability of LSD. Pharmacol. Biochem. Behav. 13: 569-574, 1980.

Winter, J.C.: Behavioral effects of N,N-dimethyltryptamine: Absence of antagonism by xylamidine tosylate. J. Pharmacol. Exp. Ther. 169: 7-16, 1969.

Winter, J.C.: Tolerance to a behavioral effect of lysergic acid diethylamide and cross-tolerance to mescaline in the rat: Absence of a metabolic component. J. Pharmacol. Exp. Ther. 178: 625-630, 1971.

Winter, J.C.: Hallucinogens as discriminative stimuli. Fed. Proc. 33: 1825-1832, 1974.

Winter, J.C.: The effects of 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethoxy-4-ethylamphetamine (DOET), d-amphetamine and cocaine in rats trained with mescaline as a discriminative stimulus. Psychopharmacologia (Berl.) 44: 29-32, 1975.

Winter, J.C.: Stimulus properties of phenethylamine hallucinogens and lysergic acid diethylamide: The role of 5-hydroxytryptamine. J. Pharmacol. Exp. Ther. 204: 416-423, 1978.

Winter, J.C.: Quipazine-induced stimulus control in the rat. Psychopharmacology 60: 265-269, 1979.

Winter, J.C.: Effects of the phenethylamine derivatives, BL-3912, fenfluramine, and Sch-12679, in rats trained with LSD as a discriminative stimulus. Psychopharmacology 68: 159-162, 1980.

Wooley, D.W. and Shaw, E.: A biochemical and pharmacological suggestion about certain mental disorders. Proc. Natl. Acad. Sci. 40: 228-231, 1954.

Yamamoto, T. and Ueki, S.: Behavioral effects of 2,5-dimethoxy-4-methylamphetamine (DOM) in rats and mice. Eur. J. Pharmacol. 32: 156-162, 1975.