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Drug Discrimination: Applications to Drug Abuse Research

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Drug Discrimination: Applications to Drug Abuse Research

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Introduction

Richard A. Glennon

The drug discrimination (DD) paradigm is essentially a drug detection procedure whereby animals are trained to recognize or discriminate the stimulus effects of a given dose of a particular training drug from those of (1) a different dose of the same training drug, (2) a different training drug, or, more commonly, (3) saline/vehicle (i.e., a nondrug condition). The most commonly employed apparatus for conducting drug discrimination studies is a two-lever operant chamber; however, several other types of test procedures, including three-lever chambers, are also being used. Several species of animals are quite popular, primarily rat, pigeon, and monkey; the use of other species, including human, is now becoming more common.

The DD paradigm, by itself, cannot be used to completely characterize a novel agent. This is true of any pharmacological procedure. However, the DD paradigm can be used to investigate a wide variety of pharmacological aspects relating to the stimulus properties of a drug. These aspects include, for example, time of onset and duration of action, mechanism of action, similarity of effect to other agents, structure-activity relationships, activity of metabolites, and identification and development of potential antagonists. Recently, the paradigm has been used to investigate the processes of tolerance and withdrawal.

There have already been several international drug discrimination symposia. For the most part, these symposia were concerned more with the technique itself than with the application of the technique for the investigation of a particular problem. Prior emphasis has been on, for example, the use of drug discrimination in central nervous system pharmacology and on the transduction mechanisms involved in the stimulus effects of drugs. Because many of the drugs used in DD studies have a potential for abuse or, indeed, are already noted for their abuse, we felt the time was right to address the application of DD in drug abuse research.

This monograph presents selected papers from the 1990 International Drug Discrimination Symposium held in Noordwijkerhout, The Netherlands, June 25-27,1990. The symposium was cosponsored by the European Behavioral Pharmacology Society and the Society for the Stimulus Properties of Drugs. The theme for the 1990 symposium was “Drug Discrimination: Applications in Drug Abuse Research.” The meeting consisted of a number of invited presentations on drug abuse research; it was also open to a general poster session. Abstracts for most of the presentations were published as a supplement to *Psychopharmacology* volume 101,1990. Unlike previous symposia, this meeting brought together members of the international academic, industrial, government, and drug enforcement communities to discuss the relevance and application of the paradigm to a single health-related issue: drug abuse. Presentations ranged from the basic science, such as mechanisms of action and structure-activity relationships, to DD studies involving human subjects, to the role of DD in the legal control of abused substances. Another topic of interest was the use of abused substances as training drugs for developing new therapeutic agents.

The consensus of the symposium participants was that the DD paradigm is an important, useful, and very versatile tool for investigating drugs of abuse. However, the committee invited several speakers to address this issue in a formal sense. A separate session devoted to this topic was entitled “Invited Perspectives.” The committee selected a member of the U.S. Drug Enforcement Administration (Dr. F. Sapienza) to address the issue from a law enforcement perspective. Two additional speakers were also requested to critique the DD paradigm and/or the symposium per se. To obtain a balanced viewpoint, one speaker (Dr. J. Appel) was selected because of his contributions to the field of DD research and because he could address the issues as an informed insider. The other speaker (Dr. L. Harris) has had extensive experience in the field of drug abuse research and is familiar with the technique of DD but does not actively conduct such studies in his own laboratory; his comments may be taken as those of an unbiased outsider. Dr. Harris was given the difficult task of critiquing the entire symposium.

The Scientific Organizing Committee for the symposium was composed of Dr. Richard A. Glennon (United States, chair), Dr. Toby Järbe (Sweden, cochair), Dr. John A. Rosecrans (United States), Dr. Ian P. Stolerman (United Kingdom), and Dr. Alice M. Young (United States). The Scientific Organizing Committee was assisted by a local organizing committee without whose help the symposium would have been impossible. The local committee consisted of Drs.

C.L. Broekkamp, A.R. Cools, M.R. Kruk (chair), J.H.C.M. Lammers, B.M. Spruyt, and A.M. Van der Poel. The Scientific Organizing Committee acknowledges the efforts of the local committee in helping make this meeting a success.

The Scientific Organizing Committee was awarded a conference grant from the National Institute on Drug Abuse. These funds secured the success of the symposium and provided awards for several young investigators on a competitive basis. The award winners include Drs. SD. Comer, J.P. Druhan, C.P. France, S. Gleeson, L.H. Gold, G.A. Rowan, S. Smurthwaite, H.F. Villanueva, and E.I. Walker.

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A Historical Perspective on Drug Discrimination

Donald A. Overton

INTRODUCTION

Drug-induced state-dependent learning (SDL), as well as the ability of physiological states to control retrieval of memories, has been known at least since 1830. Until 1950, however, understanding of this area was based primarily on clinical descriptions of fugue states, somnambulism, dream recall, and cases of multiple personality. Since 1950, a series of experimental demonstrations of the properties of SDL and drug discriminations (DDs), along with progressive modifications of the DD procedure-each change in itself relatively inconsequential-has led to the development of the DD paradigm that is being employed now. Its properties make it an extremely useful tool for preclinical investigation of a variety of pharmacological and psychological questions. These technical and conceptual developments have allowed widespread acceptance of the DD paradigm as a method for conducting preclinical research. This paper reviews the 19th- and 20th-century history of concepts, experiments, and clinical observations that led to the current status of knowledge about drug-induced SDL and DDs.

SDL IN THE 19TH CENTURY

Throughout the 19th century there was widespread interest in hypnosis, fugue states, somnambulism, multiple personality, and other forms of amnesia (Ellenberger 1970). Various explanations for these clinical phenomena were put forward, including (after 1830) the idea that the physiological state of the organism determined, at each instant in time, which memories were accessible to consciousness. Combe (1830, pp. 520-522) wrote as follows.

> The patient was a girl of 16 [who had episodic somnambulistic attacks]. . . . The circumstances [events] which occurred during the

> paroxysm were completely forgotten by her when the paroxysm was over, but were perfectly remembered during subsequent paroxysms.
>
> Dr. Abel informed me of an Irish porter to a warehouse, who forgot, when sober, what he had done when drunk; but being [again] drunk, again recollected the transactions of his former state of intoxication. On one occasion, being drunk, he had lost a parcel of some value, and in his sober moments could give no account of it. Next time he was intoxicated, he recollected that he had left the parcel at a certain house, and there being no address on it, it had remained there safely, and was got on his calling for it.
>
> The only conclusion which seems to arise . . . is that before memory can exist, the organs [have] to be affected in the same manner, or to be in a state analogous to that in which they were, when the impression was first received.
>
> These facts cannot be accounted for in a satisfactory way; but by communicating a knowledge of their existence, attention will be drawn to them, and future observations and reflection may ultimately throw light upon the subject.

Combe's report was a direct extension of the zeitgeist of the time. It had been known for a century that memories for the hypnotic state could not be retrieved in the normal waking state, although they clearly persisted in unconscious form (Chastenet de Puységur 1809). The similarities between hypnosis and somnambulism were sufficient so that hypnosis was often called artificial somnambulism (Ellenberger 1970). Somnambulistic patients were also known to have state-dependent recall sometimes, as indicated in the first sentences of the preceding quotation. However, the inclusion of a drug state (alcohol) as a determiner of memory retrieval was probably a novel contribution by Combe, because the case described above was quoted during the next half century as evidence for drug-induced SDL and no other previous (or subsequent) evidence was ever cited.

For many years after Combe's "Irish porter" case was reported, the idea that alcohol could produce SDL was carried forward through the medical literature (Elliotson 1840, p. 646; Macnish 1834, p. 78; Macnish 1835, p. 30; Winslow 1860, p. 338). In 1868, Wilkie Collins incorporated SDL produced by drugs into the plot of his novel *The Moonstone.* The novel was published in serialized form

and widely read, thus making the concept of SDL even more available to the public.

INTEGRATION OF SDL INTO THEORIES OF LEARNING AND RECALL

The period 1880-l910 saw a major change in the status of SDL, in that it was integrated into comprehensive theories of memory retrieval and personality. Ribot proposed that the control of memory retrieval by bodily state was specifically mediated by the mechanism of "organic sensations," which he enumerated, and which were essentially equivalent to stimuli later denoted by the term "interoceptive stimuli" (Ribot 1891, pp. 23-30). Ribot was also somewhat more explicit than previous writers in asserting that the stimulus effects of the normal no-drug state were equally important as those of abnormal states, and that the memory retrieval in the no-drug (N) state could occur only as long as N cues were present. Hence he made an explicit assertion that N-state cues were as salient as drug (D)-state cues, and that equally large impairments in memory retrieval would be produced by D→N and by N→D state changes (Ribot 1882, pp. 108-l15). This was the first mechanistic theory for SDL, and it was a stimulus theory.

Semon explicitly integrated ethanol-induced SDL into his comprehensive model for memory formation and retrieval (Semon [1904] 1921, pp. 144-145; [1909] 1923, p. 180); see discussions of Semon (Schacter et al. 1978; Schacter 1982, p. 185). Coriat (1906) conducted experiments to show that memories for periods of alcoholic blackout could sometimes be retrieved if special techniques were employed, and he mentioned the Irish porter case as showing that such memories sometimes became available if the subject again became intoxicated. In 1914, Prince published a comprehensive description of dissociation and unconscious processes, which included the following statement.

> We may. . . lay it down as a general law that during any dissociated state, no matter how extensive or how intense the amnesia, [memories of] all the experiences that can be recalled in any other state, whether the normal one or another dissociated state, are conserved and, theoretically at least, can be made to manifest themselves. And, likewise and to the same extent, during the normal state [memories of] the experiences which belong to a dissociated state are still conserved, notwithstanding the existing amnesia for those experiences. (Prince 1914, p. 78)

It should be noted, however, that Prince did not explicitly refer to SDL in a fashion comparable to Ribot, Semon, or Cornbe; instead, he mentioned reintoxication only as a method of assisting retrieval for periods of alcoholic blackout without proposing state equivalence as the mechanism that was responsible for this retrieval (p. 81). Prince's book was reprinted at least as late as 1929. Semon's books were translated into English in the 1920s, and some of Ribot's books were reprinted as late as 1910, thereby making the SDL concept available at that time.

DISAPPEARANCE OF THE CONCEPT OF SDL

Although drug-induced SDL had been accepted as a property of the brain for three-quarters of a century, I have not been able to trace the concept past the first quarter of the 20th century. Although I am not very well informed about intellectual trends during this period, I will venture a few comments about this development.

SDL had been an integral part of the exaggerated interest in the unconscious that developed during the 19th century. For example, Whyte (1960, p. 168) states that popular acceptance of the idea of unconscious determination of behavior progressed from being "credible" in 1700 to being "topical" by 1800 to being "fashionable" by 1875 (as reflected by the presence of SDL in *The Moonstone).* Ellenberger (1970) characterizes the 19th century as the era of the "first dynamic psychiatry," which was primarily concerned with phenomena of somnambulism, catalepsy, multiple personality, hysterical symptoms, and hypnotism (p. 111); he also reports that this school of thought was actively rejected starting in 1880 (p. 171); the usually accepted date for its demise was 1900 (p. 174).

During the transition phase before the "new dynamic psychiatry" was firmly established by Freud and others, some workers still accepted the existence of SDL, as indicated by mention of SDL in the writings of Ribot and Semon. However, the phenomenon was not considered as important as it had been 50 years earlier, as indicated by its very brief mention in books published after 1900. Incidentally, it is not certain that Semon or Prince had even read the original case report by Combe, because they cited Ribot as the source of the Irish porter case and Ribot in turn described the Irish porter case as "well known," but did not explicitly cite Combe (Ribot 1882, p. 115).

Later, psychologists were influenced by Freud's idea that most dissociation occurred because of avoidance of one sort or another, that is, because of

strong emotional reactions leading to repression. This idea apparently led them to reject the earlier tendency of viewing all dissociative phenomena as reflecting the operation of physiological processes. Janet, for example, characterizes the attempt to explain somnambulism (which term he used to refer to multiple personality and hysterical symptoms), hypnotic suggestibility, and automatic writing (other techniques previously accepted as routes to the unconscious) in terms of physical brain function as "pure childishness" and "useless reveries" (Janet 1907, p. 63). Possibly, as new ideas about the origins and contents of the unconscious were developed, SDL just fell through the cracks and was lost. SDL clearly was not a dynamic process, and it was not a frequently observed physiological process. Indeed, no second case of drug-induced SDL had ever been reported, and so the whole existence of the phenomenon rested on the single case report by Combe.

Subsequently, experimental psychology apparently became increasingly disinterested in dissociation of all types. Hilgard (1977, p. 10) has commented on other factors that possibly underlay the rapid loss of interest in dissociation that apparently occurred in the newly developing behavioral psychology in the 1930s; in his view, interest waned more by social consensus than because of any new data that explicitly reduced the significance of unconscious processes. For whatever reasons—although knowledge of dissociation, somnambulism, fugues, and multiple personality persisted—this writer has not found instances after Prince (1914) in which state-dependent retrieval was mentioned in the literature.

PRECURSORS OF REDISCOVERY

Goodwin (1972) pointed out that Charlie Chaplin's 1931 movie "City Lights" depicts events remarkably resembling ethanol-induced SDL (McDonald et al. 1965), although alternative explanations for the events portrayed in the film are possible. If the film depicts SDL, then where did Chaplin get the idea? Chaplin's autobiography does not clarify the issue (Chaplin 1964, p. 325); indeed, his wording suggests that the film was not intended to portray SDL. Unfortunately, Chaplin's autobiographical statements are reported to be highly unreliable (Geduld 1985) which leaves us with substantial doubts about what the film was intended to portray. All that can be concluded is that "City Lights" may possibly have reflected a knowledge of SDL on Chaplin's part.

In 1937, Girden and Culler reported an experimental demonstration of drug-induced dissociation between the drug state produced by raw curare and the no-drug condition. Initially they studied the conditioned leg flexion response

in dogs. Later they expanded their work to include dissociation in other response modalities. Girden's experiments had serious methodological difficulties resulting from his use of a drug that paralyzed the experimental subjects. Although Girden tested a number of dogs, only a few were tested by means of paradigms that would allow evaluation of whether SDL had occurred or not, and Gardner's replication effort was largely unsuccessful (Gardner 1961; Gardner and McCullough 1962). Nonetheless, Gitden's work was apparently accepted by the scientific community and appeared in textbooks of physiological psychology for 20 years after it was reported (e.g., Morgan and Stellar 1950, p. 450). Girden did not cite the 19th-century literature on SDL. Although few of the individuals who studied the stimulus properties of drugs during the following 30 years acknowledged that their work was influenced by Girden's findings, his studies mark the starting point of modern experimental work on SDL and DDs.

STATUS AS OF 1950

By 1950, the following ideas had been published: (1) Current physiological state determines which memories are retrievable at any instant in time. (2) Both the no-drug state and abnormal states have equally important influence on memory retrieval. (3) Control of recall by bodily states may or may not be mediated by the mechanism of interoceptive sensations. (4) Environmental contextual cues also determine recall in a fashion analogous to the control of recall by interoceptive stimuli. (5) Drugs may induce dissociation by functionally decorticating the animal (Girden and Culler 1937).

The data underlying these assertions included many clinical observations of hypnosis, somnambulism, fugue, dissociation, and multiple personality collected during the 19th century, along with one reported case of SDL in a delivery man who got drunk on the job around 1830, and some muscle spindle twitches in Girden's dogs.

Developments that directly underlie current DD methods started in 1951, and the ensuing 25 years saw a progressive increase in the amount of experimental attention devoted to stimulus properties of drugs. These studies can be organized according to various themes. Some studies dealt with theoretical models for SDL. Some measured whether significant amounts of SDL were produced by clinically used doses of tranquilizers. Many were intended to investigate neurochemical issues. The remainder of this paper will selectively

review this history, focusing on developments that contributed to the development of the DD method as the practically applicable investigational tool it is now.

THE FIRST DRUG DISCRIMINATION STUDY

Conger (1951) reported the first DD study. He was trying to study the effects of alcohol on approach and avoidance behavior and realized that the effects he was observing could be caused either by the intrinsic effects of ethanol or by stimulus generalization deficits resulting from a change in drug state between training and testing, that is, by SDL. In his words:

> The avoidance was established under the condition of sober; one group was then tested under the . . . condition of sober, and the other group under the different condition of inebriation. Thus it is logically possible that the decrease in the avoidance response might be due solely to a change in the animal's condition (regardless of the direction of the change) rather than to any specific effect of alcohol [because] it seems likely that a change from sobriety to inebriation (or vice versa) produces a change in the animal's stimulus situation. (Conger 1951, p. 15)

Conger then made an unprecedented contribution by pointing out that if ethanol did have stimulus effects, then the existence of these effects could be demonstrated by using a discrimination training procedure. In an approach and avoidance task, Conger's rats learned to approach when drunk and avoid when sober, or vice versa, thus becoming the first animals in history to learn a DD in a laboratory setting. It is interesting that Conger's study never answered the question that led him to perform it. By showing that ethanol could exert discriminative control, the rats indicated that stimulus generalization effects might occur and that the intrinsic effects of ethanol might be confounded with SDL in the experiments that Conger had conducted. However, his results never answered the questions of whether or to what degree such confounding actually had occurred. In Conger's report, no prior literature was cited as indicating that drugs could act as stimuli.

THE 2x2 EXPERIMENTAL DESIGN

In the same year, Auld (1951) published the first study I know of that used a 2x2 experimental design to evaluate drug stimulus effects. His experiment tested

the effect of tetraethylammonium (TEA) on escape and avoidance performance. No SDL effects were found, which is reasonable because TEA primarily produces effects outside the brain. Why was Auld concerned enough about stimulus effects to use a 2x2 design?

In subsequent years, Miller repeatedly proposed the 2x2 experimental design as a method for determining the relative strength of SDL effects and other drug effects (Grossman and Miller 1961; Miller 1957; Miller and Barry 1960). He actively promoted use of the 2x2 paradigm, arguing that it was unwise to attempt to determine the intrinsic effects of drugs without including the extra experimental groups that would demonstrate stimulus generalization decrements if they were present. For reference, the following table describes the structure and properties of that design.

Table 1. *The 2 by 2 experimental design*

Group	Training Session State	Test Session State	Effects Present in Test Session Data
1	N	N	None
2	N	D	Retrieval deficit + performance deficit + SDL
3	D	N	Memorization deficit + SDL
4	D	D	Memorization deficit + retrieval deficit + performance deficit

The quantitative size of the SDL effect is computed from test session performance by groups 1 + 4 - 2 - 3. Any effects of drug during training trials on memory consolidation is computed from test performance of groups 1 + 2 - 3 - 4. Depressant drug effects on performance during test trials cannot be distinguished from drug-induced impairments of memory retrieval, and the combined size of these two effects is computed from test performance in groups 1 + 3 - 2 - 4. The design assumes that all effects are linearly additive and that SDL is symmetrical with equally large decrements after D→N and N→D state changes. If any effects other than the postulated ones are present, then the computed effect sizes will be incorrect.

STUDIES ON SDL

Conger had demonstrated discriminative control only after a moderately prolonged series of training trials. However, a number of other investigators soon tested whether the stimulus effects of drugs might be strong enough to produce the generalization decrements postulated by Conger; they conducted 2x2 studies, many of which yielded evidence for weak SDL effects (Barry et al. 1962; Belleville 1964; Heistad 1957; Heistad and Torres 1958; Murphy and Miller 1955; Otis 1964; Shmavonian 1956). Not all SDL studies from this era resulted from the hypothesis that drugs produced stimulus effects; other models for brain function, later termed “neurological” models by Bliss (1974)) also predicted SDL, and several SDL studies were conducted by investigators seeking to test these models (Overton 1964; Sachs 1966). In addition, Holmgren (1964a, 1964b) reported SDL without, apparently, having any theoretical predilection about where the phenomenon came from. Among these studies, Shmavonian obtained the first results actually indicating the occurrence of stimulus generalization decrements.

SOURCES OF REBIRTH OF INTEREST IN SDL

It appears that investigators in the 1950s and 1960s had no direct awareness of the 19th-century literature on SDL and that their studies derived from other sources. We should note that Guthrie’s stimulus elements theory was well known in 1950 (Guthrie [1935] 1960). It was significantly similar to the theories previously developed by Ribot and Semon, which had been devised in the first place to explain SDL, among other phenomena. Hence, the possibility of SDL was certainly not contrary to theories of learning extant in 1950, and it is hardly surprising that investigators, even if they did not accept Guthrie’s model, at least wanted to test whether their results might reflect its operation.

To clarify this issue, this writer recently interviewed (by telephone) several investigators of that era. Girden reports that he had no knowledge of the earlier published reports of SDL. Auld reports that he used the 2x2 design because Miller encouraged him to do so, but without any particular expectation on his part of finding drug stimulus effects. Conger attributes the stimulus change explanation for his results as arising from the interest in drive stimuli that was extant at the time and that made it appear reasonable to him that drugs also could produce analogous stimulus effects. Barry’s thesis research was on the effects of changes in the level of hunger on performance in a straight alley, and he subsequently used a similar design when testing the effects of drugs on

performance in the same task. Auld, Conger, and Barry were students of Neal Miller, and their publications report their research with him. Miller, then, was centrally important in causing the experiments that rediscovered DDs and SDL and he reports that he had no knowledge of the 19th-century literature on drug-induced SDL. Barry reports that Miller encouraged him to use a 2x2 design to test for state change effects but discouraged him from performing DD studies. Heistad reports that he was substantially influenced by Guthrie and entertained the idea that a substantial portion of the effects of drugs on behavior might result from stimulus generalization decrements. Shmavonian reports that he used the 2x2 design, not as a tool to see stimulus effects, but because it would allow detection of carryover effects. Otis had conducted an interesting Ph.D. thesis on the possibility that drive stimuli might act as conditioned stimuli and might, if paired with punishment during infancy, elicit anxiety as a conditioned response later in life (Otis 1956). He later viewed drugs as a convenient method to induce comparable changes in internal state and for this reason used them in his 1964 study.

All the preceding reports are based on personal communications in 1990 and, obviously, do not reflect the entire range of recollections by which these investigators were influenced at the time they did their work. Nonetheless, the investigators I contacted were unanimous in denying any direct knowledge of the 19th-century work, and it seems reasonable to conclude that the interest in SDL that reappeared in the 1950s and 1960s did not result from direct knowledge of the 19th-century literature. Instead, the concept was apparently reinvented on the basis of ideas prevalent at the time. Some of these ideas, in turn, can be traced back to the 19th century, when they were first invented to explain SDL and other dissociative phenomena.

The difficulty in evaluating the influence of prior ideas and findings is well illustrated by the experience of this writer. My own “rediscovery” of SDL occurred because of a neurological model (Overton 1964) that occurred to me during a lecture while I was a graduate student. It seemed to me a novel prediction of SDL based on ideas derived from the behavior of electronic feedback systems, which I had recently studied as a student of engineering. The idea led me to perform experiments that demonstrated SDL produced by pentobarbital (Overton 1964). However, those experiments occurred about 12 months after I had read about (and forgotten) Girden’s work. I also probably had read and forgotten Hebb’s 1949 (p. 201) paragraph on state-dependent cell assemblies. So was my theory really novel, or was it a reevocation, in altered form, of ideas that I had previously read? Subjectively, I was surprised when I

later learned of the work of Girden, Hebb, Conger, and Auld. Nonetheless, many precedents for my ideas existed in the literature, and I had read some of them.

PROLIFERATION OF THEORIES FOR SDL

Combe's initial description of SDL did not propose a mechanism by which SDL occurred; it was simply a statement of the fact that SDL did occur. As far as we know, Ribot provided the first mechanistic explanation for SDL by postulating that the physiological state of the body was reflected in "organic" sensations and that recurrence of these sensations was a prerequisite for memory retrieval. Girden proposed a different model based on the assumption that drugs could functionally decorticate the animal (Girden and Culler 1937). Conger, Auld, Miller, Otis, and others accepted a stimulus generalization model for SDL (similar to Ribot's model). Hebb proposed that cell assemblies should be state specific; this was not a sensory model. Indeed, once the scientific world was convinced again that SDL really existed, a plethora of mechanisms for it were proposed; toward the end of this period I published a review paper that summarized 22 different models that had been suggested as responsible for SDL (Overton 1978).

These various competing theories have for the most part never been explicitly tested. However, by the process of voting, the scientific community has come to support a stimulus theory predominantly. Probably this support has come about largely because the stimulus theory has proved so flexible in accommodating to the very diverse and complicated phenomenology that DD experiments have increasingly elucidated.

REDISCOVERY OF HISTORY

It appears that not one of the investigators who contributed to the experimental development of the SDL and DD paradigms between 1937 and 1980 knew explicitly of the 19th-century interest in SDL. Several writers related DDs and SDL to multiple personality and other forms of dissociation, but not one single citation of the early work was made. Finally, this amnesia about our history was lifted by Siegel (1982) after someone attending one of his lectures pointed out that the plot of *The* Moonstone implied knowledge of (1) contextual control of retrieval, (2) SDL, and (3) one-trial tolerance. In *The Moonstone,* Collins quoted the Irish porter case verbatim and mentioned Combe's name; this allowed

Siegel to find part of the 19th-century literature on SDL and publish a description of it (Siegel 1982,1983).

SYMMETRICAL TASKS

The first improvement in DD methodology after Conger's study was that of using a symmetrical task (Overton 1961). In such a task, stimulus effects of drugs are reflected by response selection instead of by response failure, and hence the rate-depressing effects of drugs are not confounded with stimulus effects to anywhere near the same degree as in a single-response go/no-go task (in which the rate of occurrence of a response is used as an index of retention). The two-response T-maze task was selected to accomplish this purpose after pilot experiments in a straight alley go/no-go maze yielded results that were difficult to interpret because drug effects on rate and SDL effects were confounded in that task.

RELATIONSHIP BETWEEN SDL AND DDS

Were the stimulus effects that produced discriminative control in Conger's experiment actually the same drug effects that produced SDL decrements in 2x2 experiments? The most convincing evidence for an affirmative answer to this question comes from my own experiments. In a shock escape T-maze task, I was able to demonstrate both SDL amnesias caused by changes in state and D versus N DDs established by repeated training trials. With very high doses of certain drugs, only two sessions were required to learn one response in the D state and an opposite response in the N state, thus demonstrating SDL. With tower doses of the same drugs, 30 or 40 training sessions were required to establish discriminative control. When intermediate doses were tested, the amount of training required to establish D versus N DDs turned out to be inversely proportional to dosage. Hence, whatever action of the drugs was producing SDL and DDs, it was apparently the same action (Overton 1974).

FURTHER IMPROVEMENTS IN THE DD PARADIGM

By 1970, it was possible to list about 20 different behavioral paradigms that had been employed in DD studies by one investigator or another (Overton 1971) and a few of these are identifiable as milestones in the development of the paradigm that is most commonly employed at present. We mentioned previously that introduction of a two-choice task was a major improvement over tasks in which only a single response was measured. The next major step was

the use of operant tasks (Harris and Balster 1966). Even the earliest results obtained in operant tasks indicated that they were sensitive to doses considerably lower than could be detected in the T-maze task, and when the symmetrical two-lever operant DD task was used, the operant paradigms provided both high sensitivity and a relatively uncontaminated indication of discriminable drug effects (Kubena and Barry 1969; Morrison and Stephenson 1969). One additional important development did not occur until 1975, when Colpaert et al. (1975, 1976) introduced the use of a fixed ratio (FR-10)-versus-extinction schedule of reinforcement. This schedule produced much higher accuracy of lever selection than previously had been observed in operant tasks, and it was soon adopted by the majority of DD investigators.

MULTIPLICITY OF DRUG CUES

In 1960 it had never been demonstrated whether more than a single drug cue existed, and investigators entertained the possibility that rats might be discriminating “normal” versus “abnormal” irrespective of what drug was used as the training compound. Hence, Overton (1966) felt obliged to conduct a series of studies designed to demonstrate that atropine and pentobarbital produced two qualitatively different states (or stimulus effects). Stewart (1962) also produced data indicating that at least two different drug states existed.

By the end of the 1960s however, a remarkable development had occurred and Overton (1971) was able to report 10 different types of drugs that were discriminable from no drug and from one another. This report led to the generalization that each different type (or class) of drugs would produce a different discriminable effect (the “one cue per pharmacological class” idea) and to the expectation that this pattern might continue to be found as additional types of drugs were tested in the DD paradigm.

Analogous studies conducted in operant DD tasks yielded an additional important result. In those tasks, after D-versus-N training with a particular drug, animals would select the no-drug lever when tested with any novel drug. Hence the no-drug lever was the default response selected by trained animals under all drug conditions except the drug condition used for training (and drug conditions closely related to the training condition). It should be noted that this pattern of results differed from the one obtained 10 years earlier by Overton. In his T-maze task, rats made 30-70 percent D choices under most novel drugs and only a few test conditions led to consistent selection of the no-drug arm of

the maze. The operant task produced a different pattern of results, however, for reasons that have never been adequately explained.

ONE MORE THEORY

In 1975, Colpaert et al. introduced an apparently minor restatement of the stimulus theory, which in fact caused a fundamental revision in the predictions of that theory. Colpaert argued that rats discriminated presence versus absence of the training drug's cues during D-versus-N DD training. Around the same time, Frey and Winter (1977) made the same proposal even more pointedly, referring to it as a "third cue" model. It followed as a prediction that the animals would consistently select the no-drug lever during tests with a novel training drug that produced cues different from the particular cues they had been trained to detect.

This model differed radically from the one that had been accepted by most SDL investigators who expected responding to be equally contingent on D cues and N cues. It was not congruent with some data obtained by this writer during tests for substitution in the T-maze DD task. However, most investigators were now using the operant task, and Colpaert's model did match the pattern of results obtained in that task; probably for that reason, the "presence-versus-absence" model has achieved wide acceptance. A variant of this model is also compatible with the often observed "asymmetrical SDL" result in which loss of the response occurs after D→|N but not after N→|D state changes (Overton 1968,1988).

SUMMARY OF PROGRESS SINCE 1975

The DD method most frequently employed after 1975 used a composite paradigm incrementally constructed during the preceding 25 years by means of the process that I have just described. By 1975, all the component pieces were in place to allow the subsequent widespread use of DDs for investigation of drug effects, causes of drug abuse, and other issues. The major components included (1) simple two-response tasks in which lever selection was primarily determined by stimulus effects—i.e., tasks with high specificity in that discriminable effects could be distinguished moderately well from depressant or other drug actions; (2) a paradigm that could be used with almost any type of centrally acting drug; (3) specificity of recognition of different stimulus effects, so that the stimulus effects of one drug could be distinguished from those of most others; (4) a rational principle for predicting what stimulus effects might be expected from a previously untested drug (the "one cue per class" model); and,

perhaps most important, (5) a simple and easy-to-understand theory (presence versus absence), which made the results of the paradigm appear simple, plausible, and interpretable. All that was now required was time for the news to get around and for members of the pharmacological community to gradually acquire confidence in the method. Stolerman has prepared a comprehensive bibliography listing more than 1,000 DD studies and showing that the number of DD publications per year has increased steadily since 1970 (slightly before the method reached its current stage of development). The majority of DD studies have been published since 1980 and have used the currently popular two-lever FR-10-versus-extinction DD training paradigm.

THE FUTURE OF DRUG-INDUCED DISSOCIATION AND DISCRIMINATIVE CONTROL

The study of dissociation, SDL, and DDs is now at an exciting juncture, having split into several distinct subfields. DDs are used to investigate the neurochemical effects of drugs, as illustrated by several other papers in this volume, and to investigate the stimulus effects of drugs that are presumed to underlie drug abuse (Overton 1987). Although DDs are believed to occur because of the "stimulus effects of drugs," very few studies intended to identify these stimuli or to elucidate the properties of stimulus control by drug stimuli are being reported (Overton 1988). Drug-induced SDL has not been extensively investigated since it was concluded in about 1980 that the doses of psychoactive drugs normally used for outpatient treatment do not produce the phenomenon to an impressive degree (Eich 1980; but see Lowe 1982). However, SDL produced by emotional states is under current investigation as a possible etiological factor in depression and other mental illnesses (Blaney 1986). Recent years have seen a dramatic resurgence of interest in the multiple personality syndrome, and the clinical syndrome has been redefined to include "super multiples" reported to have as many as 50 distinct personalities, each partially dissociated from the others. At the same time, something approaching 40 distinctively different drug stimuli have now been identified in the DD literature. These various studies are being carried out by investigators in several different fields, and a continuing challenge exists to identify findings in one field that may have ramifications in another. We can optimistically hope (with Schacter 1982, p. 263) that a "social amnesia" such as the one that prevented our 19th-century predecessors from more directly influencing work conducted between 1937 and 1980 will not dissociate too completely the various fields in which work is currently being conducted on the stimulus properties of drugs and on other forms of dissociation.

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Discriminative Stimulus Properties of Hallucinogens and Related Designer Drugs

Richard A. Glennon

CLASSICAL HALLUCINOGENS

Hallucinogenic agents are of several categories (see table 1). The present review considers only those agents referred to as classical hallucinogens and certain structurally related designer drugs. Most of the nonclassical hallucinogens are treated separately elsewhere. The stimulus properties of the classical hallucinogens have been previously reviewed (Glennon et al. 1982, 1983*a).*

Nature of the Stimulus

Several examples of hallucinogens have been widely used as training drugs in drug discrimination (DD) studies. These include the simple tryptamine 5-methoxy-N, N-dimethyltryptamine (5-OMe DMT), the ergoline (+)lysergic acid diethylamide (LSD), the phenethylamine mescaline, and the phenylisopropylamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Glennon et al. 1983*a*). Stimulus generalization occurs between these four agents regardless of which is used as the training drug. This is part of the justification for treating the classical hallucinogens as a group. On the other hand, stimulus generalization does not typically occur between the classical hallucinogens and the nonclassical hallucinogens, regardless of which is used as the training drug. Indeed, the classical hallucinogens appear to share a common mechanism of action, whereas (1) their mechanism of action seems to be different from that of the nonclassical hallucinogens, and (2) each type of nonclassical hallucinogen probably possesses its own distinct mechanism of action. Also the DD procedure of using animals trained to discriminate a classical hallucinogen from saline does not represent an animal model of hallucinogenic activity (Glennon 1991). Stimulus effects of the classical hallucinogens involve, at least in part, a mechanism of serotonin (5-HT); thus,

TABLE 1. *Classification of hallucinogenic agents*

Classical Hallucinogens
Indolealkylamine hallucinogens
Simple tryptamine (e.g., DMT, 5-OMe DMT, psilocin)
a-Methyltryptamines (e.g., a-MeT, 5-OMe a-MeT)
β- -Carbolines (e.g., harmine, harmaline, 6-OMe harmalan)
Ergolines (e.g., (+)LSD, other lysergic acid analogs)
Phenalkylamines
Phenethylamines (e.g., mescaline)
Phenylisopropylamines (e.g., DOM, DOB, certain DMAs and TMAs)
Other Hallucinogens/Psychotomimetics
Cannabinoids
Phencyclidine (PCP)-related agents
Certain opiates
Certain cholinergic agents
Miscellaneous

stimulus generalization may occur between a classical hallucinogen stimulus and stimuli produced by, for example, nonhallucinogenic agents capable of acting as direct or indirect 5-HT agonists. For example, an LSD stimulus and/or DOM stimulus generalizes with the 5-HT-releasing agent fenfluramine and the nonselective 5-HT agonist quipazine. Both the LSD stimulus and the DOM stimulus generalize to the nonselective 5-HT/dopamine (DA) agonist lisuride. And yet, animals can be trained to discriminate between LSD and lisuride in a three-lever paradigm (Callahan and Appel 1990). Although the DOM stimulus (1 mg/kg) generalizes to 0.6 mg/kg of lisuride, administration of 0.01 mg/kg of lisuride in combination with the training dose of DOM results in attenuation by 50 percent of the DOM stimulus (see figure 1; higher doses of lisuride in combination with DOM result in disruption of behavior). It appears, then, that lisuride may be a partial agonist, and Colpaert et al. (1982) have indeed shown that at sufficiently high doses a variety of 5-HT “antagonists” can in fact mimic the LSD stimulus, suggesting that they too may be acting as partial agonists.

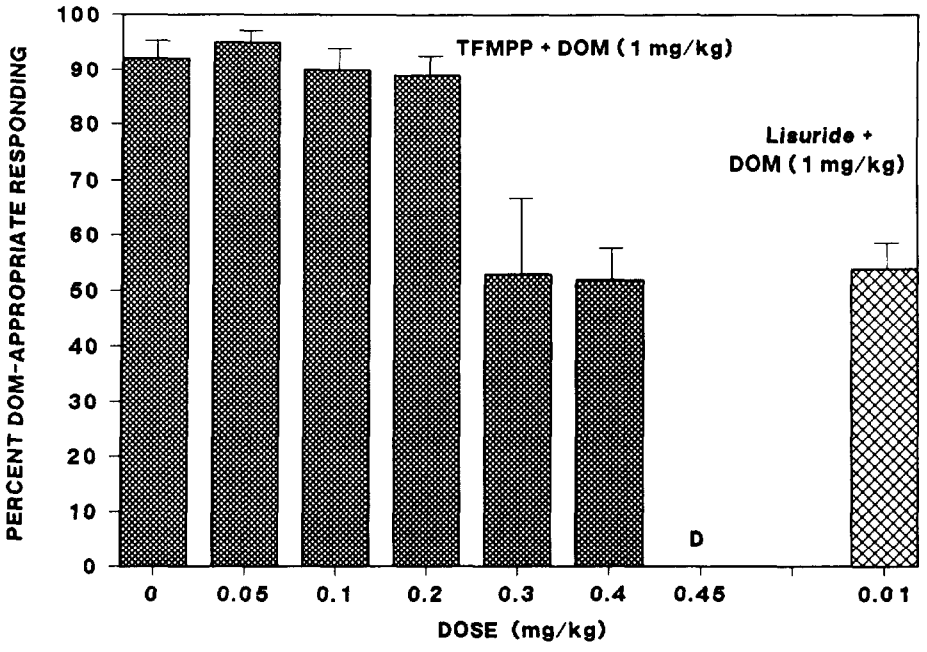


FIGURE 1. *Effect of TFMPP and lisuride administered in combination with DOM in rats trained to discriminate 1 mg/kg of DOM from saline. Doses of TFMPP and lisuride greater than 0.4 and 0.01 mg/kg, respectively, in combination with DOM result in disruption of behavior.*

5-OMe DMT and LSD may produce their stimulus effects via a multiple (and perhaps dose-dependent) serotonergic mechanism; DOM seems to produce a more selective stimulus, and R(-)DOB may be even more selective than DOM (Glennon 1988). In addition to any serotonergic involvement, recent work by Meert et al. (1990) suggests that the stimulus effects of LSD involve a catecholaminergic mechanism.

Mechanism of Action

Early studies with LSD, 5-OMe DMT, mescaline, and DOM concluded that these agents act via a 5-HT agonist mechanism. For example, certain 5-HT agonists mimicked the stimulus effects of hallucinogens, whereas known 5-HT antagonists were capable of antagonizing these effects. Curiously, however, the 5-HT antagonist cinanserin was a relatively weak LSD antagonist and was

much more effective in attenuating the stimulus effects of mescaline (Glennon et al. 1983*a*). initial arguments related these differences to the effectiveness of the hallucinogens as discriminative stimuli. Furthermore, 5-OMe DMT and LSD seemed to produce somewhat different stimulus properties depending on their training dose (Glennon 1988). In addition, although hallucinogen stimuli generalized with some 5-HT agonists, generalization did not occur with certain other 5-HT agonists. These and other studies raised the possibility of some subtle differences between the stimulus effects produced by indolealkylamine hallucinogens and phenalkylamine hallucinogens.

Puzzling as it was at the time, we now know that there are multiple populations of central 5-HT receptors (e.g., 5-HT_1, 5-HT_2,5-HT_3). In 1983, Glennon et al. (1983*b*) demonstrated that the stimulus effects of DOM, and DOM-stimulus generalization to examples of other categories of classical hallucinogens such as LSD, 5-OMe DMT, and mescaline, were potently antagonized by the 5-HT_2 antagonist ketanserin. Colpaert et al. (1982) had reported 1 year earlier that pirenperone was a specific LSD antagonist; pirenperone is now recognized as a 5-HT_2 antagonist. Other 5-HT_2 antagonists also potently inhibit the DOM stimulus. These findings led to the hypothesis that hallucinogens produce their stimulus effects via a 5-HT_2 agonist mechanism (Glennon et al. 19836). Later, with the use of LSD-trained rats, the LSD stimulus was also potently antagonized by various 5-HT_2 antagonists (for a review see Cunningham and Appel 1988).

On the basis that the DOM stimulus was a “cleaner” cue than that produced by indolealkylamine hallucinogens, detailed mechanistic and structure-activity relationship (SAR) studies were performed using DOM as the training drug. Subsequent studies revealed the following:

- DOM and DOM-related agents such as DOB and DOI are 5-HT_2 agonists (or at least partial agonists).
- Indolealkylamine hallucinogens are nonselective 5-HT_2 agonists (i.e., although they can act as 5-HT_2 agonists, they bind at various subpopulations of 5-HT_1 sites with high affinity and also act as 5-HT_1 agonists).
- Hallucinogen-induced stimuli can be attenuated by a wide variety of 5-HT_2 antagonists.
- DOM-stimulus generalization does not occur with serotonergic agents that are selective for other populations of 5-HT receptors (e.g., the 5-HT_{1A} agonist 8-OH DPAT).

- For a wide variety of hallucinogens, DOM-stimulus generalization potency is significantly correlated with their affinity for central 5-HT_2 receptors (for a review see Glennon et al. 1984).

Stimulus effects produced by DOM are distinct from those produced by the 5-HT_{1A} agonist 8-OH DPAT (Glennon 1988); in fact, in DOM-trained rats, low doses of 8-OH DPAT (e.g., ≤0.1 mg/kg) produce less than 10 percent DOM-appropriate responding. However, because we have recently demonstrated that 5-HT_1, agonists can modulate certain behavioral effects of 5-HT_2 agonists (Glennon et al. 1990), we were interested in determining the effect of 8-OH DPAT on the DOM stimulus. As shown in figure 2, 50 µg/kg produces a shift to the left of the dose-response curve for DOM in DOM-trained animals (ED_{50} = 0.45 vs. 0.19 mg/kg). Apparently 8-OH DPAT can augment the stimulus effects of DOM. This is currently under further investigation.

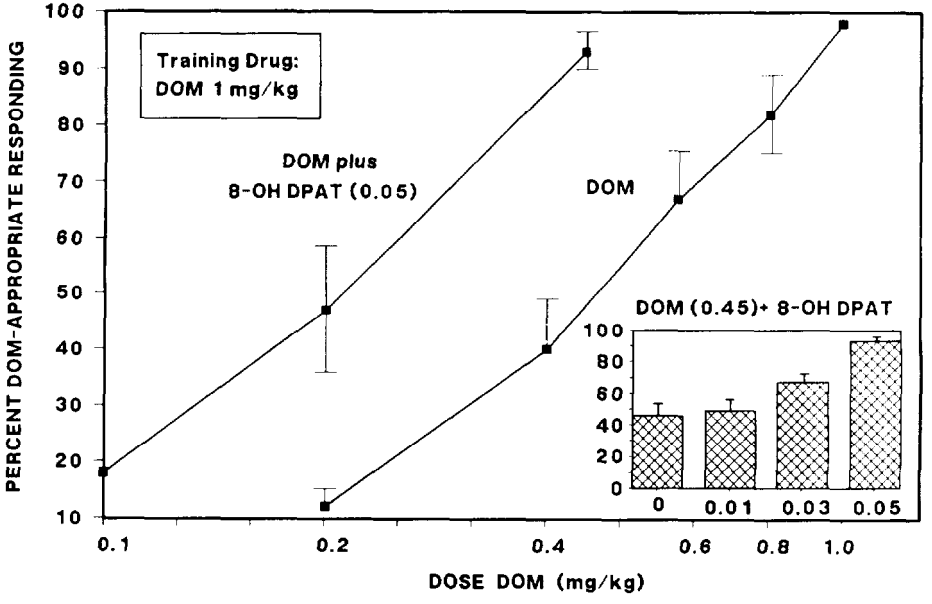


FIGURE 2. Effect of 50 µg/kg *of 8-OH DPAT on the dose-response curve of DOM in DOM-trained rats. 8-OH DPAT was administered 5 min prior to DOM and DOM was adminisfered 15 min prior to testing. Inset shows the effect of different doses of 8-OH DPAT in combination with the ED_{50} dose of DOM.*

Hartig (1989) has demonstrated a significant homology between 5-HT_{1C} and 5-HT_2 receptors. Indeed, although DOM-like agents are quite selective for 5-HT_2 receptors, they also bind (with a tenfold to fiftyfold lower affinity) at 5-HT_{1C} receptors (Titeler et al. 1988). This raises the possibility that the stimulus effects of certain classical hallucinogens may involve a 5-HT_{1C} component. To date, 5-HT_{1C}-selective agents are unavailable to test this hypothesis. 1-(3-trifluoromethylphenyl)piperazine (TFMPP) is a 5-HT_{1B}/5-HT_{1C} agonist; stimulus generalization does not occur between DOM and TFMPP regardless of which is used as the training drug. TFMPP also binds at 5-HT_2 sites, and there is evidence that it may be a 5-HT_2 antagonist. In stimulus antagonism studies using rats trained to discriminate 1 mg/kg of DOM from saline, 0.3 and 0.4 mg/kg of TFMPP attenuate the DOM stimulus by about 50 percent; higher doses (0.45 to 0.7 mg/kg) of TFMPP administered in combination with DOM produce disruption of behavior. Most 5-HT_2 antagonists such as ketanserin are also 5-HT_{1C}antagonists, and their affinities for 5-HT_{1C} receptors and 5-HT_2 receptors are rather similar. The one exception is the DA antagonist spiperone; this agent is a more effective 5-HT_2 antagonist than a 5-HT_{1C} antagonist. Tests of stimulus antagonism were conducted with spiperone using DOM-trained rats (figure 3); doses of up to 0.1 mg/kg were without effect and higher doses produced disruption of behavior. Thus, at this time it is not possible to rule out 5-HT_{1C} involvement in the stimulus effects of DOM. It might be noted that because of its DA antagonist actions, spiperone was able to attenuate the stimulus effects of S(+)amphetamine in amphetamine (AMPH)-trained animals.

Because DOM is a phenylisopropylamine, it has been speculated for years that DOM might produce stimulus effects similar to those of the structurally related phenylisopropylamine stimulant AMPH. In fact, it was once thought that DOM, like AMPH, might be acting via a dopaminergic mechanism. However, it has been demonstrated that DOM and AMPH produce distinct stimulus effects regardless of which is used as the training drug. Nevertheless, certain other DOM analogs to which the DOM-stimulus generalizes (i.e., 2,4,5-trimethoxy analog [TMA], 3,4,5-TMA, 2,5-dimethoxy analog [DMA]) produce as much as 46 percent AMPH-appropriate responding in S(+)AMPH-trained animals (Glennon et al. 1985). In fact, these agents may produce some central stimulant effects in humans (Shulgin 1978). As a consequence, we sought to determine if these agents would produce AMPH-like effects in AMPH-trained rats when the 5-HT_2 response was blocked. Tests of stimulus generalization were conducted in S(+)AMPH-trained rats that had been pretreated 45 min earlier with a dose of ketanserin (0.5 mg/kg), which completely attenuates the effect of DOM in

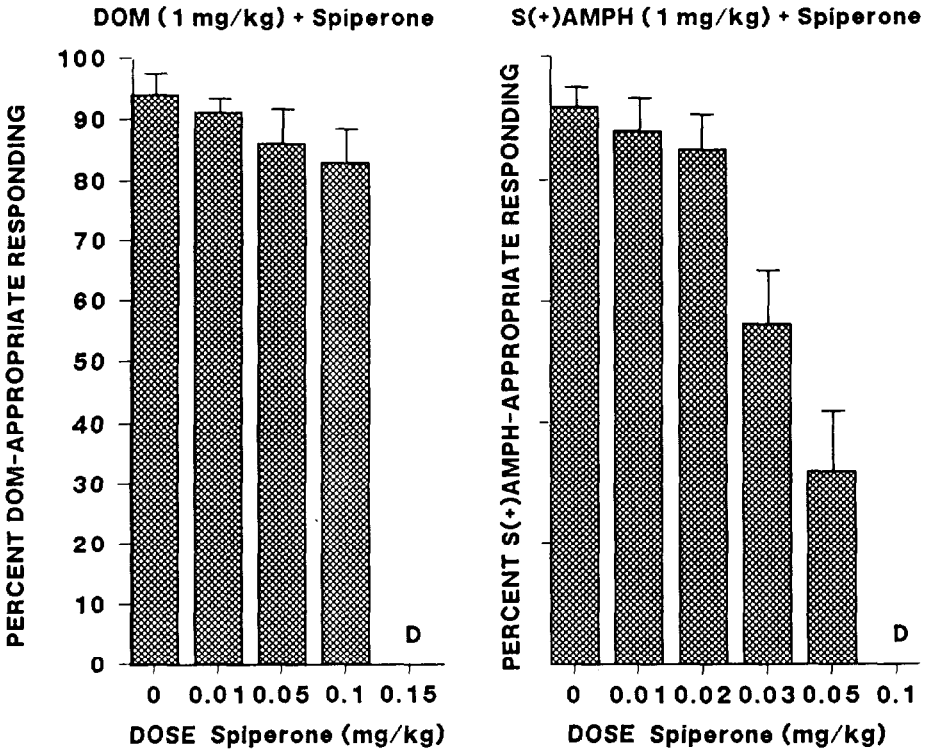


FIGURE 3. *Tests of stimulus antagonism with spiperone in rats trained to discriminate either 1 mg/kg of DOM or S(+)AMPH from saline.*

DOM-trained rats. Under these conditions, DOM failed to elicit more than 33 percent AMPH-like responding at doses of up to 2.7 mg/kg (figure 4); higher doses resulted in disruption of behavior. Likewise, 2,4,5-TMA and 3,4,5-TMA failed to produce more than 25 percent AMPH-appropriate responding (figure 5). Doses of 2,4,5-TMA greater than 15 mg/kg produced disruption of behavior. 3,4,5-TMA was evaluated at doses of up to 40 mg/kg; although at the higher doses the animals' response rates were reduced by about 60 percent, responding was essentially saline-like.

Similar results were obtained with 2,5-DMA (figure 4); doses of up to 40 mg/kg failed to engender more than 45 percent AMPH-appropriate responding. N-Monomethylation of AMPH-like agents usually enhances their amphetamine-like properties; consequently, the N-monomethyl analog of 3,4,5-TMA was evaluated (figure 5); here too, doses of up to 40 mg/kg resulted

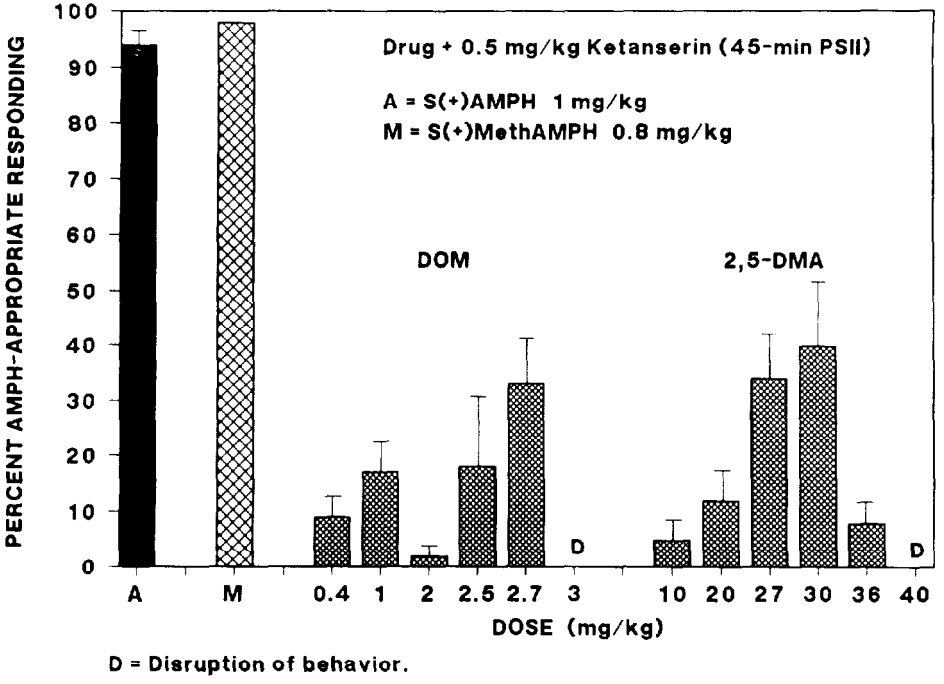


FIGURE 4. *Tests of (+)amphetamine generalization to (+)amphetamine (A), (+)methamphetamine (M), DOM, and 2,5-DMA in rats pretreated with 0.5 mg/kg of the 5-HT$_2$ antagonist ketanserin.*

in AMPH-appropriate responding that did not exceed 11 percent. Although ketanserin binds at D2 DA receptors and may behave as a DA antagonist at high doses, the dose of ketanserin used in the present study had no effect on the AMPH stimulus; that is, 0.5 mg/kg of ketanserin did not attenuate the effect of S(+)AMPH in AMPH-trained animals, nor did this dose of ketanserin interfere with AMPH-stimulus generalization to 0.8 mg/kg of S(+)methAMPH (figure 4). The results suggest that these agents do not produce AMPH-like stimulus effects at the doses evaluated and further support lack of significant dopaminergic involvement in their mechanism of action.

Pharmacokinetic Investigations

The DD paradigm can be used to investigate the pharmacokinetic and biodispositional properties of hallucinogenic agents. Unfortunately, relatively little has been reported in this regard.

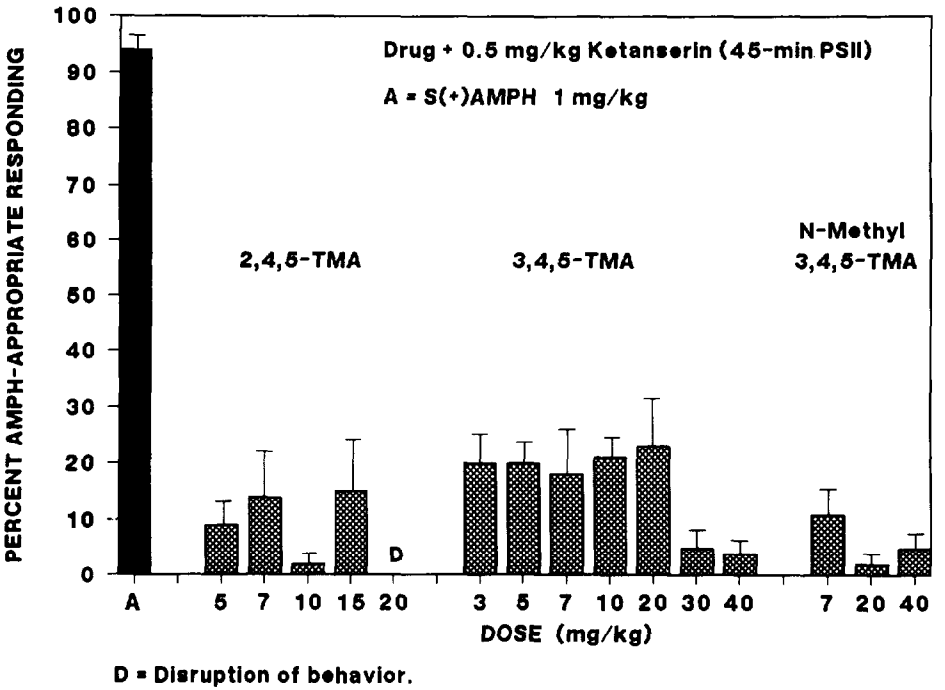


FIGURE 5. *Tests of (+)awhetamine stimulus generalization to 2,4,5-TMA, 3,4,5-TMA, and N-methyl 3,4.5-TMA in rats pretreated with 0.5 mg/kg of ketanserin. Rats were trained to discriminate 1 mg/kg of (+)amphetamine from saline.*

Locus of Action. The specific locus of action mediating the stimulus effects of hallucinogenic agents is unknown. However, it seems likely that hallucinogens produce their stimulus effects via a central mechanism. Several lines of reasoning support this notion, For example, xylamidine (a 5-HT_2 antagonist that does not readily penetrate the blood-brain barrier) is ineffective in attenuating the stimulus effects of the classical hallucinogens. Also, although 5-OH DMT and 5-OMe DMT share similar 5-HT_1/5-HT_2 binding profiles, the 5-OMe DMT stimulus only partially generalizes to 5-OH DMT; the latter agent is of low lipophilicii and is known to poorly penetrate the blood-brain barrier. Another agent that should not penetrate the blood-brain barrier is the quaternary analog of DOB (i.e., QDOB). In animals trained to discriminate R(-)DOB from saline, stimulus generalization does not occur with QDOB. However, it has been demonstrated that QDOB does not bind at 5-HT_2 sites. Minnema et al. (1980) conducted DD studies in which animals were implanted

with indwelling cannula; in this manner, hallucinogens could be administered directly into the brain. Although this technique has not been widely used to investigate such agents, it could prove valuable for investigating (1) agents that do not readily penetrate the blood-brain barrier and (2) specific locations in the brain that might be responsible for mediating the stimulus effects of hallucinogens.

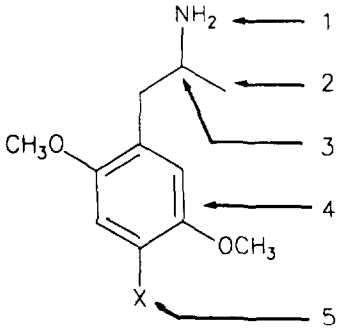
Temporal Properties. The time of onset and the duration of action of the stimulus effects of various hallucinogenic agents have been examined. Of course, these vary from agent to agent; the interested reader is referred to the primary literature for details of such investigations.

Action of Metabolites. Although relatively little work has been done, there is no evidence that the stimulus effects of classical hallucinogens are due primarily to their metabolites. Phenylisopropylamines undergo parahydroxylation in vivo, and it has been speculated that 4-OH 2,5-DMA might be a metabolite of 2,5-DMA. The 4-COOH derivative of 2,5-DMA is a metabolite of DOM. Neither of these agents produces DOM-like stimulus effects in rats, nor do they bind at 5-HT_2 receptors. Iodinated compounds can undergo a rapid deiodination in vivo; thus, the possibility exists that DOI, the iodo counterpart of DOM, may be metabolized to 2,5-DMA. In DD studies, DOI is significantly more potent than 2,5-DMA and the potencies of both compounds are consistent with their affinities for 5-HT_2 receptors. It appears unlikely that the stimulus effects of DOI are due to its deiodinated derivative 2,5-DMA. DD may be a useful procedure for examining the activity of metaboliies or potential metabolites, particularly if this approach is coupled with cannulation studies (as mentioned above) in order to avoid potential problems associated with penetration of the blood-brain barrier.

Structure-Activity Relationship (SAR)

That the SAR of LSD has been neglected is probably a direct consequence of the unavailability of LSD analogs. On the other hand, extensive SAR studies have been conducted with 5-OMe DMT and DOM as training drugs; indeed, the SAR of no other training drug has been as widely investigated as that of DOM. The 2,5-dimethoxy substitution pattern of DOM is important. In general, little can be done to increase the potency of DOM-like agents; the only structural modifications that result in more potent DOM-like agents are replacement of the 4-methyl group of DOM with an ethyl (DOET) or n-propyl (DOPR) group, or with certain halogens such as bromo (DOB) and iodo (DOI). The R(-)isomers of

DOM-like agents are several times more potent than their S(+)enantiomers, whereas the reverse is true for derivatives of a-methyltryptamine. In neither series does stereochemistry play a major role. The SARs of these agents have been reviewed in detail (Glennon 1989*a*) and are summarized in figure 6. Readdressing the structural similarity between the DOM-like agents and the phenylisopropylamine stimulant AMPH, a separate and distinct SAR has been formulated for AMPH-like stimulus effects; these are summarized in figure 7.



STRUCTURE - ACTVITY RELATIONSHIPS (SAR)

1. Terminal amine:
- **Primary amine optimal**
- **Alkyl substituents decrease potency**

2. α-Methyl group:
- **Removal decreases potency by 10-fold**
- **Homologation reduces potency**

3. Chiral center:
- **R-isomers 5 (3-10) times more potent than S**

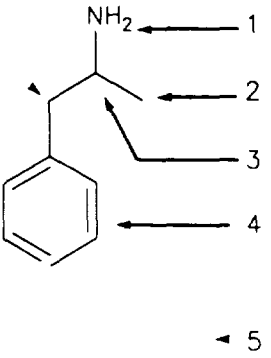
4. Aromatic substituents:
- **Mono-methoxy derivatives are inactive**
- **Of the six possible dimethoxy analogs, only the 2,4- and 2,5-DMAs are active**
- **All of the six possible tri-methoxy analogs (TMAs) are active**

5. Para Substituents:
- **Methyl significantly more potent than H (e.g. DOM > > 2,5-DMA)**
- **Other alkyl analogs (Et, nPr) more potent (e.g. DOET, DOPR > DOM)**
- **Certain halogen analogs (Br, I) most potent (e.g. DOB, DOI > DOM)**
- **Polar substituents (e.g. OH, COOH) inactive**

FIGURE 6. *Summary of selected structure-activity relationships important for DOM-like stimulus effects.*

Relationship With Human Hallucinogenic Activity

When DOM-trained animals are used, there is a significant correlation between the stimulus generalization potencies of variius classical hallucinogens and their human hallucinogenic potencies. In fact, this was the first example of a correlation between discrimination-derived data and any human measure of activity. Because of the relationship between generalization potency and 5-HT_2



STRUCTURE - ACTIVITY RELATIONSHIPS FOR AMPHETAMINE - LIKE ACTIVITY

1. Terminal amine:
- **Secondary amine (N-Me) optimal (e.g. methamphetamine)**
- **Substituents larger than Me less potent (Me > H > Et)**

2. α-Methyl group:
- **Seems optimal**
- **Removal reduces potency**

3. Chiral center:
- **S-isomers more potent than R isomers**

4. Aromatic substituents:
- **Decrease potency**
- **Mono-methoxy analogs weakly active (MMA > OMA > PMA > AMPH)**
- **Di-methoxy analogs (DMAs) inactive**
- **Tri-methoxy analogs (TMAs) inactive**

5. Benzylic substituents:
- **Hydroxy groups reduce potency**
- **Carbonyl group has no effect (e.g. cathinone)**

FIGURE 7. *Summary of selected structure-activity relationships important for AMPH-like stimulus effects.*

receptor affinity, there should also be a correlation between receptor affinity and human hallucinogenic potency; such a correlation has been reported (Glennon et al. 1984). Initially, the correlation was demonstrated using rat cortex as the source of 5-HT_2 receptors; this study has now been replicated using human cortex as the source of tissue for the binding studies (Sadzot et al. 1989). Thus, DD studies were directly responsible for aiding our understanding of the mechanism of action of human hallucinogenic activity of the classical hallucinogens.

Role in Drug Development

At first, one might think that DD studies using animals trained to discriminate a classical hallucinogen from saline serve only the investigation of other hallucinogenic agents. This is not the case; hallucinogen-trained animals can be employed in several different applications.

Investigation of Basic Neurochemical Mechanisms. DOM-related agents are fairly selective 5-HT_2 agonists. indeed, it was speculated that these agents were the first examples of 5-HT_2-selective agonists on the basis of DD studies. SAR for DOM stimulus generalization was later found to parallel SARs for the binding of these agents at 5-HT_2 sites. DOB and DOI are commonly used now as 5-HT_2 agonists, and [^{3}H]DOB and [^{125}I]DOI are commercially available for radioligand binding and autoradiographic studies. DD studies using animals trained to discriminate DOM, DOB, or DOI from saline may serve, then, as a functional behavioral model of central 5-HT_2 receptor activation.

Indicator of Abuse Potential. Hallucinogen-trained animals can be used by the pharmaceutical industry to evaluate the abuse potential of new therapeutic entities. Stimulus generalization to a new agent suggests that the new agent be further evaluated in other tests to determine whether it has any abuse liability. To date, there are no examples of classical hallucinogens that are not recognized by animals trained to discriminate DOM from saline. As mentioned above, however, DD cannot be considered a model of human hallucinogenic activity.

New Drug Development. There is evidence that 5-HT_2 antagonists possess neuroleptic, antianxiety, and antidepressant properties. Thus, animals trained to discriminate a 5-HT_2 agonist could be useful tools for identifying novel 5-HT_2 antagonists. Indeed, examples of this approach have already appeared in the literature (e.g., Meert 1989; Meert and Awouters 1990).

DESIGNER DRUGS

Hallucinogen-related designer drugs were a topic of several recent symposia, and the discriminative stimulus properties of these agents have been reviewed (Glennon 1989*a*, 1989*b*, 1990; Nichols and Oberlender 1989). Separate and distinct SARs have been formulated for the stimulus properties of phenalkylamine hallucinogens and phenalkylamine stimulants (figures 6 and 7). While in the process of formulating the SAR of phenylisopropylamines, we became interested in 3,4-methylenedioxyamphetamine (3,4-MDA, MDA, “Love Drug”). Because the methylenedioxy group was not in our SAR data base, and because MDA had been popular during the 1960s as a mild hallucinogenic agent with central stimulant properties, we investigated this agent and its isomers in DOM- and S(+)AMPH-trained animals. Consistent with its street reputation, MDA was recognized by both groups of animals. Its R(-)-isomer is primarily responsible for DOM-like effects, whereas its S(+)-isomer is primarily

AMPH-like. Likewise, Nichols et al. (1989) have reported that racemic and R(-)MDA, but not S(+)MDA, result in stimulus generalization in LSD-trained rats. To date, MDA is the only phenalkylamine demonstrated to produce both types of effects. Subsequently, animals were trained to discriminate MDA from saline (table 2); confirming the foregoing observations, the MDA stimulus generalizes both to DOM-like and AMPH-like agents.

TABLE 2. *Drug discrimination studies involving methylenedioxy derivatives of amphetamine* as *training drug in male rats*

Drug and Study	Route	Schedule	PSII	Dose (mg/kg)
MDA				
Glennon et al. 1982	IP	VI-15	15	1.50
Glennon and Young 1984	IP	VI-1 5	15	1.50
R(-)MDA				
Appel et al. 1990	IP	FR-10	15	1.50
S(+)MDA				
Appel et al. 1990	IP	FR-10	15	1.50
MDMA				
Glennon et al. 1986	IP	VI-15	15	1 .00
Schechter 1987	IP	FR-10	20	1.50
Oberlender and Nichols 1988	IP	FR-50	30	1.75
Glennon and Misenheimer 1989	IP	VI-15	15	1.50
MDE				
Boja and Schechter 1987	IP	FR-10	20	2.00
(+)MBDB				
Nichols and Oberlender 1989	—	—	—	1.75

NOTE: PSII = Presession injection interval (min); MDA = 1-(3.4-Mefhylenedioxyphenyl)-2 -aminopropane; MDMA = N-Methyl MDA or N-Methyl-1-(3,4-methylenedioxyphenyl)-2 -aminopropane; MDE = N-Ethyl MDA or N-ethyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane; MBDB = N-methyl-1-(3,4-methylenedioxyphenyl-2-aminobutane; VI-15 = variable interval 15-second schedule of reinforcement; FR-10 = fixed ratio (10) schedule of reinforcement.

Using DOM- and S(+)AMPH-trained animals, we examined several other methylenedioxy phenalkylamines including 2,3-MDA, MMDA (2-methoxy-4,5-methylenedioxy amphetamine), and MDMA (the N-monomethyl derivative of MDA). During the early to mid-1980s MDMA became a rather popular street drug ("Adam," "Ecstasy"); once it became a scheduled (Schedule I) substance, a series of MDMA-related designer drugs appeared on the clandestine market. Two of the more popular agents include the ethyl homolog of MDMA (MDE, "Eve") and the N-hydroxy analog of MDA (N-OH MDA). We had earlier demonstrated that MDMA differs from MDA in that it produces AMPH-like, but not DOM-like, effects. MDA and MDMA also produce AMPH-like stimulus effects in pigeons (Evans and Johanson 1986) and monkeys (Kamien et al. 1986) trained to discriminate S(+)AMPH from saline, and MDMA produces similar effects in rats trained to discriminate the phenylisopropylamine stimulant cathinone from saline (Schechter 1987). Stimulus generalization occurs between AMPH and cocaine regardless of which is used as training drug, and Broadbent et al. (1989) showed that MDA and MDMA produce cocaine-like effects (though not necessarily complete stimulus generalization, depending on the training dose of cocaine) in rats trained to discriminate cocaine from saline. The S(+)MDA stimulus generalizes to cocaine but only partially generalizes to (+)AMPH (Appel et al. 1990). Oberlender and Nichols (1988) failed to observe AMPH stimulus generalization to MDMA or to either of its isomers; on the basis that the number of animals selecting the drug-appropriate lever never exceeded 25 percent, it was claimed that MDMA lacks AMPH-like character. However, they showed that the MDMA stimulus generalizes to S(+)AMPH, and we have shown that the MDMA stimulus partially generalizes to S(+)AMPH and S(+)methAMPH (Glennon 1990). Although some of the reported inconsistencies may be attributable to differences in technique, there is ample evidence from discrimination studies and others that MDMA is capable of producing some AMPH-like effects. On the other hand, it has never been claimed that MDMA, AMPH, or cocaine produce identical effects. (For example, see Goudie in this volume for a comparison of the stimulus effects of AMPH and cocaine.) On the contrary, all instances of AMPH-stimulus generalization to MDMA have been accompanied by a significant decrease in animals' response rates. Taken together, these results suggest that, although AMPH and MDMA may share some similarities, there also appear to be some differences in their stimulus effects.

MDE and N-OH MDA reportedly produce effects in humans that are similar to those of MDMA. Neither of these agents produces stimulus generalization in AMPH-trained or in DOM-trained animals. However, both agents produce

MDMA-like effects in MDMA-trained rats (Glennon and Misenheimer 1989). These results support the overall contentions of Oberlender and Nichols (1988) that methylenedioxy derivatives of AMPH may possess properties that are distinct from those considered simply AMPH-like or hallucinogen- (e.g., DOM-)like. In contrast to their conclusions, however, it seems very likely that MDA and MDMA possess some AMPH-like qualities. MDE and N-OH MDA, on the other hand, appear to lack significant AMPH-like and DOM-like character.

Relatively little has been reported regarding the SAR of MDMA analogs. The a-ethyl homolog of MDMA (i.e., MBDB) is another MDMA-like agent that lacks AMPH-like and LSD-like stimulus properties; several conformationally restricted analogs have also been evaluated (for a review see Nichols and Oberlender 1989). Recently, we examined a new agent that has been confiscated from several clandestine laboratories: PMMA. PMMA is a structural relative of MDMA that possesses a 4-methoxy group in place of the 3,4-methylenedioxy bridge. This agent, like MDE and N-OH MDA, fails to produce AMPH-like or DOM-like stimulus effects, but it does produce MDMA-like effects (Glennon 1990). On a molar basis, PMMA (ED_{50} = 0.2 mg/kg) is about 3.5 times more potent than MDMA (ED_{50} = 0.76 mg/kg). Consequently it appears that the methylenedioxy group is not essential for MDMA-like activity and that an entirely new SAR may need to be investigated.

SUMMARY

Animals trained to discriminate classical hallucinogens from saline have been used in the past decade to examine other hallucinogenic agents. Time course (onset, duration of action) and locus of action have been studied, SARs have been formulated, and mechanism of action has been investigated in detail. On the basis of DD studies in animals, it was proposed that hallucinogenic agents may produce their actions in humans via a 5-HT_2 agonist mechanism and that certain phenalkylamine hallucinogens such as DOM and DOB might constitute the first known examples of 5-HT_2 agonists. This led to the development of $^{[3]}$HDOB and [^{125}I]DOI for use in radioligand binding and autoradiographic studies and to the use of hallucinogen-trained animals as a functional behavioral model of 5-HT_2 receptor activation. Animals trained to classical hallucinogens are more recently being used to evaluate novel designer drugs. It can be seen, then, that this paradigm, using hallucinogenic agents as training drugs, has proven to be quite useful for the investigation of hallucinogens and nonhallucinogens alike.

Note added in proof: We have recently found that both the DOM stimulus and the MDMA stimulus can be attenuated by pretreatment of the animals with very small doses of the 5-HT_3 antagonist zacopride. Zacopride (0.001 mg/kg) in combination with the training doses of DOM and MDMA (1 and 1.5 mg/kg, respectively) reduces drug-appropriate responding from >90 percent to <25 percent in both groups of animals. These findings offer entirely new insight into the mechanism of action of hallucinogenic agents and designer drugs as discriminative stimuli; they suggest that 5-HT_3 antagonists are capable of modulating the effects of these agents and that they may be of value for antagonizing the effects of these agents in a clinical setting.

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Discriminative Stimulus Properties of Amphetamine, Cathinone, and Related Agents

A. J. Goudie

INTRODUCTION

The drug discrimination (DD) research reviewed here concentrates on amphetamine (AMPH) and cathinone (CATH). The review excludes research on cocaine, although it is known that AMPH, CATH, and cocaine have similar cue properties. The justification for excluding cocaine comes from evidence that the cue properties of AMPH and cocaine are not identical (Stolerman and D'Mello 1981). It is actually possible to train rats to discriminate between AMPH and cocaine (Goudie and Reid 1988). In these studies rats were trained, over a substantial number of sessions (about 140), to discriminate a constant dose of AMPH (1 mg/kg) from varying doses of cocaine (5-12.5 mg/kg). In tests with saline in trained animals, at the lowest training dose of cocaine (5 mg/kg) animals selected the cocaine-associated lever. These animals therefore interpreted saline as more like cocaine at 5 mg/kg than like AMPH at 1 mg/kg, implying that this discrimination was the equivalent of a *quantitative* low dose (cocaine) versus a high dose (AMPH) discrimination. However, at higher cocaine training doses, saline tests produced "random" (50 percent) responding. These data accord with predictions from Järbe and Swedberg's (1982) model of DD learning for a qualitative discrimination. Although it is not known how such qualitative discriminations are learned, that it is possible to train them suggests that conclusions about "stimulant" cues (Nielsen and Scheel-Kruger 1988) should be considered with some caution if different stimulants are used as training stimuli.

Nielsen and Scheel-Kruger (1988) and Barrett and Appel (1989) suggested that AMPH and cocaine cues may be differentially sensitive to manipulations of catecholamine systems, providing a further reason for considering these cues as being different; parametric analyses directed at this question are clearly needed, however. Woolverton and Cervo (1986) reported that, in AMPH-trained rats, 6-OHDA lesions shifted the AMPH generalization curve to the right but had no effect on cocaine's ability to substitute for AMPH, again suggesting that the cue properties of cocaine and AMPH may differ in some way. Schechter and Boja (1988*a*) also suggested that the cue properties of cocaine may be more complex than those of AMPH, involving significant local anesthetic actions (see Zacany and Woolverton 1989). McElroy and O'Donnell (1989) reported that although AMPH substituted for the cue induced by the beta-adrenergic agonist clenbuterol, cocaine did not, again suggesting that these drugs may have subtly different cue properties. However, because AMPH and CATH are closely related structurally—CATH is the beta-keto side chain analog of AMPH (Young and Glennon 1986; Goudie et al. 1986)-and because the pharmacology of CATH resembles that of AMPH in many assays (Nencini and Ahmed 1989), it seems reasonable to assume that CATH and AMPH possess very similar cue properties and, therefore, to review research on these agents together.

Most of this review concentrates on studies with AMPH, because this drug has been studied more extensively. However, to our knowledge, no studies have shown important differences between CATH and AMPH in their cue properties, although Schechter (1989) suggested that the CATH cue may have a more rapid onset and a shorter duration of action than the AMPH cue. This issue needs to be studied more systematically.

In this review we have chosen to exclude human studies of the AMPH cue. This decision was taken to simplify the data to be reviewed. The validity of human studies will ultimately have to be evaluated against the data base reviewed here.

Amphetamine has been extensively studied in DD assays, and previous reviews have summarized basic findings (Nielsen and Scheel-Kruger 1988; Young and Glennon 1986). This review concentrates on more recent work in this area. However, before reviewing this literature it is relevant to reiterate the main conclusions about the AMPH and CATH cues derived from earlier work. The AMPH and CATH cues show the following properties.

1. They are dose dependent, being centrally mediated at "high" doses, and possibly peripherally mediated at "low" doses (Colpaert et al. 1976; Goudie

et al. 1986). The extent to which drugs generalize to the cues may be critically determined by the training dose (Stolerman and D'Mello 1981).

2. Catecholamine (specifically dopamine [DA]) systems are thought to be involved in mediating the AMPH and CATH cues, both agents being indirect DA agonists. Intracerebral AMPH injections in the nucleus accumbens generalize to the cue produced by systemic AMPH. This generalization is blocked by intra-accumbens sulpiride. However, AMPH injected into the striatum does not produce such generalization (Nielsen and Scheel-Kruger 1986) suggesting that the we is mediated by mesolimbic DA systems (see Wood and Emmett-Oglesby [1989] for similar results with cocaine). The nucleus accumbens may therefore play a critical role in the AMPH cue. In support of this hypothesis, it has been shown that typical and atypical neuroleptics block the AMPH cue (Nielsen and Jepsen 1985). Because atypical neuroleptics are believed to act at mesolimbii rather than striatal sites, these data support the hypothesis that the AMPH cue is mediated by the mesolimbii DA system. It has consequently been proposed that the AMPH cue can be used to screen for neuroleptics, including atypical agents devoid of extrapyramidal side effects (Nielsen and Jepsen 1985). Although much evidence indicates that the AMPH and CATH cues are in part DA mediated, roles for other neurotransmitters in these cues have not been excluded (see "The Involvement of Noradrenergic (NA) Systems in the Amphetamine Cue," in this paper). Furthermore, suggestions that endogenous phenylethylamine mediates the cue properties of stimulants (Colpaert et al. 1980; Goudie 1982) have not been followed up in recent years.

3. The cue properties of AMPH are not related to its action in stimulating behavior, because the cueing actions of drugs that generalize to AMPH do not correlate with their stimulant actions (Nielsen and Scheel-Kruger 1988). This is perhaps not surprising if the AMPH cue is mediated by mesolimbic DA, because motoric effects of stimulants are thought to be mediated predominantly at striatal sites.

4. The AMPH cue shows stereoselectivity, d-AMPH being 3-5 times more potent than l-AMPH. Because d- and l-AMPH are equipotent in peripheral actions but d-AMPH is more potent in its central actions, these data accord with the idea that, at doses typically studied, the AMPH cue is centrally mediated. The CATH cue is also stereoselective (Schechter 1986) and centrally mediated (Goudie et al. 1986).

5. A wide range of phenylethylamine and nonphenylethylamine drugs of abuse substitute for AMPH and CATH, although other abused drugs (LSD, morphine, ethanol) do not substitute. Such data led to the suggestion that the AMPH cue may be used to assess novel drugs for abuse potential of the AMPH type (Porsoft et al. 1984).

6. The structure-activity relationships for the AMPH and CATH cues have been reviewed in detail by Young and Glennon (1986) and Glennon and Young (1987). The interested reader is directed to these excellent reviews for information on this topic.

The foregoing material summaries established knowledge about the AMPH and CATH cues. We now turn to more recent research, which has built on this basic information.

INVOLVEMENT OF DA SYSTEMS

General Studies

Recent studies provide further evidence that DA is involved in mediating the AMPH and CATH cues. Schechter (1986) reported that in rats haloperidol caused a parallel shift to the right in the CATH generalization curve, although he noted that this shift was seen only with large haloperidol doses. He tentatively suggested that non-DA systems also mediate the CATH cue. Goudie et al. (1986) reported that haloperidol produced only 50 percent antagonism of the CATH cue (and of AMPH substitution) in studies with the highest dose of haloperidol that could be tested. It is not known whether more robust antagonism of the CATH we with DA antagonists can be obtained under other conditions or whether DA is less important in mediating the CATH cue than is the AMPH cue (Schechter 1986).

In studies with AMPH, Schechter and Boja (1988*a*) examined the effect of CGS 10746B, which inhibits DA release but does not affect postsynaptic receptors. The AMPH cue was antagonized by CGS 10746B, as was the ability of CATH to generalize to AMPH. Schechter (1990) later reported that CGS 10746B blocked CATH discrimination in animals trained on CATH. In addition, it blocked acute tolerance to CATH (induced by CATH pretreatment), suggesting that DA is involved in both the CATH cue and the tolerance that can develop for ft.

Nencini and Woolverton (1988) examined the effects of haloperidol and the calcium channel blocker nimodipine on the AMPH cue. Nimodipine antagonized the cue, although not to the extent haloperidol did. It was suggested that the antagonism seen with nimodipine was due to the inductbn of a third state (unlike AMPH or saline) rather than to true antagonism. Such an interpretation raises the question of how to differentiate specific from nonspecific antagonism in DD studies. Nencini and Woolverton (1988) proposed that a specific antagonist should (1) shift the generalization curve to the right, (2) possibly antagonize effects of the drug tested on response rate, and (3) induce complete antagonism at some dose. They noted that by these stringent criteria even haloperidol only approximates being a specific antagonist of the AMPH cue (see Goudie et al. 1986 for similar comments about the CATH cue).

Dworkin and Bimle (1989) showed that 6-OHDA lesions of the nucleus accumbens cause a shift to the right in the AMPH generalization curve. These data suggest that DA accumbens systems mediate the AMPH cue, in agreement with earlier findings.

The foregoing studies further implicate mesolimbic DA in mediating the AMPH and CATH cues. However, it is clear that (1) the criteria for assessment of pharmacological antagonism in DD studies need to be clarified and (2) the parametric conditions for obtaining maximal antagonism of the AMPH and CATH cues are not established; it is possible that antagonism studies may be influenced by pharmacokinetic factors related to agonist-antagonist interinjection intervals (Callahan et al. 1991).

Studies With Partial DA Agonists

Further evidence for DA involvement in the AMPH cue comes from studies with partial D_2 agonists with high affinity but low efficacy for DA receptors. Partial D_2 agonists are interesting drugs because they have inhibitory partial agonist actions on autoreceptors but buffering actions on postsynaptic receptors (Coward et al. 1989,1990). Typically, such agents act postsynaptically as DA antagonists because of their low efficacy and high affinity. However, because of their partial agonist actions at postsynaptic receptors, they maintain sufficient DA activation to prevent the extrapyramidal side effects that normally result from full postsynaptic DA receptor blockade (Coward et al. 1989,1990). Exner et al. (1989) studied the effects of such partial agonists on the AMPH cue. Complete blockade was seen with the prototypical partial agonist terguride (transhydrolisuride). However, only incomplete blockade, with no increase in

antagonism over a very wide (eightfold to sixteenfold) dose range, was seen with the partial agonists preclamol [-3PP] and SDZ 208-911. Exner et al. (1969) concluded that the failure of SDZ 208-911 and preclamol to antagonize the AMPH cue fully may have been due to postsynaptic agonist actions of these agents, preclamol having been reported to substitute for a selective D_2 agonist cue (Appel et al. 1986). (Presumably, terguride does not possess such agonist actions to the same extent, for it blocked the AMPH cue fully and acted like a classical D_2 antagonist.) Because some partial D_2 agonists inhibited, but did not fully block, the AMPH cue, and because they do not induce catalepsy (Coward et al. 1989), Exner et al. (1989) suggested that these agents may be useful in treating psychotic states without inducing extrapyramidal side effects. These data obviously further implicate DA in mediating the AMPH cue. They also develop earlier ideas about the potential use of the AMPH cue to screen for neuroleptics (Nielsen and Jepsen 1985).

In limited studies with partial D_1 agonists SKF 38393 and SKF 75760, Nielsen et al. (1989) reported that these agents had very little effect in antagonizing the AMPH cue. Because partial D_2, but not D_l, agonists inhibit the AMPH cue, these preliminary data suggest that D_2 rather than D_1 receptors may play the primary role in the AMPH cue.

Involvement of D_1 and D_2 Receptors In the AMPH Cue

Recent years have seen much research into the roles of D_1 and D_2 receptors in mediating the AMPH cue. These studies have generated a fairly consistent pattern of data, although the interpretation of the data remains controversial. Typically, D_1 and D_2 antagonists block the AMPH cue. However, although D_1 agonists do not substitute for AMPH, D_2 agonists do. The relevant empirical data are summarized briefly as follows.

Studles With D_1 Agonists. Most of these studies have been conducted with SKF 38393, which has been found universally not to generalize to AMPH in rats (Arnt 1988; Callahan et al. 1990; Furmidge et al. 1989*a*; Nielsen et al. 1989; Smith et al. 1989) and monkeys (Kamien and Woolverton 1969). Nielsen and Scheel-Kruger (1988) noted that the failure of SKF 38393 to generalize could be due to its partial agonist actions . However, the full D_1 agonist SKF 81297 also does not generalize fully to AMPH (Furmidge et al. 1989*a*; Nielsen et al. 1989) thus D1 agonists clearly do not substitute for AMPH.

Studies With D_1 Antagonists. Virtually all these studies have been conducted with SCH 23390, which antagonizes the AMPH cue in rats (Arnt 1988; Callahan et al. 1990; Exner et al. 1989; Nielsen and Jepsen 1985; Nielsen et al. 1989; Smith et al. 1989) and monkeys (Kamien and Woolverton 1989). The D_1 antagonist SKF 83566 also blocks the AMPH we (Furmidge et al. 1989*a*). There is consequently reliable evidence that D_1 antagonists block the AMPH cue.

Studies With D_2 Agonists. In the vast majority of studies reported, various D_2 agonists substitute for AMPH. In rats quinpirole has repeatedly been found to substitute (Arnt 1988; Callahan et al. 1990; Furmidge et al. 1989*a*; Nielsen et al. 1969; Smith et al. 1989) as have D_2 agonists such as RU 24213 (Furmidge et al. 1989*b*), (–)-NPA, and pergolide (Arnt 1988; Nielsen et al. 1989). At low doses quinpirole antagonizes the AMPH cue (Furmidge et al. 1989*c*), an effect thought to be mediated by actions on D_2 autoreceptors because in the same study higher doses showed the typical generalization seen with quinpirole. In pigeons, pirebedil also generalizes (Evans and Johanson 1987). However, Kamien and Woolverton (1989) reported that in monkeys pirebedil, bromocriptine, and propylbutyidopamine did not substitute for AMPH. The reason or reasons for the discrepant monkey data are not clear.

Studies With D_2 Antagonists. Various D_2 antagonists (including haloperidol, raclopride, YM 09151-2, spiroperidol, sulpiride, and clebopride) block the AMPH cue in rats (Amt 1988; Callahan et al. 1990; Furmidge et al. 1989*a*; Nielsen and Jepsen 1985; Nielsen and Scheel-Kruger 1986; Nielsen et al. 1989). Studies in pigeons (Järbe 1982) have also reported antagonism with haloperidol. However, Kamien and Woolverton (1989) reported that pimozide antagonized the AMPH cue in only one of three monkeys and raciopride antagonized the cue only in one of two. Again, the cause or causes of the discrepant monkey data are unclear.

If one ignores the monkey data, these studies suggest that D_2 receptors play a primary role in the AMPH cue (Arnt 1986; Callahan et al. 1990; Nielsen et al. 1989) and that D_1 stimulation is a necessary but insufficient condition for generalization to AMPH to occur. This pattern of data accords with a common hypothesis about the nature of some D_1-D_2 receptor interactions. D_1 receptor activation may serve an “enabling” role, allowing some effects of D_2 stimulation to be observed (Clark and White 1967; Waddington 1989). Thus D_1 antagonists may block the AMPH cue because of inhibition ("disenabling”) of tonic D_1 activation by endogenous DA. In support of this hypothesis, Smith et al. (1989) reported that in AMPH-trained rats, generalization of a low dose of AMPH was

potentiated by SKF 38393. These data can be interpreted as demonstrating that D_1 stimulation enhances the effect of D_1 activation caused by the DA-releasing action of AMPH.

However, although the “enabling” hypothesis accounts for much of the available data from studies with AMPH, it does not accommodate ail published findings. For example, in studies with D_1 and D_2 agonist combinations, SKF 38393 did not significantly potentiate the degree of AMPH generalization induced by quinpirole (Smith et al. 1989). These data do not readily fit in with the enabling hypothesis, because D_1 activation did not potentiate effects of D_2 stimulation. Smith et al. (1989) also examined the ability of quinpiroie to substitute for AMPH after treatment with SCH 23390. Substitution by quinpirole was not blocked by SCH 23390, although it was by haloperidol, suggesting that, as far as quinpiroie substitution for AMPH is concerned, no D_1-D_2 receptor interactions occurred. In a similar study, Nielsen et al. (1989) examined the ability of SCH 23390 to block substitution for AMPH by the D_2 agonist pergolide. SCH 23390 failed to block such substitution, whereas the D_2 antagonist raclopride was effective. At the same doses, SCH 23390 blocked pergolide-induced motor stimulation. Nielsen et al. (1989) suggested that effects of D_1 and D_2 receptors are coupled in their actions on activity; however, in the AMPH DD assay, no receptor coupling occurred. This conclusion was supported by other studies in which combinations of D_1 and D_2 agonists were investigated. SKF 38393 and SKF 81297 did not potentiate the ability of pergolide and quinpirole, respectively, to substitute for AMPH, again suggesting the absence of D_1-D_2 receptor coupling.

The precise role of D_1 receptors in the AMPH we is obviously difficult to specify. Many findings are compatible with the enabling hypothesis, although other data do not support it. Furthermore, there is evidence from studies with selective agonists that it is possible to train distinct D_1 and D_2 agonist cues so that, after training with a D_1 agonist, the cue is blocked only by D_1 antagonists, not by D_1 antagonists. Conversely, after training with a D_2 agonist, the we is blocked only by D_2 antagonists, not by D_1 antagonists (Appel et al. 1988; Arnt 1988; Kamien et al. 1987). Such data suggest that in the DD assay there may be little D_1-D_2 receptor coupling when specific agonists are used as training stimuli (Clark and White 1987). Studies reporting that SCH 23390 does not block the cue induced by the D_2 agonists pirebedil (Kamien et al. 1987), (-)-NPA (Amt 1988) and apomorphine (Schechter and Greer 1987; Woolverton et al. 1987; but see Tang and Code 1989) are difficult to reconcile with a simple form of the enabling hypothesis. It is also perhaps surprising on the basis of the

enabling hypothesis that AMPH does not substitute fully for the cue induced by D_2 agonists such as quinpirole (Appei et al. 1988) and (-)-NPA (Arnt 1988). it might be expected that AMPH would substitute for a D_2 agonist, either because of endogenous DA tone or because of stimulation of D_1 receptors resulting from AMPH-induced DA release. It is consequently possible that to observe D_1-D_2 receptor interactions in the DD procedure, it may be necessary to train and (of critical importance) also to test animals with an indirect nonselective DA agonist such as AMPH rather than with direct agonists such as apomorphine or with specific D_1 or D_2 agonists (Waddington 1989). However, it is clearly necessary to investigate this issue further before definitive conclusions can be reached. Furthermore, it seems important to attempt to clarify why the training and test drugs may be critical in determining the extent of DA receptor interactions observed in DD studies.

INVOLVEMENT OF NORADRENERGIC (NA) SYSTEMS IN THE AMPHETAMINE CUE

Snoddy and Tessel (l983) reported that mice discriminating low doses of AMPH generalized to the selective NA uptake inhibitor nisoxetine. These data are surprising in the light of earlier studies showing that NA receptor blockers such as phentolamine and propranolol typically do not block the AMPH we (Young and Glennon 1986). However, Snoddy and Tessel (l985) replicated their earlier results. In addition, they showed that the AMPH we was blocked by the α_1 antagonist prazosin and potentiated by the α_2 antagonist yohimbine. The potentiating actions of yohimbine were attributed to blockade of presynaptic α_2 autoreceptors, which were thought to be activated by AMPH-induced NA release, which reduced net NA activation after AMPH treatment. Most significant, Snoddy and Tessel (1985) reported that pimozide dii not affect the AMPH we at doses that decreased AMPH-induced stimulation. Thus, these data suggest that the AMPH we may be mediated by α_1 receptors and not by DA receptors.

A number of studies have followed up these reports. Evans and Johanson (1987) found that nisoxetine generalized to AMPH in pigeons. Similarly, Kamien and Woolverton (1989) found that nisoxetine generalized to AMPH in monkeys. However, in these monkeys the *a-NA* antagonists phentolamine and prazosin did not consistently block the AMPH cue. Arnt (1988) also reported that prazosin does not antagonize the AMPH cue in rats. Schechter and Boja (1988*b*) and Nielsen and Scheel-Kruger (1988) reported that nisoxetine does not generalize to AMPH in rats, although both authors commented that generalization with nisoxetine in mice was seen only with doses larger than

those studied in rats. Sanger (1988) reported that the α_2 antagonists yohimbine and idazoxan, which may act at NA autoreceptors and thus potentiate NA functioning, did not generalize to AMPH, suggesting no role for NA in the AMPH cue in rats.

These data obviously show substantial inconsistencies, for which there are a number of potential explanations. First, there may be species differences in the involvement of NA in the AMPH cue (Schechter and Boja 1988*b*; Snoddy and Tessel 1985). Second, the effects of nisoxetine may be mediated by DA systems. Although nisoxetine is a relatively selective NA uptake inhibitor, it may modify DA systems to some extent (Kamien and Woolverton 1969). However, this explanation is difficult to reconcile with the finding (Nielsen and Scheei-Kruger 1988) that the selective DA uptake inhibitor GBR 12909 produces only partial generalization to AMPH in rats. Third, the training dose of AMPH may be critical, because Snoddy and Tessel (1983) found that mice trained to discriminate the high dose of 3.2 mg/kg of AMPH did not generalize to nisoxetine. The high-dose AMPH cue may be DA mediated and the low dose NA mediated. Finally, Snoddy and Tessel (l985) suggested that studies with nonselective a-NA antagonists such as phentolamine may be confusing because α_1 and α_2 antagonists have opposite effects on the AMPH cue. Thus, if nonselective antagonists are used, the actions on α_1 and α_2 receptors may cancel each other out and no antagonism will be seen.

The data reviewed clearly suggest some role for NA in the AMPH cue. However, the inconsistencies in the literature will probably be resolved only by parametric studies involving a number of training doses of AMPH in various species. It also seems desirable that such studies be accompanied by neurochemical analyses of the effects of nisoxetine on NA and DA levels (Schechter and Boja 1988*b*).

STUDIES ON FENFLURAMINE GENERALIZATION TO THE AMPHETAMINE CUE

Evans and Johanson (1987) reported that pigeons discriminating AMPH generalized to a range of anorectics and stimulants. These data are not surprising, because they are in accord with earlier studies. However, the nonstimulant serotonergic anorectic fenfluramine substituted fully for AMPH in two pigeons, partially in a third, and not at all in a fourth. Evaluation of these data is complicated by the relatively small number of pigeons studied and thus depends on the significance attributed to data from subjects showing individual

differences in DD studies. However, if attention is paid to patterns of data generated by individual subjects, these data are surprising because studies in various species have shown that fenfluramine does not typically substitute for AMPH (Young and Glennon 1986). Evans and Johanson (1987) suggested that the observed generalization was mediated by anorectic properties shared by fenfluramine and AMPH. However, De La Garza and Johanson (1987) reported that although AMPH generalized to a range of anorectics in monkeys, fenfluramine did not. On the basis of these data De La Garza and Johanson (1987) concluded, in contrast to the pigeon studies, that the AMPH cue was not related to the drug's anorexic action.

The findings with fenfluramine in pigeons are potentially important because it has often been suggested that generalization to AMPH may indicate that a drug possesses abuse potential of the AMPH type (De La Garza and Johanson 1987; Evans and Johanson 1989; Goudie et al. 1988; Grant and Woolverton 1989; Porsolt et al. 1984). However, there is minimal evidence that fenfluramine is either a stimulant or a drug of abuse. The generalization to AMPH seen in pigeons therefore represents a potentially important example of a "false positive" in terms of screening for drug abuse potential.

As a followup of their earlier work, Evans et al. (1990) developed a three-choice discrimination in pigeons among AMPH, fenfluramine, and saline. They demonstrated that an assay could be developed that differentiates the cue properties of fenfluramine and AMPH in pigeons. In tests with fenfluramine, subjects always responded on the fenfluramine lever; even at low doses they did not generalize to AMPH. Conversely, in tests with AMPH, subjects responded on the AMPH lever; they did not generalize to fenfluramine. Thus it was possible to develop a DD assay that consisted of an exclusive discrimination between AMPH and fenfluramine, in contrast to the earlier two-choice results in pigeons (Evans and Johanson 1987). This is probably not surprising, in that a three-choice discrimination would be expected to force animals to attend to the differences between the cue properties of the two drugs in much the same way animals can be trained to discriminate AMPH from cocaine, even though they typically generalize between these agents (Goudie and Reid 1988). Therefore, although it was possible to dissociate the cue properties of AMPH and fenfluramine in pigeons, the original findings should be considered false positives in terms of screening for abuse potential.

The tendency for some anorectics (mazindol and phendimetrazine) to generalize to AMPH does not always predict whether the agent in question is

self-administered (De La Garza and Johanson 1987). The generalization to AMPH seen by some authors with nisoxetine also seems to represent an example of a false positive in terms of screening for abuse potential, because this agent is not self-administered (Kamien and Woolverton 1989). Thus the finding that a drug generalizes to AMPH does not necessarily mean that it will be prone to abuse of the AMPH type. There is a clear need for refinements of methodology, similar to those recently developed by Evans et al. (1990), for abuse potential prediction. However, it should also be noted that, besides generating a few false positive's in screening for abuse potential, studies of the AMPH cue have also generated very many true positive findings; the false positive findings should be kept in perspective.

SUMMARY

Although the AMPH and CATH cues have been studied extensively, inconsistent findings remain that need to be investigated. It is not known to what extent, if at all, AMPH and CATH cues differ and how they differ from the cocaine cue. The extent to which D_l and D_2 receptor systems are coupled in AMPH DD assays requires further investigation. The inconsistent findings in substitution studies with nisoxetine need to be addressed in parametric studies in various species. Finally, the possibility that the AMPH cue may generate false positives in screening for drug abuse potential needs to be evaluated.

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Discriminative Stimulus Effects of Cocaine

William L. Woolverton

These reviews address four aspects of the pharmacology of the discriminative stimulus (DS) effects of various compounds: (1) assessment of novel compounds, (2) investigations of mechanism of action, (3) structure-activity relationships, and (4) problems that remain to be solved. This paper deals with cocaine. In fact, the first three aspects can be considered part of the same issue (i.e., mechanism of action). I hope to identify problems remaining to be solved in each of these areas. At this time there are approximately 30 papers in the literature that involve investigation of various aspects of the DS effects of cocaine. Each of them has contributed to this review.

ASSESSMENT OF NOVEL COMPOUNDS

A large number of compounds have been evaluated for their ability to substitute for cocaine as a DS. To summarize these data, I have somewhat arbitrarily divided the compounds into three groups: a group found to substitute for cocaine (81-100 percent drug lever responding), a group found to partially substitute for cocaine (21-80 percent drug lever responding), and a group that failed to substitute for cocaine (0-20 percent drug lever responding). Within a category, the compounds *are* grouped according to predominant pharmacological mechanism of action or into a group of miscellaneous compounds. The data points for these figures are the maximum percentage of cocaine-appropriate responses that have been found for each compound in any study. This value cuts across species and procedures and therefore suffers any limitations that such a summary may impose. In addition, the value equally weights drugs for which several studies are available and drugs that have been evaluated in only a single study. Finally, where study results differed for a particular compound, the maximum value may not accurately represent the consensus for that compound. In fact, consensus was rare; I will point out where study findings differed. With those caveats in mind, the summaries are a reasonable representation of the extant literature.

Among the compounds found to substitute for cocaine are a variety of psychomotor stimulants and anoretics (see figure 1). Compounds such as methamphetamine, amphetamine, methylphenidate, and diethylpropion have consistently been found to substitute for cocaine as a DS. Gauvin et al. (1989) reported that a caffeine-ephedrinephenylpropanolamine combination substituted for cocaine in rats. A second group of compounds that are generally known as selective dopamine (DA) reuptake blockers (nomifensine, bupropion, GBR 12909) have been found to substitute fully for cocaine as a DS. A number of cocaine analogs (e.g., WIN 35062-2) engendered 100-percent drug responding at some dose in cocaine-trained animals. Colpaert et al. (1980) evaluated the ability of monamine oxidase (MAO) inhibitors to substitute for cocaine. In fact, several of the compounds substituted for cocaine. The effect was correlated with their potency as MAC-B inhibitors, leading Colpaert et al. (1980) to postulate that endogenous 8-phenethylamine was involved in the DS effects of cocaine. This interesting effect has not, apparently, been pursued and is an issue that remains to be solved. The direct DA agonists quinpirole and apomorphine, primarily D_2 agonists, have been reported to fully substitute for cocaine. This is one point where summary data may misrepresent the literature: the more general finding has been that DA agonists partially substituted for cocaine. Phencyciidine (PCP), dexetimide, and procaine are grouped together, not because of pharmacological similarity, but because they do not fii particularly well into any of the other pharmacological categories or into our notions of what should substitute for cocaine. Colpaert et al. (1979) reported that PCP and the anticholinergic dexetimide substituted for cocaine and postulated that the indirect DA actions of PCP accounted for the substitution. Järbe (1984) reported that procaine substituted for cocaine, an effect that was also found in some rhesus monkeys (de la Gatza and Johanson 1983). Moreover, Woolverton and Balster (1982) found that cocaine substituted for procaine as a DS. The behavioral commonalities between some local anesthetics and cocaine clearly warrant further investigation, as do the substitutions of PCP and dexetimide for cocaine.

Figure 2 summarizes drugs that have been found to substitute partially for cocaine and includes agents from a number of different pharmacological classes. The major point to be made from these data is that the DS effect of cocaine is pharmacologically selective. The monoamine uptake inhibitors that partially substituted for cocaine (pargyline, nialamide) have less MAO-B activity that those that substituted for cocaine (figure 1). Nicotine, caffeine, and strychnine are stimulants that, in contrast to the traditional psychomotor stimulants shown in figure 1, only partially substituted for cocaine. Benztropine

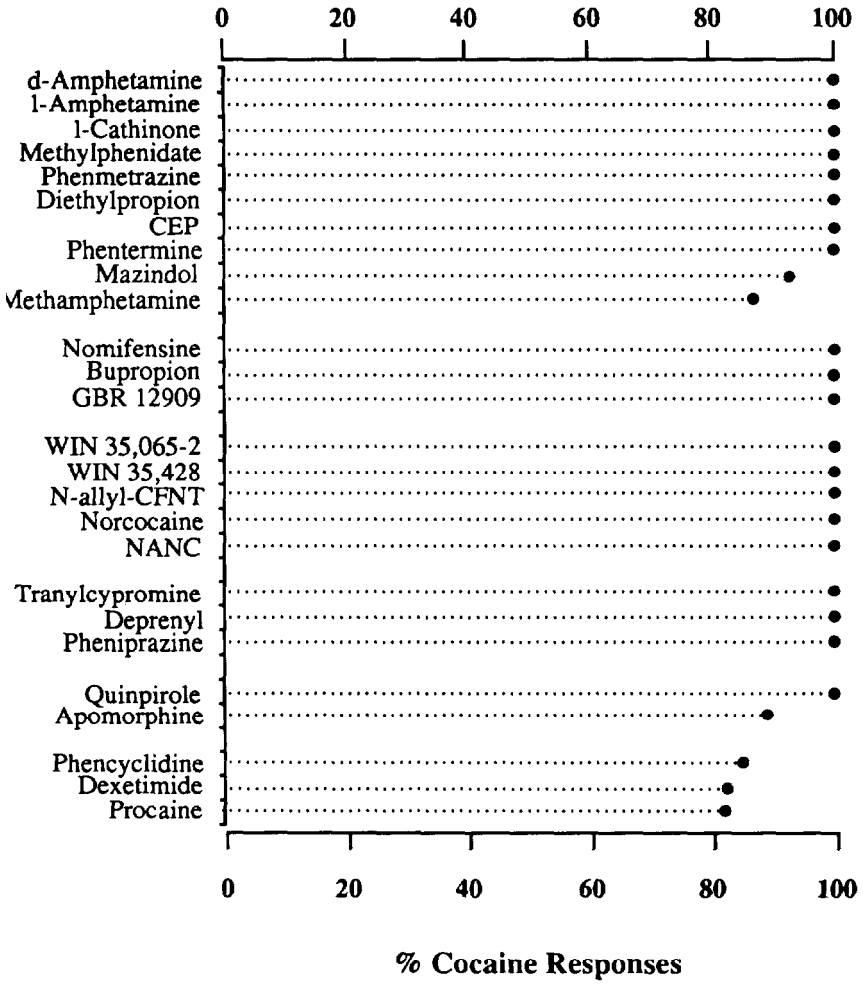


FIGURE 1. *Drugs found to substitute for cocaine*

and amantadine are indirect DA agonists that only partially substituted for cocaine, apparently because they have actions in addition to their indirect DA agonist actions (e.g., the antichlolinergic actions of benztropine). it seems that failure of a compound to substitute for cocaine can be due to lack of an action

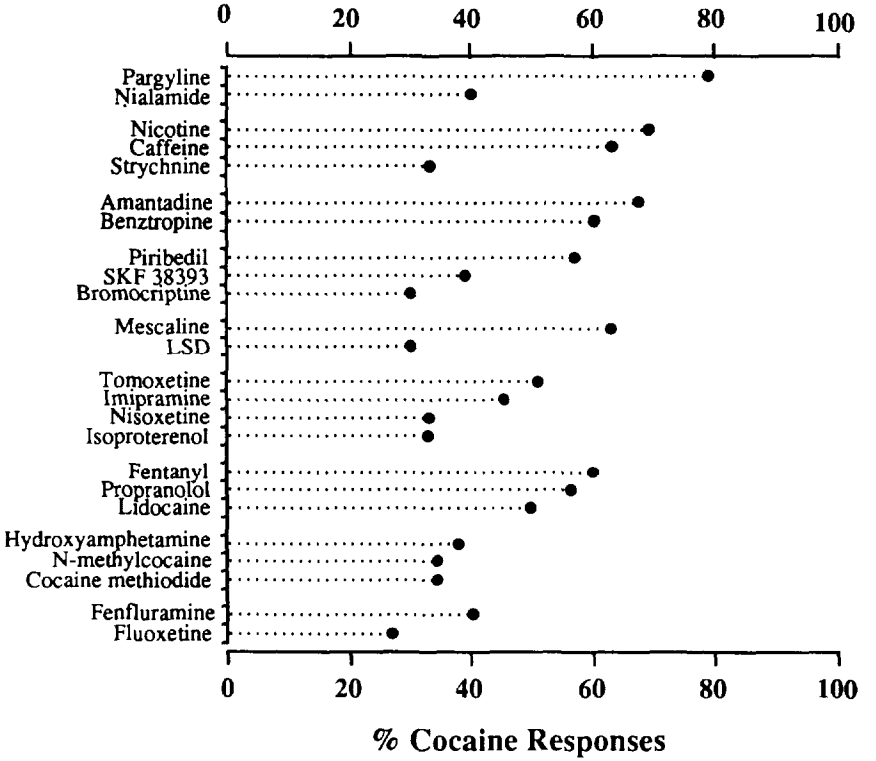


FIGURE 2. *Drugs found to partially substitute for cocaine*

found in cocaine (indirect DA actions) or presence of an action lacking in cocaine (e.g., anticholinergic effects). SKF 33393, piribedil, and bromocriptine present a more typical picture of the direct DA agonists than noted in figure 1 (i.e., partial substitution for cocaine). Hallucinogens and norepinephrine (NE) agonists have been found to substitute partially for cocaine. Partial substitution with the local anesthetic lidocaine probably indicates that the local anesthetic effect of procaine is not responsible for its substitution for cocaine. Cocaine analogs that do not cross the blood-brain barrier do not engender cocaine-appropriate responding, consistent with the belief that the DS effects of cocaine involve an action in the brain Indirect serotin (5-HT) agonists only partially substituted for cocaine, indicating that 5-HT actions of cocaine do not play a primary role in this behavioral effect.

Compounds that failed to substitute for cocaine (figure 3) include MAO-A inhibitors, delta-9-tetrahydrocannabinol, metabolites of cocaine, and one of the isomers of cocaine. In addition, sedatives, hypnotics, anxiolytics, and anticholinergics do not substitute for cocaine. The main point to be made from these data is that the DS effect of cocaine, like that of other drugs, is pharmacologically selective.

STRUCTURE-ACTIVITY RELATIONSHIPS

Although some work has been done with the DS effects of cocaine analogues, the available data are limited. Figure 4 shows the structures of the cocaine analogs that have been evaluated in cocaine-trained animals and their order of potency as discriminative stimuli, again compiled across studies. The compounds include modifications at three points of the molecule: the ester linkage between the tropane and the phenyl ring, the phenyl ring itself, and the amine group. The most potent compound, WIN 35428, does not have the ester

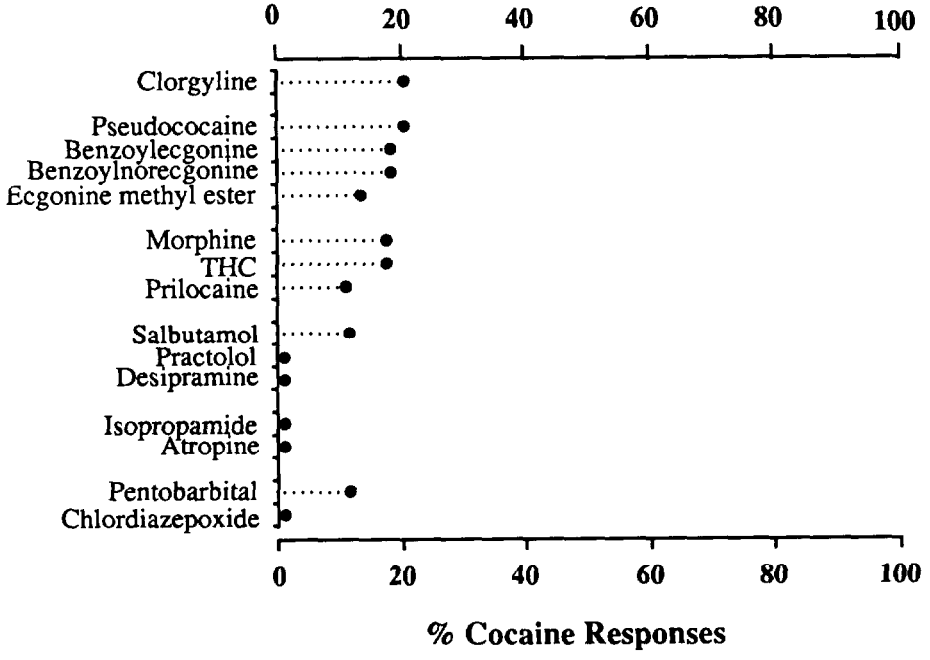


FIGURE 3. *Drugs that failed to substitute for cocaine*

linkage between the tropane and the phenyl ring and has a fluorine substituent on the ring. Although Woolverton and Balster (1982) postulated that the ester linkage was an important structural compound of the focal anesthetics with cocaine-like effects, this compound demonstrates that ester linkage is not essential for activity and that its presence can reduce potency. The second most behaviorally potent compound is norcocaine, a compound in which the methyl group has been removed from the terminal amine. Adding an N-allyl group to the amine of CFT to produce CFNT, or to norcocaine to produce N-allyl norcocaine, decreases potency relative to the methylated parent compounds. Metabolites of cocaine (figure 5) do not engender cocaine-appropriate responding.

MECHANISM OF ACTION

Table 1 is a comparison of relative potencies of various compounds at the [^{3}H]cocaine binding site in monkey brain and as a cocaine-like DS in rats, pigeons, and rhesus monkeys. In rats, the potency order is identical except that norcocaine is somewhat more potent as a DS than would be predicted based on the binding data. In pigeons the potency order is similar, again with the potency of norcocaine as a DS slightly greater than predicted. In monkeys the potency relationships across preparations are also similar except that the potency of cocaine as a DS is enhanced relative to the other compounds. In short, although the potency orders are not identical across preparations, they are remarkably similar considering the many ways in which pharmacokinetics could alter the *in vivo* effects of cocaine. Madras et al. (1989) examined the relative potency of blocking DA, NE, and 5-HT uptake with potency at the cocaine binding site. The correlation between cocaine binding potency and DA uptake blockade was quite high (r = 0.93) whereas the correlations with NE and 5-HT uptake blockade were low (r = 0.52 and 0.38, respectively). Therefore, the available data implicate cocaine binding sites and blockade of DA uptake as important components of the mechanism of action of cocaine as a DS. It should be noted that potency comparisons based on four or five compounds may be unreliable and that additional analysis of this sort would make this a more valid conclusion.

There is substantially less information concerning antagonism of the DS effects of cocaine. The available data are summarized as the maximum reduction in drug lever responding with the training dose of cocaine, again cutting across studies (figure 6). We have found that D_1 dopamine antagonists consistently reduce the effects of the training dose of cocaine from 100 percent to 0 percent (Kleven et al. 1990; Vanover et al. 1989). SCH 23390, SCH 39166, and A

Compounds Found To Substitute For Cocaine

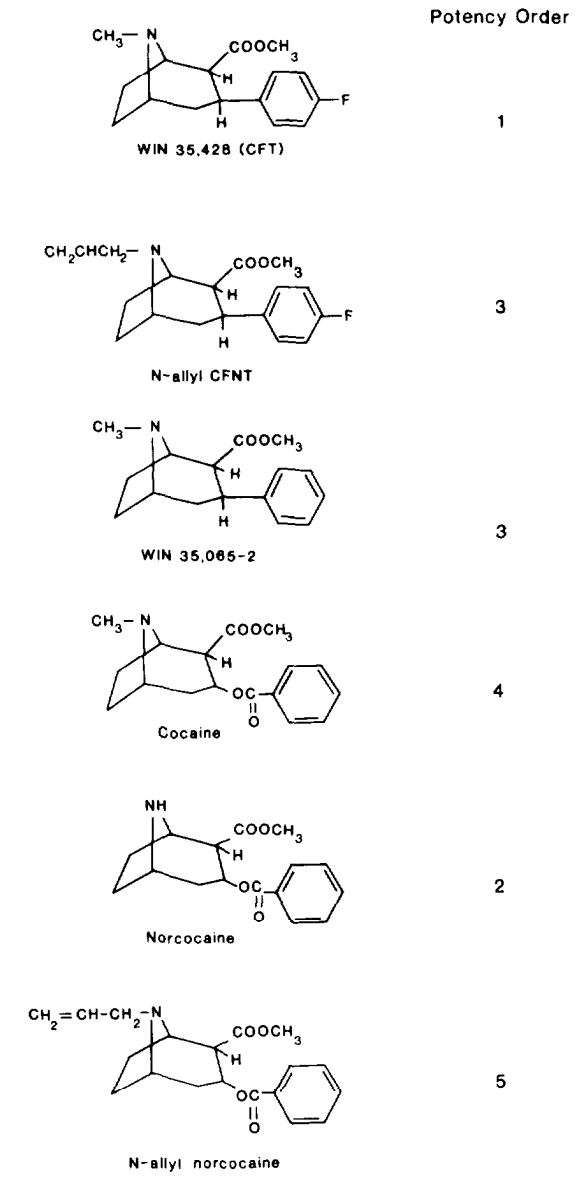
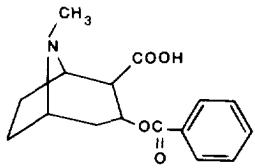
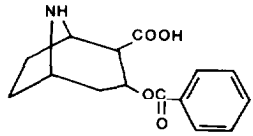


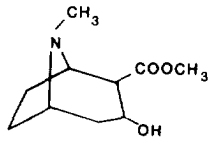
FIGURE 4. *Structures of compounds found to substitute for cocaine*



Benzoylecgonine



Benzoylnorecgonine



Ecgonine Methyl Ester

FIGURE 5. *Structures of compounds that failed to substitute for cocaine*

66359 are all D_1 antagonists and are from different chemical classes. It should be noted, however, that Barrett and Appel (1989) evaluated SCH 23390 as an antagonist of the DS effect of cocaine in rats and, although there was evidence of blockade, it was not as clear-cut as in the monkey. A substantial number of DA antagonists with primarily D_2 activity have been evaluated as antagonists of the DS effects of cocaine. The most common finding has been that these compounds partially block the DS effects of cocaine to between 20 percent and 80 percent cocaine-appropriate responding at the training dose. The potency order for binding at haloperidol sites is identical to that for reducing the effects of the training dose of cocaine to 50 percent cocaine-appropriate responding (table 2) suggesting some involvement of D_2 receptors in the DS effects of cocaine. Again, it should be noted that there is very little evidence on which to base this conclusion (see also the chapter by Goudie). NE and 5-HT antagonists do not alter the DS effects of cocaine.

In most experiments, antagonists have been combined only with the training dose of cocaine, a strategy that does not allow detailed comparisons between

TABLE 1. *Relative potency of several compounds at cocaine binding site and as cocaine-like discriminative stimuli (DS)*

Cocaine binding site[a] :	Nomifensine > methylphenidate > cocaine > norcocaine
Cocaine DS in rat[b]:	Nomifensine > methylphenidate > cocaine = norcocaine
Cocaine binding site[a]:	WIN 35428 > 35065-2 > cocaine > norcocaine
Cocaine DS in pigeon[c]:	WIN 35428 > norcocaine > WIN 35065-2 > cocaine
Cocaine binding site[a]:	Mazindol > nomifensine > GBR 12909 > cocaine > bupropion
Cocaine DS in monkey[d]:	Cocaine >2 mazindol > nomifensine > GBR 12909 > bupropion

[a]Relative potency displacing [^{3}H]cocaine binding in monkey caudate-putamen (Madras et al. 1989).
[b]Relative potency substituting for cocaine as a DS in rats (Bedford et al. 1981; Colpaert et al. 1979; Ho et al. 1976; Järbe 1984; McKenna et al. 1979).
[c]Relative potency substituting for cocaine as a DS in pigeon (Järbe 1981).
[d]Relative potency substituting for cocaine as a DS in rhesus monkeys (Kleven et al. 1990).

antagonists. On the few occasions in which the entire dose-response function for cocaine has been determined in the presence of a D_2 antagonist, the effect appears similar to noncompetitive antagonism (e.g., see Ho and Silverman 1978). Besides partial antagonism of the effects of cocaine, this effect could be the result of one or more of a number of drug effects, such as loss of stimulus control of behavior in general, that have nothing to do with the blockade of cocaine. This effect should be contrasted with the effects of D_1 antagonists in monkeys (Kleven et al. 1990; Vanover et al. 1989). Cocaine dose-response functions have been consistently shifted parallel to the right in combination with D_1 antagonists. In one case with SCH 39166, that shift was as large as sixteenfold (monkey 8409; figure 7). There also was a mutual antagonism between drugs in terms of the effect on response rate. Species differences may contribute to these apparent differences between D_1 and D_2 antagonists in their interaction with cocaine. In fact, we have found a small (approximately twofold) shift to the right in the cocaine dose-response function in monkeys pretreated with haloperidol (Kleven et al. 1990).

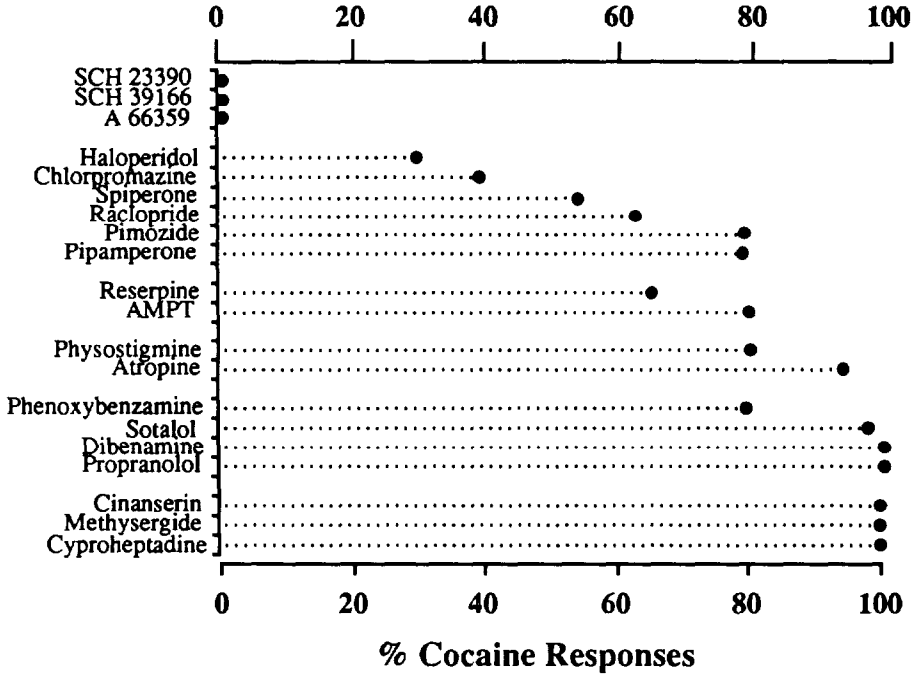


FIGURE 6. *Effects of antagonists*

Although these data clearly suggest that D_1 and D_2 receptors play a significant role in the DS effects of cocaine, it remains to be established what that role may be. For D_1 receptors, an intriguing possibility is that the permissive role that has been postulated for D_1 receptors in the expression of DA-mediated behaviors holds for the DS effects of cocaine. That is, blockade of the indirect DA agonist effects of cocaine at D_1 receptors may function as an on-off switch for the effects of cocaine. In fact, we have found that several other behavioral effects of cocaine (e.g., stereotypy and effects on food intake) can be blocked by D_1 antagonists (Rapoza and Woolverton 1988; Rapoza et al. 1990). Another possibility is that the cocaine DS is a complex stimulus that includes a D_1 component and that, when that component is blocked, the remaining stimulus is sufficiently unlike cocaine (but not neutral) to engender saline lever responding.

TABLE 2. *Relative potency of several compounds binding at the haloperidol binding site or reducing cocaine-appropriate responding to 50 percent at the training dose*

Haloperidol binding site[a]:	Spiperone > haloperidol > pimozide > chlorpromazine (CPZ)
Cocaine DS in rat[b]:	Spiperone > haloperidol > pimozide > CPZ

[a] Inhibition of [^{3} H]haloperidol binding in calf striatal membranes (Creese et al. 1976).
[b] Relative potency reducing the effect of training dose of cocaine to 50 percent (Colpaert et al. 1976, 1978; Järbe 1978).

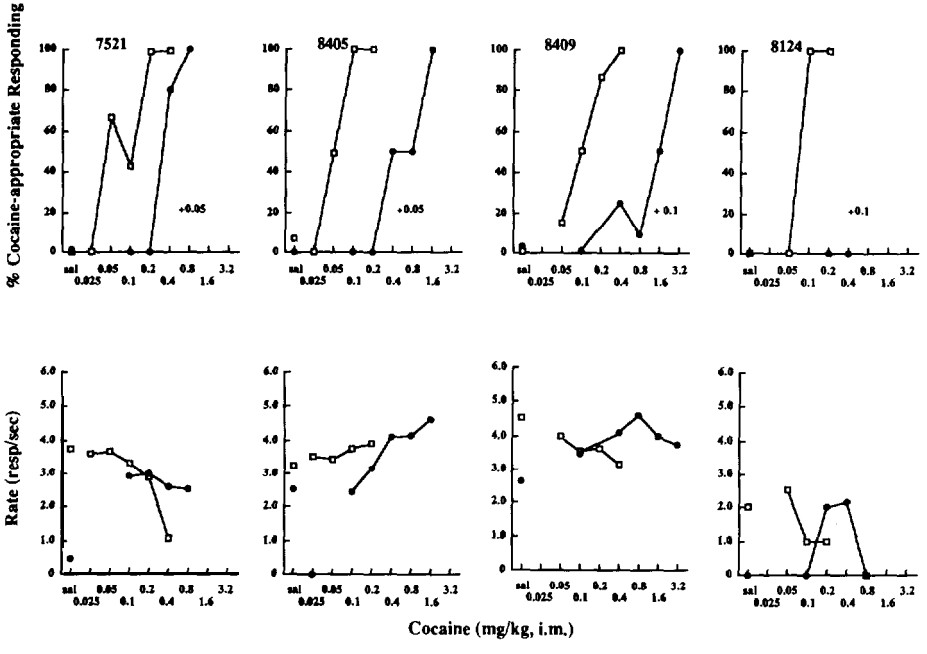


FIGURE 7. *SCH 39166 + cocaine*

Finally, it will be of interest to know whether D_1 antagonists can block the subjective effects of cocaine in humans.

Two additional points should be made with regard to problems remaining to be solved, one pharmacological and one behavioral. Our efforts to this point with cocaine have concentrated on DA function in the nucleus accumbens (e.g., see Wood and Emmett-Oglesby 1989). The nucleus accumbens is not an isolated unit in the brain; it has connections to other brain regions, connections involving the action of several other neurotransmitters. By investigating the role of DA and DA receptors, I think we have discovered something important about the neuronal actions of cocaine, particularly its DS effects. However, the role of other brain structures and neurotransmitters remains to be established. Finally, I would like to reiterate a point made previously by Schuster. There have been no investigations of the role of behavioral-environmental variables in the DS effects of cocaine. This is clearly a gap that needs to be filled.

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Discriminative Stimulus Functions of Cannabinoids/Cannabimimetics

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INTRODUCTION

The psychoactive effect of cannabis is unique. This cannabimimetic effect is primarily responsible for cannabis abuse and for the unwanted side effects of cannabis therapy. To isolate the chemical components that induce this psychoactive property, experimental methods for detecting and quantifying cannabimimetic activity in animals have been developed. Most of these methods use measures of spontaneous behaviors such as locomotor activity, ataxia, sleep time, and posture. However, one method specifically measures detection of a cannabimimetic effect. That method is drug discrimination learning (DDL). In DDL procedures, the effects of cannabinoids are used as discriminative stimuli (DS) for a choice between response alternatives. These studies are important for identifying commonalities in mechanisms between cannabinoids and cannabimimetics, investigating factors that contribute to cannabis dependence, and assessing the therapeutic potential of newly synthesized cannabinoids (for previous reviews see Balster and Ford 1978; Järbe et al. 19896; Krimmer and Barry 1977; Weissman 1978). As outlined below, these compounds are easily discriminated and their effects are pharmacologically specific.*

*A possible exception to pharmacological specificity of the tetrahydrocannabinol (THC) cue is the observation that THC-discriminating rats at feast partly generalize the THC-appropriate response to the stimulus effects of the benzodiazepine diazepem (Browne and Weissman 1981; Mokler et al. 1986). However, for THC-diicriminating pigeons and gerbils, very little generalization of the THC response to benzodiazepines, such as diazepam and Ro 11-3128, has been found (Järbe and Hiltunen 1988; Järbe et al. 1988*a*). Nevertheless, it has been reported that THC shows some affinity for benzodiazepine receptor sites (Sethi et al. 1986; Sung and Jacubovic 1987) thus indicating a possible neurochemical mechanism for THC response generalization to benzodiazepine administration.

Two central concepts in DDL are discrimination and generalization. Discrimination refers to the ability of an organism to distinguish between at least two stimulus values that differ either qualitatively or quantitatively. The training methods that are used to establish a drug discrimination have been substantially reviewed elsewhere (Colpaert 1987; Järbe 1987; Järbe and Swedberg 1982; Overton 1987; Overton et al. 1986). The most commonly used procedure uses an operant task and a fixed ratio schedule of reinforcement (e.g., Colpaert 1987; Lal and Emmett-Oglesby 1983; Mathis and Emmett-Oglesby 1990; Spealman 1985; Woolverton et al. 1987). Once discrimination of a cannabinoid has been established, generalization tests are conducted to examine cannabimimetic effects.

Generalization reflects the inverse of discrimination, that is, it is the ability of an organism to perceive other stimulus values as similar to the training stimulus (e.g. Järbe 1986,1989). Thus, generalization of a cannabinoid discrimination to the stimulus or stimulus complex produced by other drugs is used to identify similarities or differences between the cannabimimetic effects of the training drug and of the test compound.

Detailed descriptions of the generalization test procedures used in our laboratory may be found in Järbe et al. (1981). Although discrimination of a cannabis stimulus is readily attained, the standard testing procedures that are used in DDL are untenable for cannabinoid research. For example, if the potency and time course of a test compound are unknown, the standard DDL procedure requires that separate tests be conducted at various times after injections of each drug dose. Thus, many tests must be conducted at several time intervals and with several drug doses. This procedure then requires large amounts of the test compound to complete these preliminary tests. For cannabinoids, large amounts of test compound are rarely available.

A procedure developed in our laboratory not only reduces the amount of compound needed to assess the potency and duration of a test compound's effect but also decreases the time needed to conduct these tests. This procedure uses a repeated test method in which both onset and duration of effect can be assessed after a single injection of the drug (Hiltunen and Järbe 1986*b*; Järbe et al. 1981, 1986). Comparisons of the data obtained using this procedure and the conventional procedure have shown that repeated testing is reliable for assessing not only cannabinoids and cannabimimetics (Hiltunen and Järbe 1986*b*; Järbe et al. 1981), but also other compounds (Haug and Götestam 1982; Holtzman 1979; Woods et al. 1981). This method has been

particularly useful for assessing the structure-activity relationship (SAR) of newly synthesized compounds with potential cannabimimetic effects.

The initial investigations of cannabinoids were limited to naturally occurring compounds, such as marijuana and hashish smoke or derived tinctures. However, once the active component or components of cannabinoids were identified and the chemical structures elucidated (Mechoulam and Gaoni 1965) synthetic compounds were available for DDL research. Thus, an interdisciplinary approach using organic chemistry and choice behavior of laboratory animals was developed to determine the chemical requirements for inducing cannabis intoxication. One outcome of these experiments demonstrated that the cannabimimetic discriminative stimulus (DS) (Weissman 1981) and the analgesic, antiemetic, and anticonvulsant activity of cannabinoids are dissociated (Mechoulam and Feigenbaum 1987).

Investigations of the DS properties of cannabinoids have focused on the tetrahydrocannabinols, including the naturally occurring compounds and their metabolites, and synthetic derivatives. As discussed below, cannabis intoxication is primarily induced by, but not limited to, the direct action of delta-9-THC.

DELTA-9-THC AS A DISCRIMINATIVE STIMULUS

For humans, the subjective, psychoactive effects of hashish and marijuana result primarily from the activity of THC. The most psychoactive of these compounds are delta-9-THC and delta-8-THC. However, delta-8-THC is found only in limited quantities in some plant materials (Bhargava 1978; Mechoulam and Edery 1973) and is less potent than the delta-9-THC isomer (Järbe and Henriksson 1974; Järbe et al. 1976,1977). The effect induced by delta-9-THC in animals is apparently directly comparable to the effect of hashish smoke.

For example, rats (Järbe and Henriksson 1974; Järbe et al. 1976) and gerbils (Järbe et al. 1975) perceive the effects induced by hashish inhalation as similar to the effects induced by IP administered delta-9-THC. When these animals were trained to discriminate delta-9-THC and were then exposed to hashish smoke, they performed the THC-appropriate response. Similarly, when rats were trained to discriminate between active smoke inhalation (hashish smoke) and placebo (chervil smoke), they performed the hashish-appropriate response after IP injections of THC. This effect was stronger for the delta-9-THC isomer than for the delta-8-THC isomer (Järbe and Henriksson 1974). In addition, other

effects of delta-9-THC (e.g., vocalization and depression of water intake) are similar to the effects of hashish smoke exposure (Henriksson and Järbe 1971; Johansson et al. 1975). Thus, most DDL investigations of cannabinoid activity have focused on training animals to discriminate the delta-9-THC stimulus.

When animals are trained to detect the presence or absence of a DS for THC, speed of acquisition is a function of training dose and species of subject. Because pigeons tend to be more sensitive than rats to the stimulus effects of THC, many investigations of THC use pigeons as subjects. Acquisition of this discrimination is fairly rapid and requires approximately 20-25 training trials with each stimulus condition once initial shaping of the operant response has been attained. Tests for stimulus generalization of the THC we result in a characteristic dose-effect relationship. Generalization from the delta-9-THC training stimulus to different doses of the training drug is illustrated in figure 1. From the figure it can be seen that the animals emit progressively fewer drug responses when tested with lowered amounts of the training drug. In addition, similar to generalization tests for exteroceptive stimulus intensity (DeWitte 1978; Heinemann and Chase 1975; Mackintosh 1974), for doses higher than the training dose there is no change in the curvature of the gradients (see also Browne and Weissman 1981). The data in this figure, obtained from pigeons trained with 1 mg/kg and rats trained with 3 mg/kg, demonstrate the good stimulus control acquired with the delta-9-THC stimulus. The correlation coefficients *(r)* and ED_{50} values for the generalization gradients were estimated by logarithmic regression analysis-for pigeons, $r = 0.935$ ($ED_{50} = 0.43$ mg/kg); for rats, $r = 0.929$ ($ED_{50} = 1.17$ mg/kg).

Similar to the ED_{50}s obtained with other drugs used as discriminative stimuli, the magnitude of the ED_{50} for delta-9-THC is affected by the training dose. This effect of training dose within one species is illustrated in figure 2. Pigeons were trained to discriminate between delta-9-THC (0.56 or 1 mg/kg) and vehicle, then tested for generalization to different doses of the training drug. The obtained ED_{50} value for the high-dose training condition was approximately three times higher than for the low-dose condition. In addition, the magnitude of an ED_{50} for delta-9-THC is affected by the route of and time since drug administration.

For example, when rats are trained to discriminate between IP injected delta-9-THC (2 mg/kg) and no drug, tests conducted with IV administration of lower doses of THC elicit more THC- appropriate responding than similar tests using IP administration. Conversely, tests conducted with PO administration of low THC doses elicit less THC-appropriate responding (Barry and Krimmer

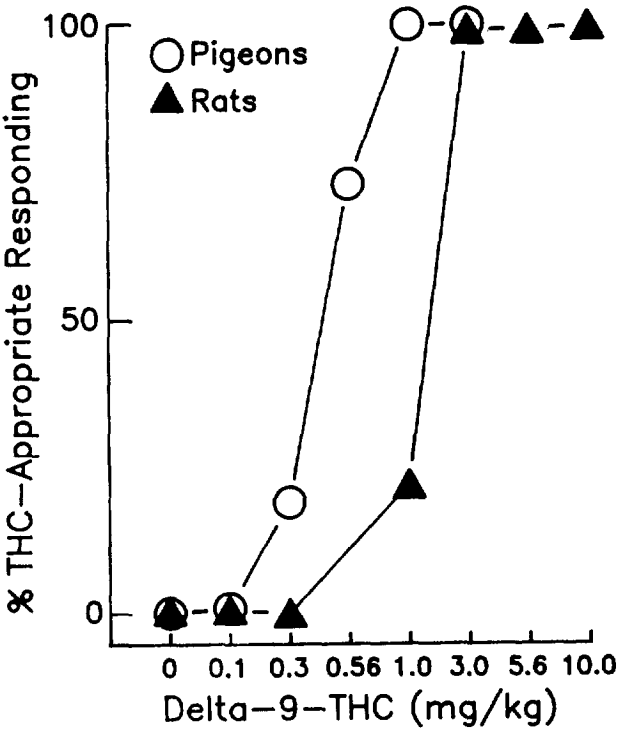

FIGURE 1. *Dose generalization gradients of delta-9-THC for pigeons and rats trained to discriminate between delta-9-THC and vehicle. Pigeons were trained with 1 mg/kg, injected IM 90 min prior to session onset. Rats were trained with 3 mg/kg, injected IP 30 min prior to sesstion onset. N = 8 at all points. (Adapted from Järbe and McMillan 1980.)*

1976). The time course for the generalization of the delta-9-THC DS is illustrated in figure 3. Pigeons were trained to discriminate between the presence and absence of 0.56 mg/kg of delta-9-THC, 90 min after IM administration of THC or vehicle. By means of a repeated test procedure, a generalization curve for doses of THC was determined at 30, 90, 270, and 540 min after drug administration. The peak of generalization to delta-9-THC generally occurred at 90 min after THC administration. These data are also similar to data obtained using a single test procedure (Hiltunen and Järbe 1986*b*).

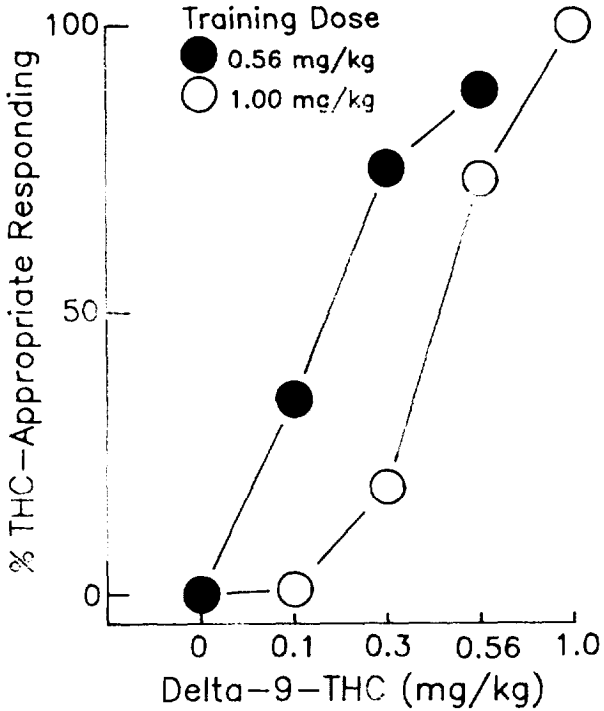

FIGURE 2. *Dose generalization gradients of delta-9-THC for pigeons trained to discriminate between delta-9-THC (0.56 mg/kg, N = 7; 1 mg/kg, N = 8) and vehicle. Training and test sessions were conducted 90 min after IM injection. Test sessions used a repeated test procedure (see Järbe et al. 1981; Hiltunen and Järbe 1986b). Data represent the average of at least two determinations for each animal for the 0.56 mg/kg training condition and the average of one determination for the 1.0 mg/kg training condition.*

The time of peak generalization of THC does not vary as a function of training dose. Pigeons were trained to discriminate delta-9-THC (0.28 mg/kg or 0.56 mg/kg) and vehicle, then tested for generalization to the drug (full or half training dose) at 1.5 and 4.5 hr after administration (figure 4). The time course of generalization did not differ, regardless of the original THC training dose. Similar effects have been found with rats (Järbe et al. 1976) and gerbils (Järbe et al. 1975).

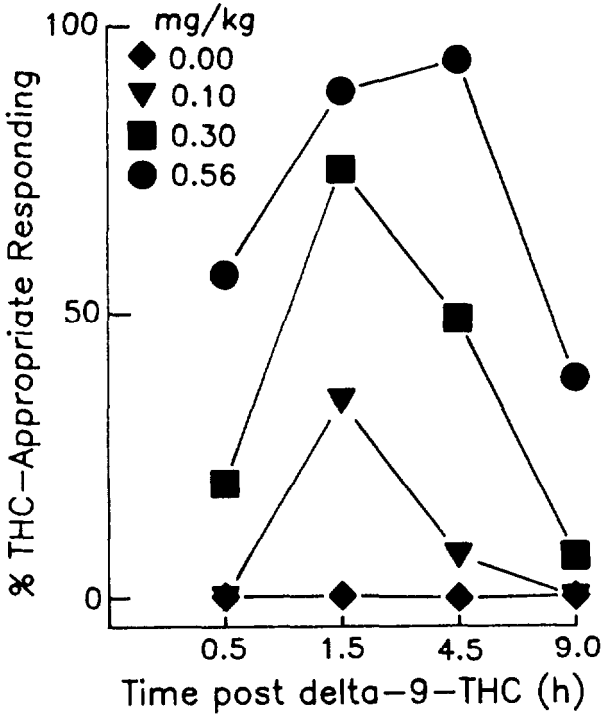

FIGURE 3. *Time course for dose generalization of delta-9-THC for pigeons trained to discriminate between the presence and absence of 0.58 mg/kg of delta-9-THC. Training sessions were conducted 90 min after IM injections. Test sessions used a repeated test procedure (see Järbe et al. 1981; Hiltunen and Järbe 1986*b), *and data represent the average of at least two determinations. Data for the 90-min test interval are the same as those shown for the lower training dose in figure 2. N = 7 at all points.*

In addition, retention of the THC discrimination is robust and tolerance to the stimulus effects of delta-9-THC has not been reported, given the commonly used DDL training and testing procedures. However, tolerance to high or noncontingent doses of this compound does affect performance. For example, when animals are trained to discriminate delta-9-THC from no drug and then administered high doses of this drug, tests conducted 24 to 48 hr afterward result in an attenuation of the delta-9-THC we (Semjonow and Binder 1985).

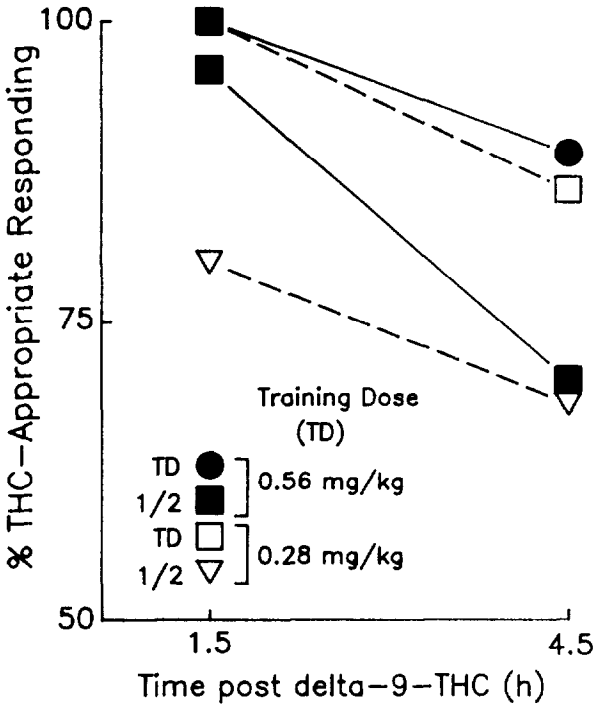

FIGURE 4. *Time course for dose generalization of delta-9-THC for pigeons trained to discriminate the presence or absence of low or high doses of delta-9-THC. Subjects were first trained to discriminate 0.28 mg/kg (low), then tested with 0.28 mg/kg training dose (TD) and 0.14 mgIkg (1/2 TD). Then subjects were retrained with 0.56 mg/kg and retested with 0.56 mg/kg (TD) and 0.28 mg/kg (1/2 TD). Training sessions were conducted 90 min after IM injections. Test sessions were conducted using a repeated test procedure (Järbe et al. 1981; Hiltunen and Järbe 1986b), and data represent the average of one determination for each animal. N = 5 at all points.*

Some attenuation of the THC stimulus after chronic, noncontingent administration of THC has also been observed (Hirschhorn and Rosecrans 1974; Järbe and Henriksson 1973). Nevertheless, because tolerance to the DS effects of THC under DDL procedures that do not administer noncontingent or high doses of delta-9-THC has not been reported, it is unlikely that tolerance to

discrimination of the THC stimulus disrupts performance during generalization testing. In addition, because the parameters controlling the magnitude of generalization to this stimulus are well established, the DDL of delta-9-THC has provided a useful model for detecting the cannabimimetic activity of other naturally occurring cannabinoids.

CANNABIMIMETIC ACTIVITY OF CANNABIDIOL (CBD) AND CANNABINOL (CBN)

A variety of other substances with potential psychoactive properties have been detected in cannabis preparations, including CBD and CBN (Kettenes-Van den Bosch et al. 1980; for an overview of these cannabinoids, see Sofia 1978). Although the cannabinoid activity of CBD may be dissociated from its cannabimimetic activity, CBN may share at least some of the cannabimimetic effects of THC.

Investigations with CBD have shown that this compound has other cannabinoid properties, such as effects on sleeping time (Monti 1977), anticonvulsive activity (Consroe et al. 1981) and operant rate-depressant properties (Hiltunen et al. 1989). However, generalization of CBD to the subjective, DS effects of THC has consistently not been found (e.g., see Hiltunen and Järbe 19866). Thus, although this compound apparently does contribute to other effects of cannabis preparations, it lacks cannabimimetic properties. In addition, the CBDs monomethyl ether and cannabichromene are not cannabimimetic (Järbe et al. 1986; Järbe, unpublished data 1982).

On the other hand, the cannabimimetic activity of CBN is more certain. For studies with animals, most authors report that the CBN stimulus is generalized from the THC discriminative stimulus (Bueno et al. 1976; Järbe and Hiltunen 1987; Järbe et al. 1977; Weissman, 1978). However, some authors report that CBN induces THC-like effects for humans (Perez-Reyes et al. 1973); others report that it does not (e.g., Hollister and Gillespie 1975). Thus, these investigations of the THC-like effects of CBD and CBN indicate that only CBN has cannabimimetic activity. However, because cannabis preparations contain all three cannabinoids (i.e., THC, CBN, and CBD), these compounds may have different effects when administered in combination. For example, administration of THC has been reported to induce anxiety in humans, but coadministration of CBD with THC reduces that anxiety (Zuardi et al. 1982).

The results obtained from studies that assessed the interaction of the stimulus effects of delta-9-THC, CBN, and CBD are summarized in tables 1 and 2. These data indicate that THC does not appear to be solely responsible for the cannabimimetic effects of cannabis preparations. The main interest of studies on cannabinoid combinations has focused on THC administered with one of the other compounds. The results of these studies indicate that the stimulus effects of THC are generally enhanced or prolonged by coadministration of either CBD or CBN. For example, when THC was given in combination with CBD, the DS effects of THC were prolonged (Hiltunen and Järbe 1986*b*. However, this interaction effect may differ among species; for example, for pigeons tested with CBD and THC, no prolongation of THC-appropriate responding was obtained (Hiltunen and Järbe 1986*b*). On the other hand, for studies in which THC was coadministered with CBN to rats, discrimination of the THC stimulus was enhanced but not prolonged (Järbe and Hiltunen 1967). Again, species differences are apparent with these compounds; for pigeons, CBN only slightly increased discrimination of the THC stimulus (Järbe and Hiltunen 1967).

Relatively fewer studies have focused on the combined effects of CBN and CBD (Hiltunen and Järbe 1986*a*; Hiltunen et al. 1988,1989). The data obtained so far indicates that CBD may reduce the stimulus effects of CBN. Some studies have suggested that when animals are trained to discriminate THC, this discrimination is generalized to the CBN stimulus (Bueno et al. 1976; Järbe and Hiltunen, 1987; Järbe et al. 1977; Weissman 1978), but not to the CBD stimulus (Henriksson et al. 1975; Hiltunen and Järbe, 1986*a*, 1986*b*; Järbe et al. 1977). When THC-discriminating rats were administered CBN and CBD in combination, this generalization of THC to CBN was attenuated (Hiltunen and Järbe 1986*a*). Thus, CBD may antagonize or modulate the THC-like, psychoactive effect of CBN. However, cannabis intoxication is not limited to the direct action of these compounds. As discussed below, cannabimimetic effects may also result partly from some metabolites of THC.

CANNABINOID METABOLITES

The metabolism of THC is complex and varies among species (Mechoulam et al. 1976). Metabolites that have been isolated and examined with THC-trained animals include the 11-hydroxy metabolites (11-OH-THCs). Collectively, the data indicate that 11-OH-THCs are major, psychotropic metabolites exerting THC-like activity of biological significance. Investigations with animals trained to discriminate between THC and the no-drug state indicate that the 11-OH THCs are similar to the delta-9-THC stimulus. The cannabimimetic activity of

TABLE 1. *Generalization*[a] *of delta-9-THC (3 mg/kg) to delta-9-THC administered in combination with cannabinol (CBN) or cannabidiol (CBD)*

Drug	Combination (mg/kg)			Time Post Administration (hr)			
				0.5	1.5	4.5	6.5
Delta-9-THC	0.0	CBD	0.0	0	0	0	0
			30.0	0	0	2	0
	0.3		0.0	27	8	0	0
			30.0	0	22	0	0
	1.0		0.0	66	41	6	0
			30.0	63	83	69	46
Delta-9-THC	0.0	CBN	0.0	0	0	0	0
			3.0	22	9	0	0
	0.1		0.0	1	0	0	0
			3.0	32	25	0	0
	0.3		0.0	28	8	0	0
			3.0	63	53	5	0
	1.0		0.0	67	41	9	1
			3.0	89	73	10	0

[a]Percentage of responding to the THC-appropriate position for 8-12 rats at each injection test interval.

SOURCES: Hiltunen and Järbe 1986*b*; Järbe and Hiltunen 1987.

11-OH-THCs (11-OH-delta-9-THC and 11-OH-delta-8-THC) has been investigated using rats (Ford et al. 1984; Järbe and McMillan 1980; Weissman 1978), and pigeons (Järbe and McMillan 1980). Both of these species generalized from the delta-9-THC stimulus to the test compounds in a dose-related manner. However, 11-OH-delta-9-THC was more potent than 11-OH-delta-8-THC. In turn, 11-OH-delta-8-THC was at least as potent as delta-9-THC.

TABLE 2. *Generalization of delta-9-THC (3 mg/kg) to combinations of cannabinol (CBN) and cannabidiol (CBD)*

Drug Combination (mg/kg)			Mean Time Post Administration[a] (hr)	
			1.0	5.5
CBN 0.0	CBD	0.0	0.0	0.0
		10.0	0.0	0.0
		30.0	0.0	0.0
10.0		0.0	31.0	22.0
		10.0	25.0	11.0
		30.0	24.0	5.0
17.0		0.0	75.0	51.0
		10.0	39.0	30.0
		30.0	30.0	12.0

[a]Average across two lime blocks (0.5 and 1.5 hr; 4.5 and 6.5 hr).

SOURCE: Hiltunen and Järbe 1986*a*

Other THC metabolites that have been studied for their cannabimimetic effect are 8-alpha-OH-delta-9-THC, 8-beta-OH-delta-9-THC, 8-alpha, 11 di-OH-delta-9-THC, and 8-beta, 11 di-OH- delta-9-THC (Ford et at. 1984; Järbe and McMillan 1980). Only the 8-beta, 11 di-OH-delta-9-THC produced complete generalization for the delta-9-THC stimulus by pigeons. The metabolite 8-beta-OH-delta-9-THC did produce partial generalization for the MC stimulus; however, this effect may reflect species differences and may be limited to pigeons (Järbe and McMillan 1980). Other investigations using rats and this compound did not find generalization for the THC stimulus (Ford et al. 1984). However, because lower doses of the test compound were used for the tests with rats, and pigeons tend to be more sensitive than rats to the the THC cue, further tests using higher doses of this compound with rats must be conducted before a conclusion about species differences can be drawn. Thus, apart from the 11-OH-THCs, the remaining metabolites may possess MC-like action, but fairly high doses are needed to produce a cannabimimetic effect (see ED_{50} values, estimated according to a logarithmic regression analysis, presented in table 3). Thus, these data suggest that the contribution of these metabolites to the psychoactive properties of cannabis in natural settings is very minor. However,

TABLE 3. *ED_{50} Values for generalization of drug-appropriate responding to the training stimulus and THC metabolites by animals trained to discriminate delta-9-THC*

THC	Species	N	The[a]	ED_{50}	(r)[b]
Delta-9	Rat[C]	8	0.5	1.15	*(0.93)*
			1.0	1.07	*(0.98)*
	Pigeon[d]	6	1.5	0.43	*(0.94)*
11-OH-delta-9	Rat	7-8	0.5	0.65	(0.88)
	Pigeon	8	1.5	0.10	(0.99)
11-OH-delta-8	Rat	8	0.5	1.12	(0.97)
	Pigeon	8	1.5	0.38	(0.97)
8beta,11-di-OH-delta-9	Pigeon	4-5	1.5	19.00	(0.99)

[a] Hr from injection until testing.
[b] The ED_{50} (mg/kg) determinations assessed from the correlation coefficient (*r*) by logarithmic regression analysis of gradients for percentage of drug-appropriate (percent RDP) gradients.
[c] Rats were trained to discriminate 3 mg/kg administered IP 30 min prior to session onset.
[d] Pigeons were trained to discriminate 1 mg/kg administered IM 90 min prior to session onset.

SOURCE: Järbe and McMillan 1980.

examinations of the chemical structure of cannabinoids have helped to elucidate the compounds contributing to cannabis intoxication.

SYNTHETIC CANNABINOIDS AND CANNABIMIMETICS

The SAR between chemical structure and drug action reveals the structural requirements for the stereoselectivity or stereospecificity of a given drug effect or action. Stereoselectivity is evidenced when a potency difference for a given effect is obtained between isomers, whereas stereospecificity refers to a complete absence of a drug effect for a particular isomer. A major purpose of the SAR investigations with synthetic cannabinoids and cannabimimetics is to eliminate the enantiomer that induces the undesirable, psychoactive properties of these compounds (stereospecificiiy for a THC-like effect) and retain the structures that induce medically useful effects. These studies have shown that the THC-like properties of these compounds are highly dependent on particular

structural configurations. (See table 4 for compounds tested in our laboratory; chemical structures are indicated in the original reports cited below.)

The relationship between the structure of cannabinoids and their stimulus effects has been studied using delta-9-(11)-THC and (3”S)-3”-OH-delta-9-THC. The results of these studies indicate that the stimulus effects of delta-9-(11)-THC are less potent than those of delta-9-THC (Semjonow and Binder 1985; see also Franke et al. 1985). However, the effect of this compound on the development of rapid tolerance to the stimulus effects of delta-9-THC is similar to the effect of the parent compound. For animals that are pretreated with high doses of either delta-9-THC or delta-9-(11)-THC, then tested for discrimination of the THC cue 24 to 48 hr after these high-dose administrations, discrimination of the THC stimulus is attenuated. Thus, though delta-9-(11)-THC may be less potent than the parent compound, it apparently does have some similar psychoactive effects.

On the other hand, (3"S)-3”-OH-delta-9-THC may be more active than delta-9-THC and the R epimer of this compound, and it may be an important stereoselective determinant of the THC cue. For example, for rats trained to discriminate delta-9-THC, (3"S)-3"-OH-delta-9-THC was approximately 5 times more potent than the training compound and approximately 7 times more potent than the R epimer (Martin et al. 1984). This difference in the effect of epimers demonstrates the stereoselectivity of cannabimimetic effects. Selectivity of the relationship between R and S epimers and cannabimimetic activity is further demonstrated by studies with delta-10-THC structures.

Recent synthesis of stereoisomers for delta-10, 10a-THC and delta-10a,6a-THC (Srebnik et al. 1984) has permitted further investigations of the relationship of R and S epimers on cannabimimetic activity. Investigations of the SAR of delta-10a,6a-THC have focused on the 9S and 9R enantiomers. In general, these studies indicate that, for this structure, although the 9S enantiomer is less potent than delta-9-THC, the 9R enantiomer may not be active. The psychoactive properties of the delta-10a,6a enantiomers have been tested in humans (Hollister et al. 1987) and pigeons (Järbe et al. 1988*b*). For humans, the 9S enantiomer had psychoactive properties that were qualitatively similar to the effects of delta-9-THC, but the effects of this enantiomer were quantitatively less potent (i.e., 1:3-6). For pigeons, discrimination of the delta-9-THC stimulus was generalized to this enantiomer. When the psychoactive properties of the 9R enantiomer were tested with humans, they were found to be not active (Hollister et al. 1987). However, for the tests with humans, this compound could

TABLE 4. *Generalization[a] of synthetic conqmmds[b] to THC-appropriate responding*

Compound	Species	N	Dose range[c]	ED_{50}	(*r*)[d]
Delta-8-THC					
(+)-Delta-8-THC	Rat[e]	4	5.6-10	none	(none)
(-)-Delta-8-THC-DMH	Rat	14-16	0.1-0.56	0.21	(0.94)
	Pigeon[1]	6-7	0.0175-0.175	0.05	(0.95)
(-)-11-OH-delta-8-THC-DMH	Rat	11	0.003-0.03	0.01	(0.99)
	Pigeon	5-7	0.0001-0.0056	0.002	(0.99)
(+)-11-OH-delta-8-THC-DMH	Rat	4-9	3-10	none	(none)
	Pigeon	4	3-10	none	(none)
Benzofuran, 7	Rat	9	up to 10	none	(none)
	Pigeon	5	1-5.6	3.57	(0.78)
Benzofuran, 8	Rat	9	0.3-3.0	0.72	(0.98)
	Pigeon	5	0.1-0.3	0.17	(0.99)
Delta-10-THC					
(9S,6aR)-delta-10,10a-THC	Pigeon	2-6	1-17.5	none	(none)
(9R,6aR)-delta-10,10a-THC	Pigeon	3-4	3-17.5	4.78	(0.92)
(9S)-delta-10a,6a-THC	Pigeon	3-4	1-10	6.68	(0.81)
(9R)-delta-10a,6a-THC	Pigeon	4	3-30	11.90	0.98)
Hexahydrocannabinols					
(–)-11-OH-HHC(equatorial)	Rat	14	0.1-1	0.24	(0.95)
	Pigeon	7	0.01-0.1	0.02	(0.87)
(-)-11-OH-HHC(axial)	Rat	9	1-3	1.58	(0.98)
	Pigeon	7	1 3	1.72	(0.97)

[a]Percentage responding to the drug-appropriate position (percent RDP) at postadministration time interval at which compound disclosed highest potency.
[b]Chemical structures are found in original reports cited in text.
[c]In mg/kg.
[d]ED_{50} (mg/kg) determinations assessed from correlation coefficient (*r*) by logarithmic regression analysis of gradients.
[e]ats were trained to discriminate 3 mg/kg administered IP 30 min prior to session onset.
[f]Pigeons were trained to discriminate 0.56 mg/kg administered IM 90 min prior to session onset.

not be tested in high doses. In contrast, for pigeons, this 9R enantiomer did generalize for the THC stimulus, but it was 2 to 3 times less potent. In addition, these animal tests used the acetate form of this compound rather than the parent phenol that was tested in humans. Thus, further tests with this enantiomer must be conducted to determine its efficacy as a cannabimimetic.

Examinations of the structural activity of delta-10,10a-THC has focused on the 9R, 6aR and 9S, 6aR epimers in their acetate form. The 9R, 6aR epimer disclosed THC-like activity (ED_{50}= 4.78 mg/kg), whereas the 9S, 6aR epimer did not, even at the highest dose tested (17.5 mg/kg) with pigeons (Järbe et al. 1988*b*). Thus, these SAR investigations of delta-10-THC compounds suggest a stereoselectivity for induction of cannabimimetic action. The effect of S and R epimers of delta-10, 10a-THC on cannabimimetic activity parallels findings obtained with the epimers of 11-OH-hexahydrocannabinol (see below). Other SAR studies of THC effects have focused on the (+)- and (-)-enantiomers, particularly with regard to delta-8-THC compounds.

The stereoselectivity of delta-8-THC may be a function of the (-)- and (+)-enantiomers. For example, (+)-delta+THC is considerably less potent (Järbe et al. 1981) than (-)-delta&THC (Järbe and Henriksson 1974; Järbe et al. 1976) in eliciting the delta-9-THC discriminative response. In addition, tests with THC-trained rats and pigeons (Järbe et al. 1981, 1989*a*; Järbe and Mechoulam, unpublished data 1983) have shown that the dimethyheptyl (DMH) homolog of delta-8-THC, i.e., delta-8-THC-DMH in its levorotation [(-)delta-8-THC-DMH] is a potent cannabimimetic with a slow onset and long duration.

In order to examine a possible dissociation between the effects of delta-8-THC enantiomers, monohydroxylated dimethyl heptyl homologs of a major metabolite of delta-8-THC (11-OH-delta-8-THC) have been studied [i.e., (-)-11-OH-delta-8-THC-DMH and (+)-11-OH-delta-8-THC-DMH]. These studies also indicated that the (-)-enantiomer may elicit the cannabimimetic effects of delta-8-THC. The compound (-)-11-OH-delta-8-THC-DMH was more potent than naturally occurring delta-9-THC by approximately 87 times in rats (ED_{50} = 0.01 mg/kg) and 73 times in pigeons (ED_{50} = 0.002 mg/kg) (Järbe et al. 1989*a*); Mechoulam et al. 1988). However, the (+)-enantiomer (+)-delta-8-THC was much less potent than the (-)-enantiomer in generalization for the THC cue. It was inactive at doses higher than the ED_{50} value by approximately 1,000 times for rats and 4,500 times for pigeons. Furthermore, similar effects of these enantiomers on cannabinoid activity have been obtained for performance on the ring test with mice and the rotarod neurotoxicity test with rats (Mechoulam and

Feigenbaum 1987; Mechoulam et al. 1988). Recently obtained data (Järbe and Mechoulam unpublished data 1990) also indicate that this stereoisomeric differentiation is important for cannabimimetic activity by naturally occurring compounds. Stereospecificity of the (+)-isomer was obtained with pigeons trained to discriminate between delta-9-THC (0.56 mg/kg) and vehicle. Tests with the (+)- and (-)-isomers of the major psychoactive metabolite 11 -OH- delta-8-THC indicated that the (+)-isomer did not induce THC-like responding after administration of doses up to 10 mg/kg. Studies with hexahydrocannabinols (HHCs) have further demonstrated the structural requirements for cannabimimetic effects, including the importance of the position of the methyl group on cannabimimetic activity.

Tests with HHCs indicate that conversion of THC to 11-OH-THC is not a necessary prerequisite to achieve a THC effect (Browne and Weissman 1981; Ford et al. 1984; Weissman 1978) and that HHCs may be highly stereoselective and stereospecific. For example, examinations of the relative potencies of the HHCs levonantradol and dextronantradol have demonstrated that levonantradol was considerably more potent than dextronantradol for eliciting the THC-appropriate response. In addition, the dextro form did not substitute for delta-9-THC at the dose of 3.2 mg/kg, whereas the levo form disclosed an ED_{50} value of 0.02 mg/kg, making it one of the more potent cannabimimetics examined so far with drug discrimination of delta-9-THC (Browne and Weissman 1981). Furthermore, two epimers of 11-OH-HHC elicited cannabimimetic activity in both rats and pigeons (Järbe et al. 1986). The difference in the potency of these isomers was determined by the position of the methyl group in relation to the plane of the cyclohexane ring. Generalization to the THC stimulus was more potent for the isomer in which the methyl group is equatorial (i.e., lies approximately in the plane of the cyclohexane ring) than for the isomer in which the methyl group was axial (i.e., protrudes from the plane of the cycbhexane ring). However, species differences in response to these compounds make elucidation of their SAR less clear. For example, the difference in the relationship between the structure of isomers and elicited cannabimimetic activity is considerably less for rats than for pigeons (for chemical structures, see Järbe et al. 1986).

Although most of the studies on the SAR of cannabinoids support a contention that a dihydrobenzopyran structure is a necessary component for cannabimimetic activity (Mechoulam and Edery 1973; Mechoulam and Feigenbaum 1987), some studies suggest that this structure is not a central requirement for THC- like activity. For example, an examination of a derivative

of 3-phenylcyclohexanol (CP 47497), which lacks this structure, indicates that this compound has potent cannabimimetic activity (Weissman et al. 1982). In addition, other tests conducted with rats and pigeons trained to discriminate delta-9-THC indicated that there was profound generalization to a DMH homolog of CP 47497 (Järbe and Mechoulam unpublished data 1990). Other compounds that lack this structure but have cannabimimetic properties are benzofuran cannabinoids. These compounds have a benzofuran, rather than the delta-9-THC benzopyran, moiety and induce marked cannabimimetic activity for both rats and pigeons (Mechoulam et al. 1990). In addition, levonantradol, a hexahydrocannabinol with potent THC-like activity, also deviates from this structural requirement.

It is not yet known why compounds that lack a dihydrobenzopyran structure have cannabimimetic properties. One suggestion might be that generalization of the THC response to the effects of these compounds may result from analgesic actions. CP 47497, levonantradol, and delta-9-THC all have analgesic properties. Thus, if analgesia is part of the stimulus effects of THC, then generalization to these compounds may reflect generalization of THC analgesia. However, the analgesic properties of THC have been shown to be dissociated from its DS effects. Other compounds having a standard cannabinoid configuration and strong analgesic properties (e.g., the dextro form of THC such as (+)-11-OH-delta-8-THC-DMH) do not have cannabimimetic properties (Järbe et al. 1989*a;* Mechoulam et al. 1988). Therefore, the possibility that the cannabimimetic properties of compounds that lack a dihydrobenzopyran structure reflect their analgesic action seems unlikely, and firm confirmation of the chemical structure needed to induce THC intoxication remains to be elucidated.

CONCLUSION

Determining the necessary chemical components for inducing the DS properties of delta-9-THC is important for understanding the factors that contribute not only to cannabis abuse but also to the unwanted side effects of cannabis therapy. By means of DDL procedures to examine naturally occurring cannabinoids, metabolites, and synthetic compounds, the cannabimimetic and medicinal properties of cannabinoids (e.g., analgesia, antiemesis, and anticonvulsive) have been dissociated, and some requirements for inducing THC intoxication have been delineated.

Studies using the DDL procedure with naturally occurring compounds have shown that cannabis intoxication is induced primarily, but not solely, by the direct action of delta-9-THC. The stimulus effects of THC may be modulated by coadministration of other cannabinoids, such as CBD or CBN. In fact, CBN may have direct cannabimimetic properties that are attenuated by coadministration of CBD. In addition, cannabimimetic activity can be induced by some THC metabolites, specifically the 11-OH- and 8-beta, 11 di-OH-delta-9-THC metabolites. However, in order to determine the chemical requirements for inducing cannabis intoxication, studies of the SAR of cannabinoids using organic chemistry and DDL are necessary.

Examinations of cannabinoid SAR have shown that the structures determining THC intoxication vary among compounds and are highly stereoselective, perhaps even stereospecific. Some of the relevant structural features that have been identified are epimerii and enantiomeric preferences (e.g., R and S epimers, (+) and (-) enantiomers), and the position of the C-11 substituent on the cyclohexane ring. For example, the (3"S")-3”-OH-delta-9-THC is more potent than the R epimer. For delta-10,10a-THC, the 9R,6aR epimer disclosed THC-like activity, whereas the 9S,6aR epimer did not. in addition, the 9s enantiomer for delta-10a,6a-THC is more potent than the 9R enantiomer.

Stereoselectivity of THC intoxication is also demonstrated with delta-8-THC enantiomers and HHC isomers. For example, generalization of delta-8-THC for the THC cue may be induced by the (-)-enantiomer but not by the (+)-enantiomer. In addition, SAR studies of HHCs have demonstrated that cannabimimetic activity also shows isomeric selectivity. For the compounds examined in both rats and pigeons, the equatorial isomer is more potent than the axial isomer (this SAR is also described above for delta-10,10a-THC). Furthermore, some SAR studies suggest that a dihydrobenzopyran structure is not a necessary requirement for cannabimimetic activity. A derivative of 3-phenylcyclohexanol that lacks this structure (CP 47497), an HHC (levonantradol), and a benzofuran cannabinoid have potent cannabimimetic activii.

Thus, the complexity of the SAR of cannabinoids and cannabimimetics is apparent. However, the utility of DDL and synthesis of new compounds as research tools for understanding this SAR are also apparent. It is only with further use of these research tools that a fin understanding of cannabimimetic activity may be gained.

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Discriminative Stimulus Properties of Nicotine: Mechanisms of Transduction

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INTRODUCTION

Cholinergic drugs, for the most part, are orphans among the drugs studied by behavioral pharmacologists. Until recent evidence was found that Alzheimer's disease might entail a cholinergic deficit, few researchers paid attention to this class of pharmacologic agents. Even the knowledge that more humans maintain nicotine (or cotinine) levels more of the time than any other drug (except perhaps caffeine) has engendered little interest in this area of research. In spite of this general lack of interest in cholinergic pharmacology, however, these drugs do produce many interesting effects that may eventually provide some answers about several disease states involving memory deficits, Parkinson's disease, and even drug dependencies. In addition, these agents might offer some unique approaches to treatment if studied more intensely.

The major goal of this review, however, is to summarize recent experiments concerning how nicotine acts at its respective receptor with a focus of learning more about how this interaction can elicit nicotine's discriminative stimulus (DS) effect. Because of the many recent reviews on nicotine's DS effects (Rosecrans 1989; Stolerman 1987; Stolerman and Reavil 1989), this paper spotlights the current state of the art and attempts to fill in the gaps where appropriate.

ARRANGEMENTS OF CHOLINERGIC NEURONS AND ACETYLCHOLINE RECEPTORS (AChRs)

Our appreciation of the physiological importance of both the autonomic and central nervous systems has relied heavily on evaluating the *in viva* effects of cholinergic drugs such as nicotine. Because drug discrimination (DD) techniques have been used for studying these drugs, it has become increasingly apparent that much is yet to be learned about the function of

central nicotinic-cholinergic receptors (n-AChRs) and muscarinic-cholinergic receptors (m-AChRs). Research in molecular biology has advanced far in the knowledge of how these drugs affect specific AChRs. We may be at a point in our own science where we can draw on this information to evaluate the cellular transduction mechanisms that permit these drugs to exert DS control of behavior.

The most important contribution made by cholinergic *in vivo* and *in vitro* neuroscience has been the knowledge that m-AChRs and n-AChRs have different brain region distributions and are not monolithic in relation to receptor type. Even though acetylcholine (ACh) appears to be the major endogenous ligand (or neurotransmitter) at both the m-AChR and the n-AChR, its function and mechanism of neuronal transduction appear uniquely different at both receptor sites (figure 1). The n-AChR appears linked to a cation channel that serves as its major transductional signal, whereas the m-AChR appears to employ a more traditional second messenger, inositol triphosphate (IP_3) to carry out its function.

From the perspective of evaluating the DS properties of drugs affecting these receptors, we find little overlap. Arecoline, for example, appears to act at only the muscarinic site and is antagonized by atropine but not by mecamylamine, whereas nicotine uniquely acts at the nicotinic site and is antagonized by mecamylamine but not by atropine (Rosecrans 1989; Stolerman 1987). In addition, only those drugs that act at the muscarinic site appear to generalize to increases in brain ACh-via the administration of the acetylcholinesterase (AChE) inhibitor physostigmine (Meltzer and Rosecrans 1988)-prompting us to rethink how nicotine might be interacting with its cholinergic receptor (figure 1).

The neuroanatomical arrangement of the cholinergic system has also been described in great detail. Cholinergic pathways appear to consist of neurons affected by a variety of cholinergic drugs, but for the most pan postsynaptic (or presynaptic) receptors of these cholinergic systems behave as though they were all muscarinic (Jung et al. 1987; 1988*a*). Thus, although these arrangements have been delineated and there seems to be some separation between n-AChRs and m-AChRs (from DD experiments; Rosecrans and Meltzer 1981), there is little evidence to intimate which neuronal pathways innervate n-AChRs or m-AChRs. Furthermore, there is little evidence to support the contention that n-AChRs are in fact innervated by specific cholinergic neurons (figure 2).

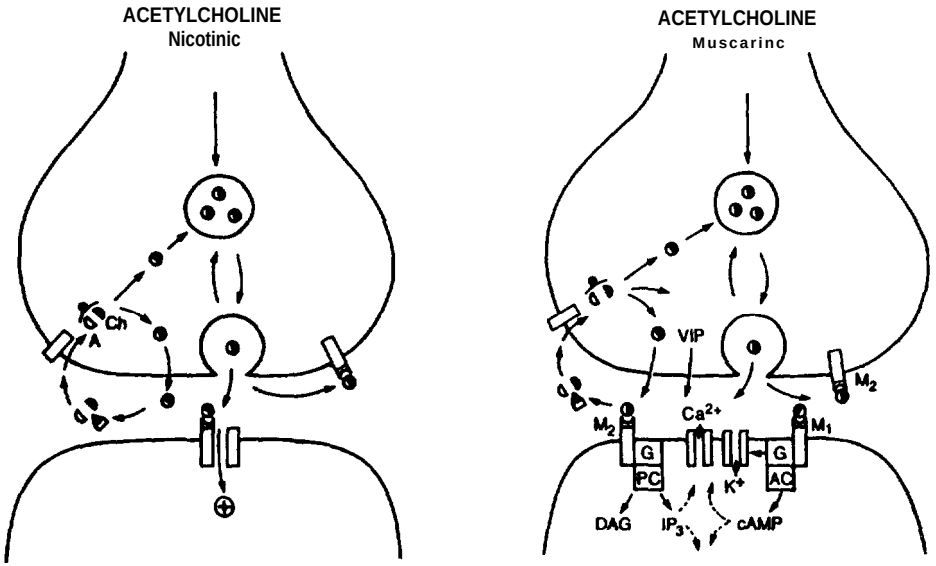

FIGURE 1. *A schematic comparison of nicotinic and muscarinic cholinergic neurons. The n-AChR is viewed as linked to a cation channel; whereas the m-AChR mediates its effects via a second messenger, IP_3 or cAMP. (Redrawn from Shephard 1988, with permission.)*

Thus the question: Is the n-AChR localized at only presynaptic sites of both cholinergic and noncholinergic neuronal systems? This important question has been posed by several investigators. The evidence that many n-AChRs are located on presynaptic dopamine (DA)-containing neurons is rather convincing at this time (Wonnocott et al. 1989). In addition, lwamoto (1989) has also provided a model in which the n-AChR appears to play a pivotal role at select presynaptic cholinergic neurons. In this model nicotine is viewed as interacting with an n-AChR, eliciting the release of ACh onto an m-AChR (figure 3). This model is engaging and reminiscent of the peripheral autonomic nervous system, the ganglia being represented by a presynaptic n-AChR. Thus, many questions remain concerning the arrangement and interrelationships between these two receptor classes.

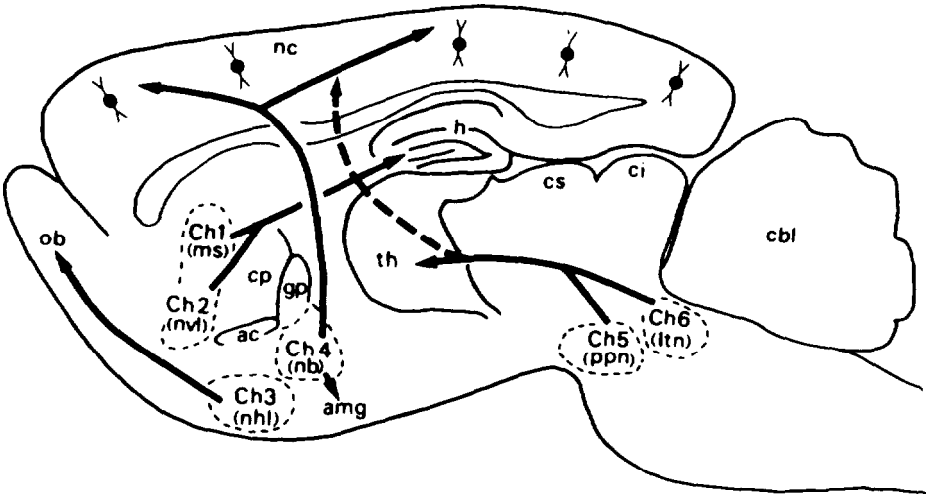

FIGURE 2. *A schematic presentation of select ascending cholinergic pathways. Cell body groups correspond to the Ch subdivisions. Abbreviations: ac=anterior commissure; amg=amygdala; cbl=cerebellum; ci=inferior colliculus; cp=caudata putamen; cs=superior collliculus; gp=slobus pallidus; h=hippocampus; ltn=laterodorsal tegmental nucleus; ms=medial septal nucleus; nb=nucleus basalis; n=neurocortex; nhl=nucleus of the horizontal limb complex; nvl=nucleus of the vertical limb complex; ob=olfactoty bulb; th=thalamus; ppn=pedunculopontine tegmental nucleus. (From Wainer et al. 1984.)*

SPECIFICITY AND SELECTIVITY OF THE NICOTINE-ELICITED DS

The ability of nicotine to exert DS control of behavior is time and dose related, and it appears to correlate well with blood and brain levels of nicotine (and analogs) even though no major brain area site appears to concentrate this drug (Rosecrans 1989; Stolerman 1987). The nicotine DS appears to be elicited at central cholinergic receptors specific to nicotine and located in at least two brain area sites, the hippocampus and reticular formation. In addition, these studies indicate that slight changes in the molecular structure of nicotine can greatly reduce its effectiveness as a DS (figure 4).

Comparing data obtained in squirrel monkeys (-)-nicotine metabolites, (-)-cotinine, and (-)-nornicotine generalized with the nicotine (0.2 or 0.4

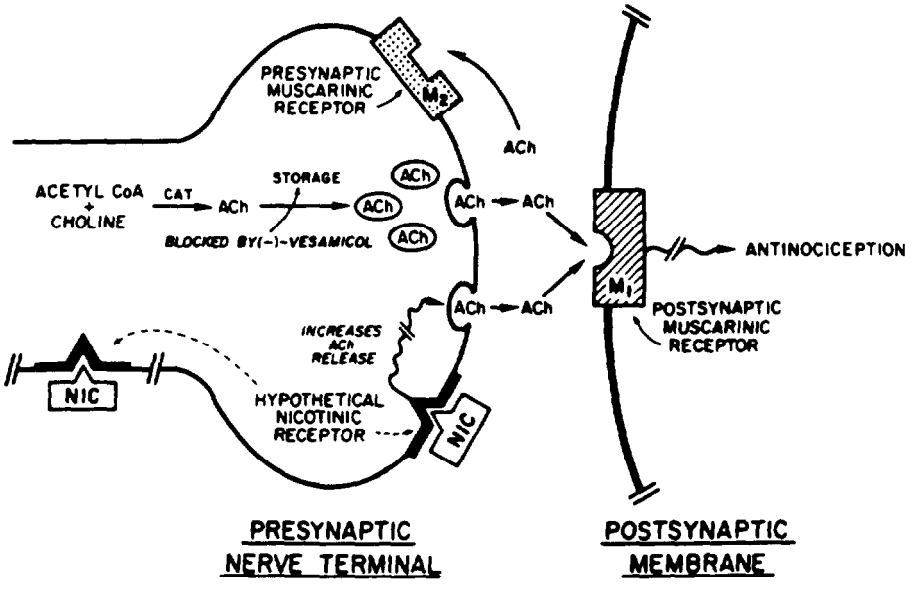

FIGURE 3. *A model of the cholinergic neuron showing the relationship between n-AChRs and m-AChRs. A model of postulated mechanisms and sites of action on nicotine-induced antinociceprion at a cholinergic nerve terminal in the mesoponrine tegmentum. (From lwamoto 1989, with permission.)*

µmole/kg IV) DS but were 29-2,000 times less potent (Takada et al. 1988). In addition, two compounds, (+)-nicotine and 3-pyridylmethylpyrrolidine-optical-positional isomers of nicotine (figure 4)-appear to have a similar pharmacology but are one-tenth as potent (Rosecrans 1989; Stolerman and Reavil 1989). These compounds, besides generalizing with nicotine, can also be antagonized by mecamylamine, indicating they also share a common molecular site of action with (-)-nicotine. Thus, the spectrum of nicotinic activity across a variety of chemical structures, from the vantage point of its DS properties, is quite limited.

Stolerman and coworkers also remind us that dose and schedule of reinforcement are important variables to be considered in nicotine generalization studies. In one such investigation, Stolerman et al. (1984) demonstrated that the nicotine analogs anabasine and cytisine were recognized

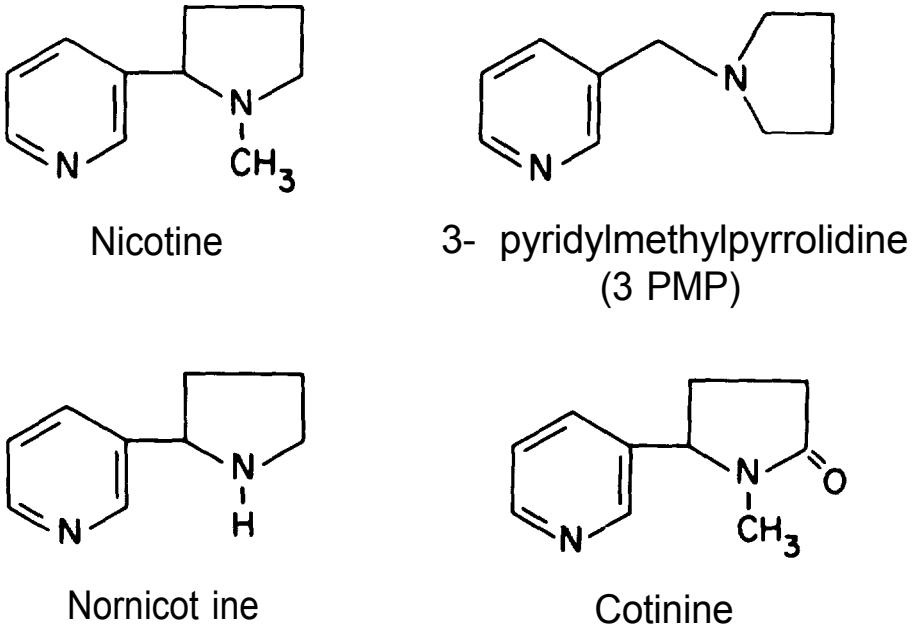

FIGURE 4. *Chemical structures of nicotine analogs or metabolites studied.*

more readily as nicotine if the training dose was reduced to 0.1 mg/kg of nicotine versus saline. More recently, Stolerman (1989) further showed that nicotine-trained rats would generalize to (+)-amphetamine (predominant DA receptor action) when the training dose was reduced from 0.4 to 0.1 mg/kg in rats trained under either a tandem or a fixed ratio schedule. Previous work indicated that nicotine would generalize only partially to (+)-amphetamine in rats trained at 0.4 mg/kg under a VI-15 second schedule of reinforcement (Chance et al. 1977). Besides demonstrating the reliability of evaluating nicotine's effects across schedules of reinforcement, this study also demonstrated the need to consider training dose when evaluating other compounds that have the potential to act at similar sites of action. Thus, this study appears to demonstrate that nicotine, while having a predominant effect at some n-AChRs, may have overlapping effects at DA receptors (Rosecrans 1988) as well. This potential DA interaction has been difficult to study in DS experiments, possibly because the cholinergic versus dopaminergic activity may dominate at higher nicotine training doses. The approach used clearly demonstrates the need to

evaluate drugs at several training doses before judging the singularity of mechanism of drug action.

MECHANISM OF ACTION OF THE NICOTINE DS

A major assumption of research involving nicotine's mechanism or mechanisms of action is that it is capable of eliciting DS control of behavior by activating specific ACh-sensitive cholinergic receptors. That is, nicotine was envisioned to act *as though* ACh were released at some cholinergic receptor, a site also antagonized by mecamylamine (figures 1 and 3). To test this hypothesis, a series of experiments was designed to determine whether the nicotine-elicited DS could be mimicked by elevating brain ACh by inhibiting AChE. Attempts to accomplish this goal, however, were negative in outcome (Meltzer and Rosecrans 1988). This approach, on the other hand, has been quite useful in demonstrating that the DS effects of the m-AChR receptor agonist arecoline are similar in action to ACh at this site.

Rosecrans and coworkers have attempted a variety of approaches to circumvent our failures, including training a group of rats to discriminate subcutaneously (SC) administered physostigmine (0.25 mg/kg) and (-)-hyoscyamine (2 mg/kg SC) as a cocktail (Rosecrans 1989). The rationale was that this drug combination would be pharmacologically equivalent to nicotine. Although theoretically sound, the drug combination did not generalize to nicotine, nor were these investigators able to antagonize the DS elicited with mecamylamine. Similar findings have been observed when physostigmine alone was used as a DS (Jung et al. 1988*b*; Tang and Franklyn 1988). Most m-cholinergic agonists generalized with the physostigmine-elicited DS. Mecamylamine was also incapable of antagonizing the physostigmine-induced DS, even though both scopolamine and atropine were potent antagonists of this stimulus. This result, in addition to the physostigmine DS being unable to generalize to nicotine, again suggests that the DS is primarily muscarinic.

These studies, therefore, suggest that in contrast to our initial cholinergic model (figure 1), nicotine may not be acting at a specific ACh-sensitive n-AChR. On the other hand, it may be that the pharmacological effects measured by our approaches, which are antagonized by mecamylamine, do not involve an n-AChR. As inferred from the research of DiChiara and Imperato (1988) nicotine could be exerting stimulus control of behavior by acting via a mesolimbii dopaminergic pathway common to several other drugs of dependence such as morphine and (+)-amphetamine. These workers, using in

vivo microdialysis techniques, demonstrated that nicotine was able to release brain DA from these areas at readily discriminable doses.

Even though nicotine could ultimately be shown to elicit DS control of behavior via a noncholinergic mechanism (such as acting at presynaptic DA n-AChRs), there is no reason to believe that all of nicotine's effects are the result of a noncholinergic neuronal interaction. Mecamylamine (a drug classified as anticholinergic), for example, appears unable to antagonize chronic nicotine-induced n-AChR up-regulation, suggesting that this antagonist is acting at a different site that is cholinergic (Schwartz and Kellar 1985). This finding is also supported by earlier work demonstrating mecamylamine's inability to compete with either ACh or nicotine at n-ACh binding sites (Schwartz et al. 1982). More importantly, mecamylamine appears to be unable to attenuate the development of tolerance to nicotine but yet readily antagonizes nicotine's acute behavioral disruptive effects in the same paradigm or in the nicotine DS (Rosecrans et al. 1989). Thus, these findings strongly suggest that nicotine is acting at two different receptors, one mecamylamine sensitive and ACh insensitive, and a second n-AChR that is ACh sensitive.

DESENSITIZATION OF THE n-AChR

The preceding discussion leads us to conclude that nicotine may be acting at either of two receptors, but we might also review what we know about the mecamylamine-nicotine interaction. As Stolerman (1987) pointed out, this drug interaction appears to be noncompetitive and may be acting presynaptically or postsynaptically at some cation channel. This concept has much support and also points out that nicotine and mecamylamine do not have to act at the similar sites of action to explain the data thus far collected. Furthermore, such a hypothesis gives much flexibility to the alternative concept that nicotine is acting at an ACh-sensitive receptor; perhaps our approaches to the problem and to mecamylamine have driven us to our current models.

We are at a stage in our quest of nicotine's molecular mechanisms to begin asking how nicotine can alter the receptors to which it binds. The research of several investigators (Marks et al. 1983; Nordberg et al. 1985; Schwartz and Kellar 1985; Wonnocott 1987; Wonnocott et al. 1989) has provided us with much evidence that nicotine may be acting partially by inducing a desensitization of the n-AChRs, especially when administered chronically. This possibility has been manifested by the ability of nicotine to induce an up-regulation of nicotinic receptors following repeated dosing. Furthermore, it

has also been established that one of the major differences between cholinergic receptors is the ability of ACh to desensitize the nicotinic receptor. Ochoa et al. (1989) in an impressive review, describe research that clearly demonstrates how ACh may be acting at the n-AChR receptor (figure 5). The major concept put forward is that ACh, after being released at the synapse, interacts with the n-AChR to form an activated state that promotes cation movement (via a channel) across the postsynaptic membrane. As receptor affinity for ACh increases, the n-AChR moves toward a deactivated (desensitized) state that closes the same cation channel. As these investigators pointed out, this mechanism may be essential to terminating the synaptic transduction effects of ACh and may be an essential mechanism to several pathological states involving learning and memory deficits as well as myasthenia gravis.

In all, a picture emerges that may assist our understanding of how nicotine is eliciting its effects at the n-AChR and, consequently, how it may act to exert stimulus control of behavior. The basic theoretical model presented views nicotine as an agonist that can induce a secondary desensitization (in *in vivo* pharmacological terms, tachyphylaxis or acute tolerance). In this conceptual model, nicotine is envisioned as mimicking ACh directly or indirectly (inducing ACh release presynaptically) at a cholinergic receptor, thus opening a channel and allowing some cation to enter the postsynaptic or presynaptic cell-possibly Ca^{++} (the transductional signal is the entrance of a specific cation). Depending on dose and time parameters, and possibly on the excitability level of the n-AChR, the nicotine levels (or increase in ACh) achieved at a specific hippocampal site or reticular formation site or both will consequently provide the neuronal mechanism (opening of the cation channel) that is important to exerting stimulus control. It is further postulated that, following the initial agonist effect, a rapid desensitization of the receptor will occur (close the channel to further cation entry), reducing the strength of the nicotine-elicited DS (figure 5).

To test this hypothesis, we initiated a series of experiments from which preliminary evidence suggested that nicotine was capable of inducing rapid *in vivo* desensitization-that is, tolerance (figure 6). Rats were initially trained to discriminate nicotine (0.4 mg/kg, SC) from saline when a VI-20 second schedule of reinforcement was used; responding was reinforced during test sessions. Following initial training, individual rats were (1) administered 0.8 mg/kg of nicotine (SC) at time zero in their home cage; (2) administered a second training dose of nicotine 15-180 min after the first nicotine challenge dose; and (3) tested for ability to discriminate nicotine during a 2-min test session (5 min after training dose) in the behavioral environment associated

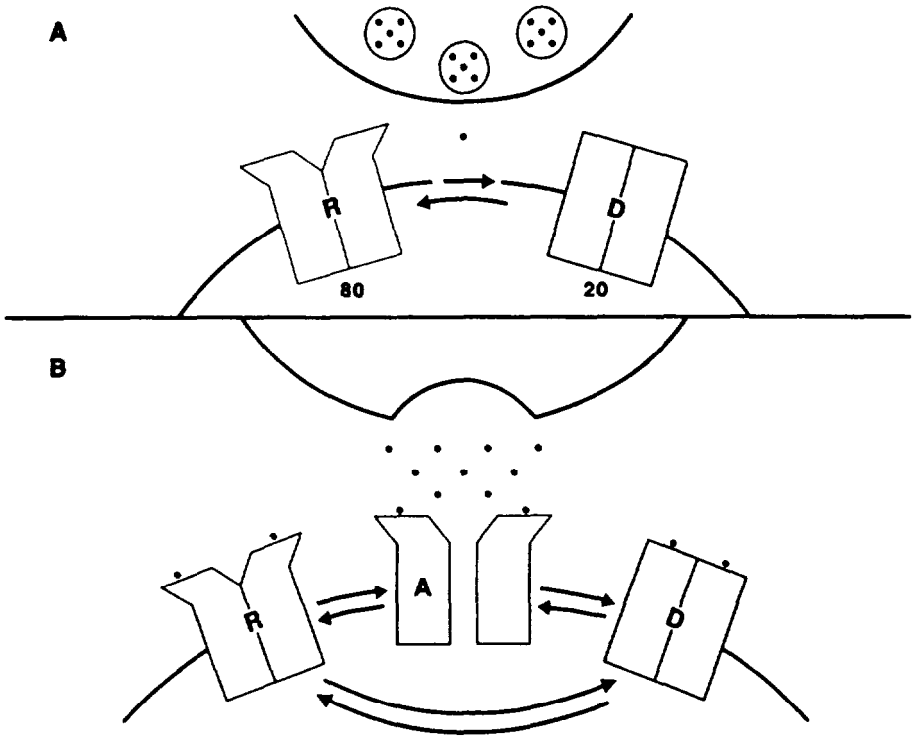

FIGURE 5. *Mechanisms of n-AChR desensitizations. Represents an idealized nicotinic cholinergic synaptic junction containing acetylcholine (black dots) contained within synaptic vesicles (in circles) and postsynaptic membrane. (From Ochoa et al. 1989, with permission.) A-Receptor exists in an equilibrium condition of a mixture of two forms: resting (R) and desensitized (D) states. B-An action potential facilitates the release of ACh. The result is a sudden increase in ACh concentration at the synaptic cleft and occupies one of the two receptor states, R and D. This induces a conformational change, which leads to the activated state (A), in which the channel opens allowing cation movements essential for the development of a postsynaptic action potential. As soon as ACh occupies its sites, the affinity of the receptors toward ACh increases and the D state is promoted, resulting in the termination of the action of ACh.*

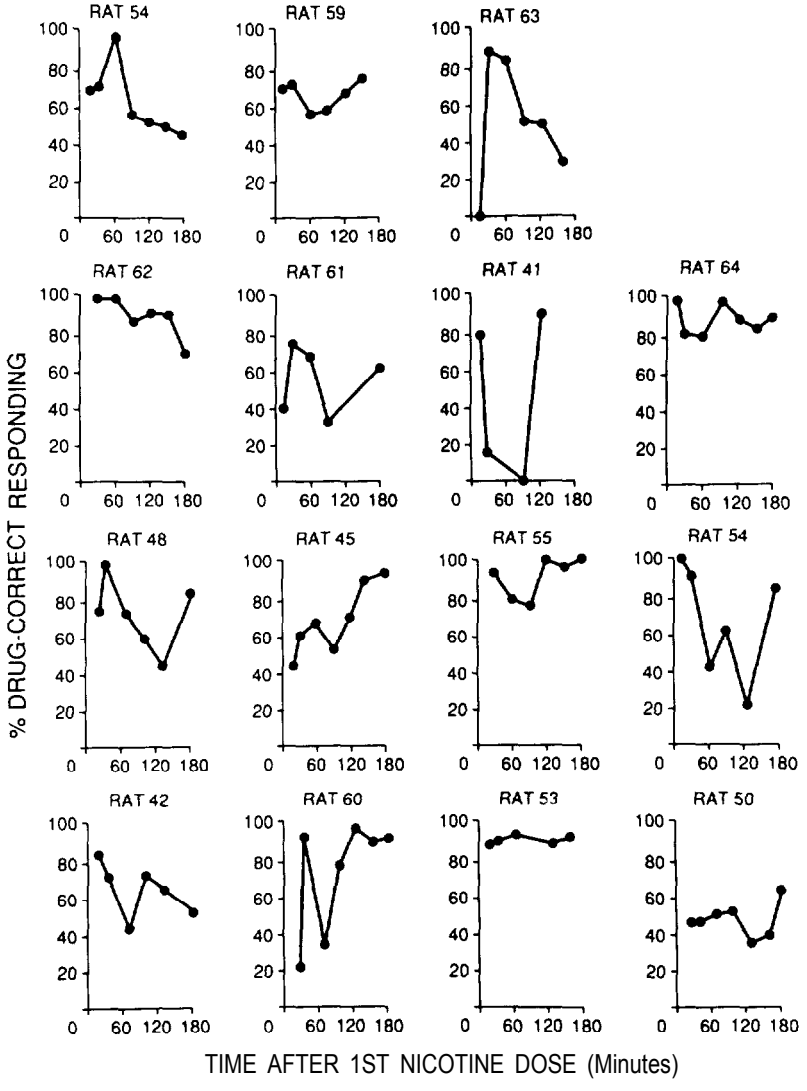

FIGURE 6. *Evidence of* in vivo *desensitization in rats trained to discriminate nicotine (0.4 mg/kg SC) from saline. Data represent nicotine-correct responding tested at various time periods following an initial challenge of nicotine (0.80 mg/kg SC). Rats were tested for two minutes and responses on both levers were rewarded.*

with learning the nicotine DS. The results of this study indicated that 11 of 15 rats exhibited tachyphylaxis (desensitization) to nicotine-elicited DS; tachyphylaxis, when observed, occurred at different intervals between nicotine challenge doses (figure 6).

These results, although preliminary, are encouraging in that they may shed some light on how nicotine exerts DS control of behavior. Of special interest is that these data may help explain why researchers (Jung et al. 1988*b*; Meltzer and Rosecrans 1988; Rosecrans 1988; Stolerman 1987) have been unable to mimic nicotine's effects by increasing brain ACh through AChE inhibition, especially if our contention is correct that nicotine is exerting its effects via an interaction at the n-AChR. This is especially puzzling because m-cholinergic agonists readily generalize to increases in brain ACh. An explanation of these observations is that n-AChRs were desensitized by physostigmine, which would produce an "antagonistic state" and prevent any generalizations in rats under nicotine-induced stimulus control. Additional studies are obviously needed to determine, whether desensitization is a mechanism common to all rats or a special case involving the ability of some n-AChRs to respond to nicotine in this manner. Thus, the experimental approach has not been at fault in demonstrating the relationship between nicotine and its n-AChR; perhaps the fault has been with the differential sensitivity of n-AChR to ACh or nicotine or both.

SUMMARY AND CONCLUSIONS

Our thinking about how nicotine might be inducing DS control of behavior has changed drastically in the past 25 years. Our first inclination was that nicotine was mimicking ACh at a variety of specific and select n-AChRs. Then several nicotine researchers suggested that nicotine might be acting via specific and select noncholinergic receptors. At present, we seem to have returned to the view that nicotine may have pronounced effects at the n-AChR (figure 1), at least in some specific cases such as in the development of tolerance and in rats trained to discriminate nicotine (figure 6).

The endogenous ligand has not changed, but the mechanism of how it affects presynaptic and postsynaptic receptors appears to have been rediscovered. The concept of rapid ACh-induced desensitization at the n-AChR is not new and appears basic to cholinergic neuronal function (figure 5). The desensitization concept has been revitalized by several investigators who also consider this mechanism important to how nicotine might act in protecting DA

neurons from neurotoxicity of chemicals such as 6-OHDA or MPTP (Janson et al. 1988). The overall concept suggests that n-AChR desensitization at presynaptic DA sites may reduce neuronal accessibility to select exogenous neurotoxins and thus attenuate neuronal destruction. This hypothesis has also been partially validated in relation to the cholinergic neuron by providing preliminary evidence that nicotine was able to reduce cholinergic neuron destruction (measured by brain ACh levels) via the intraventricular administration of the neurotoxin AF64A, an ACh nitrogen mustard (Villanueva et al. 1990). Thus, nicotine or compounds acting like nicotine could possibly be beneficial to patients exhibiting the select neurological problems observed in Parkinson's and Alzheimer's diseases.

The process of desensitization might also be useful to understanding why humans choose to smoke tobacco products. Perhaps the ability of nicotine to induce such neuronal effects at a specific n-AChR makes it reinforcing (or aversive) to behavior. Such a mechanism of action might explain why nicotine appears to both increase and decrease arousal levels in animals (or humans) exhibiting differential basal level of excitability (Hendry and Rosecrans 1982), or why some people never become dependent on nicotine.

How nicotine alters the n-AChR may also be beneficial to our understanding of the subtle nature of cholinergic neuronal function in learning, memory, and other behavioral states. The abiliiy of ACh (or nicotine) to induce an activation or attenuation of some cholinergic or noncholinergic neuron or both may be important to these brain processes. It addition, there are important interrelationships between cholinergic and noncholinergic pathways that may assist in understanding how cholinergic receptors control behavior. Robinson (1983, 1984) and Robinson et al. (1979), for example, have shown that both dopaminergic- and serotonergic-projecting neurons play an important role in controlling ACh turnover in oholinergio-rich brain areas (hippocampus and frontal cortex) that may affect the level of m- and n-AChR excitability. Conversely, we should also consider how cholinergic presynaptic receptors located on DA- and serotonin-containing neurons are able to control a variety of behavioral states. The importance of central ACh-containing neurons to behavior (cognitive and affective), therefore, may be as important as these neurons are to the autonomic nervous system (Pomerleau and Rosecrans 1989).

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Discriminative Stimulus Properties of Benzodiazepines and Several New Anxiolytics*

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INTRODUCTION

The clinical efficacy of the benzodiazepines in treating anxiety is well documented. Although they are relatively safe drugs, they possess a number of unwanted side effects, such as sedation. In recent years compounds have emerged as purported “anxioselective” agents. This quest was given considerable impetus by two factors: the discovery of benzodiazepine receptors in the brain, and the emergence of the serotonergic anxiolytic busiprone and related compounds, which appear to constitute a novel mechanistic class of anxiolytic agents.

The drug discrimination (DD) paradigm is a sensitive procedure for evaluating the interoceptive stimulus effects produced by a drug. For example, using a typical two-lever operant procedure, animals can be trained to press one lever following administration of an anxiolytic drug and the other lever after administration of vehicle. Once the animals have been trained, tests of stimulus generalization (substitution) can be conducted. The occurrence of stimulus generalization suggests that a challenge drug is capable of producing behavioral (stimulus) effects similar to those produced by a particular training drug.

This chapter is organized into three main parts: (1) a review of DD studies concerning benzodiazepine stimulus effects, including studies of the activity of benzodiazepine metabolites and stereoisomers, antagonism tests, and correlations of DD data with benzodiazepine binding data and human

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therapeutic potency; (2) the stimulus effects of newer compounds that are chemically unrelated to benzodiazepines but most likely interact with benzodiazepine receptors (i.e., benzodiazepine receptor mediated agents); and (3) the stimulus effects of pyrimidinylpiperazines, such as buspirone, ipsapirone, and gepirone, which seem to produce their effects through a serotonergic mechanism. Table 1 lists some of these different classes of drugs that have been used as training stimuli.

BENZODIAZEPINES

Several benzodiazepine derivatives have been used as discriminative stimuli in animals. Early studies (table 1) sought to determine whether the stimuli produced by these drugs differed from those produced by members of other classes of agents active in the central nervous system; these studies have been previously reviewed (see Colpaert et al. 1976) and are not discussed here. More recent studies with benzodiazepines as discriminative stimuli have been directed, for the most part, toward two goals: (1) characterizing the benzodiazepine stimulus and (2) determining a possible commonality of effect with newer types of purported anxiotytic agents. In general, DD studies have shown that stimulus control established with one psychoactive benzodiazepine generalizes to other benzodiazepines. These studies have also shown that there are several important positions on the benzodiazepine molecule where the presence of substituents can have a significant effect on activity or potency. Although the structure-activity relationship (SAR) for the benzodiazepines was relatively well established many years ago (for a review see Sternbach 1973), it should be noted that the SAR generated from DD studies parallels known SAR generalities for the benzodiazepines (Young and Glennon 1967).

Metabolites

The activity and potency of metabolites have been shown to be important considerations in evaluating the stimulus properties of benzodiazepines. For example, major metabolites of diazepam include desmethyldiazepam, temazepam, and oxazepam. In the rat, significant quantities of the hydroxylated metabolites 4′-hydroxydiazepam and 4′-hydroxydesmethyldiazepam are also produced. Young et al. (1966) examined the activity and potency of these metabolites in rats trained to discriminate diazepam from vehicle. Generalization was found to occur with temazepam and oxazepam, which were nearly equipotent with diazepam, and also with desmethyldiazepam, which was about half as potent as diazepam. The two hydroxylated metabolites

TABLE 1. *Benzodiazepines and newer types of purported anxiolytics that have been used as discriminative stimuli in animals*

Training Drug	Reference
Benzodiazepines	
Chlordiazepoxide	Colpaert et al. 1976
Clorazepate	Dantzer and Perio 1962
Diazepam	Haug and Gotestam 1962
Flurazepam	Overton 1976
Lorazepam	Ator and Griffiths 1966
Midazolam	Garcha et al. 1985
Oxazepam	Hendry et al. 1983
Pyrazoloquinolines	
CGS 9696	Bennett 1985
Imidazopyridines	
Zolpidem	Sanger and Zivkovic 1986
Pyrimidinylpiperazines	
Buspirone	Hendry et al. 1983
Ipsapirone	Spencer and Traber 1987

4′-hydroxydiazepam and 4′-hydroxydesmethyldiazepam were inactive. These results indicate that benzodiazepine metabolites may contribute to the discriminative stimulus (DS) effects of a benzodiazepine.

Stereoisomers

Optical isomers of biologically active compounds often display differences in potency and sometimes can also display differences in effect. Unfortunately, very few pairs of benzodiazepine optical isomers have been studied in DD tests. The effects of (±)-, R(-)-, and S(+)-3-methytflunitrazepam have been examined in animals trained to discriminate diazepam from vehicle (Shannon and Herling 1983*b*; Young et al. 1984). Generalization of the diazepam stimulus was found to occur to (±)- and S(+)-3-methyiflunitrazepam, with the S(+)-isomer being twice as potent as the racemate. The administration of

R(-)-3-methylflunitrazepam did not produce significant drug lever responding, even at 8-10 times the dose of S(+)-Smethylflunitrazepam that produced diazepam generalization. However, Hiltunen and Järbe (1986) have shown that animals trained to a S(+)-3-methylflunitrazepam stimulus generalize to R(-)-3-methylflunitrazepam, with the S(+)-isomer being 10 times more potent than the R(-)-isomer. S(+)-3-methylflunitrazepam is also recognized by animals trained to discriminate chlordiazepoxide from saline (Hiltunen and Järbe 1986). More recently, Järbe et al. (1986) trained animals to discriminate both diazepam and the S(+)-isomer of meclonazepam from vehicle. The administration of the R(-)-isomer of meclonazepam did not produce significant drug lever responding in either group of trained animals. Taken together, these limited data suggest benzodiazepine stereoselectivity, with the S(+)-isomers of 3-methylflunitrazepam and meclonazepam being more potent than their corresponding R(-)-isomers.

Antagonism Studies

Evidence that the DS effects produced by benzodiazepines are mediated by benzodiazepine receptors is derived from the finding that these effects can be blocked by the administration of benzodiazepine antagonists such as flumazenil (Ro 15-1788) and CGS 6216 (Herling and Shannon 1982; Young and Dewey 1982). Flumazenil may also possess agonist activity, because stimulus generalization occurs in clorazepate- or chlordiazepoxide-trained animals (Dantzer and Perio 1962; De Vry and Slangen 1966). In fact, flumazenil itself has been used as a training drug (Bennett et al. 1985). Generalization of the flumazenil stimulus was found to occur to CGS 8216 and diazepam. In that same study, animals were also trained to discriminate CGS 8216 from saline. Generalization did not occur to flumazenil, indicating an asymmetrical cross-generalization between the two training drugs. CGS 8216 stimulus generalization did occur to the purported anxiogenic agent pentylenetetrazol. The authors concluded that the stimuli produced by the two “antagonists” are qualitatively different, in that flumazenil has partial agonist (benzodiazepine-like) activity and CGS 8216 has inverse agonist (i.e., anxiogenic) activity.

Correlation Studies

A significant correlation (r= .78) exists between potencies of benzodiazepines in a DD task and their affinities for benzodiazepine binding in rat cerebral cortex (Shannon and Herling 1983*b*). More recently, Young and Glennon (1987)

compared discrimination-derived ED_{50} values for a series of benzodiazepines with data on displacing aft inities (K_i values) for ^3H-diazepam brain binding in humans, and with human therapeutic potency. A significant correlation (r = .88) was found between benzodiazepine binding affinities and ED_{50} values derived from the discrimination assay. A significant correlation (r= .92) was also found between DD ED_{50} values and human therapeutic potencies (figure 1). Thus, a relationship might exist between benzodiazepine-induced stimulus effects in animals and benzodiazepine-induced subjective effects in humans.

BENZODIAZEPINE RECEPTOR-MEDIATED COMPOUNDS

The triazolopyridazine CL 218,672 (3-methyl-6-[3-trifluromethylphenyl]-1,2,4-triazolo [4,3-b] pyridazine) displays high affinity for benzodiazepine receptors. Indeed, it was the first nonbenzodiazepine to selectively displace brain-specific benzodiazepine binding at a potency level comparable to the benzodiazepines (Lippa et al. 1979). Further studies on the binding properties of CL 218,672 suggest that it has a high affinity for a distinct subpopulation of receptors in the cerebellum, termed BZ_1, and a lower affinity for a subpopulation of receptors in the hippocampus, termed BZ_2 (Klepner et al. 1979). Behaviorally, CL 218,872 possesses the anxiolytic-anticonvulsant properties typical of a psychoactive benzodiazepine, but if appears to lack the sedative-hypnotic and muscle-relaxant effects of benzodiazepines (Klepner et al. 1979; Lippa et al. 1979). Taken together, these data led to the proposal that the BZ_1 receptor might mediate anxiolytic activity, whereas the BZ_2 receptor might mediate the side effects (i.e., sedation, ataxia) associated with the benzodiazepines. However, based on historical brain structure and function relationships, that proposal seems paradoxical. That is, ataxic-sedative effects would be thought to be more closely associated with the activity of the cerebellum (BZ_1), whereas anxiolytic activity might be more intimately involved with the function of the hippocampus (BZ_2). Consistent with the latter proposal is the activity of zolpidem (see below).

The pyrazoloquinoline CGS 9896 (2-(4-chlorophenyl)-2,5-dihydo-3H pyrazolo [4,3-c] quinolin-3-one), the pyrolopyrazine zopiclone, and the imidazopyridine zolpidem are also potent inhibitors of benzodiazepine binding to rat brain receptors (Julou et al. 1985; Sanger et al. 1987; Yokoyama et al. 1982). CGS 9696 and zopiclone show no selectivity between BZ_1 and BZ_2 receptors, whereas zolpidem displays a preference for benzodiazepine receptors in the cerebellum (BZ_1) over those in the hippocampus (BZ_2). All three wmpounds produce the anxiolytic-anticonvulsant effects associated with benzodiazepines (e.g., Bernard et al. 1985; Julou et al. 1985; Sanger et al. 1987). In addition,

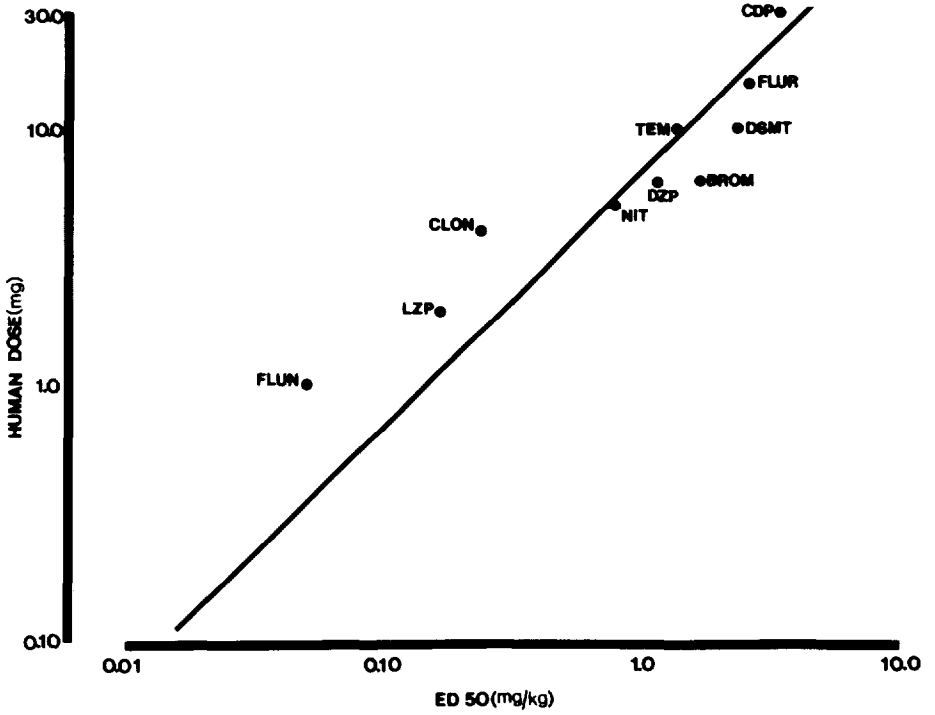

FIGURE 1. *Correlation between human therapeutic potency and discrimination-derived ED_{50} values using diazepam as training drug (r = .92). Compounds are bromazepam (BROM), chlordiazepoxide (CDP), clonazepam (CLON), desmethydiazepam (DSMT), diazepam (DZP), flunitrazepam (FLUN), flurazepam (FLUR), lorazepam (LZP), nitrazepam (NIT), and temazepam (TEM). (Data from Young and Glennon 1987.)*

CGS 9896 may be a mixed agonist-antagonist, because it blocks diazepam-induced rotorod impairment in animals (Bernard et al. 1985). CGS 9896 is purported to be an anxioselective agent, whereas zopiclone and zolpidem are reported to be sedative-selective agents (Bernard et al. 1985; Julou et al. 1985; Sanger et al. 1987).

In DD studies, animals have been trained to discriminate either chlordiazepoxide, diazepam, lorazepam, midazolam, CGS 9896, or zolpidem

from vehicle (table 2). In tests of stimulus generalization, all of those training drugs generalized to chlordiazepoxide or CL 218,872 (e.g., Ator and Griffiths 1986; Bennett 1985; Leidenheimer and Schechter 1988; McElroy and Feldman 1982; Sanger and Zivkovic 1986; Spealman 1965; Young and Dewey 1982; Young et al. 1967). The stimuli also generaliied to zopiclone or diazepam (e.g., Ator and Griffiths 1986; Bennett 1985; Colpaeft et al. 1976; Sanger and Zivkovic 1986; Sanger et al. 1965; Spealman 1985; Young et al. 1987), although zopiclone has not been tested in CGS 9896-trained animals and diazepam has not been tested in zolpidem-trained animals. To date, there has not been any detailed report of zopiclone or CL 218,872 as a training drug.

The activities of CGS 9896 and benzodiazepines in DD studies have not always produced consistent results. For example, stimulus generalization occurs between CGS 9896 and chlordiazepoxide when either drug is used as the training stimulus (e.g., Leidenheimer and Schechter 1988; Sanger et al. 1985). *In* comparison, lorazepam-trained animals reportedly do not generalize to CGS 9896 (Ator and Griffiths 1986), whereas diazepam-trained animals reportedly do not (Shannon and Herling 1983*a)* and do (Young et al. 1987) generalize to CGS 9896. Finally, diazepam is recognized by animals trained to discriminate CGS 9896 from vehicle (Bennett 1985). These apparent discrepancies may perhaps be accounted for by procedural differences in the experiments (Young et al. 1987) or by the purported mixed agonist-antagonist properties of CGS 9696 (Bernard et al. 1985; Sanger and Zivkovic 1987), or both.

TABLE 2. *Results of generalization studies in animals trained to discriminate chlordiazepoxide, diazepam, lorazepam, midazolam, CGS 9896, and zolpidem from vehicle*

	Training Drug					
Test Agent	Chlordiazepoxide	Diazepam	Lorazepam	Midazolam	CGS 9896	Zolpidem
Diazepam	Gen	Gen	Gen	Gen	Gen	—
Chlordiazepoxide	Gen	Gen	Gen	Gen	Gen	Gen
CL 218872	Gen	Gen	Gen	Gen	Gen	Gen
CGS 9896	Gen	(Gen-NGen)	NGen	—	Gen	NGen
Zopiclone	Gen	Gen	Gen	Gen	—	Gen
Zolpidem	PGen	—	—	—	—	Gen

NOTE: See text for references. Gen = generalization; NGen = no generalization; PGen = partial generalization; — = not tested.

The sedative-selective profile of zolpidem has been emphasized in DD experiments by Sanger and Zivkovic (1986, 1987). For example, in zolpidem-trained animals, stimulus substitution occurs to chlordiazepoxide, but in chlordiazepoxide-trained animals, zolpidem produces only partial (i.e., 70-75 percent chlordiazepoxide-appropriate responding) generalization with significant decreases in response rates (Sanger and Zivkovic 1986). The authors suggest that zolpidem has chlordiazepoxide-like stimulus properties only at doses that are highly sedative. A zolpidem stimulus does not generalize to CGS 9896. However, CGS 9896 does antagonize a zolpidem cue (Sanger and Zivkovic 1987). Because zolpidem shows selectivity for BZ_1 receptors and CGS 9896 diaplsys high affinity for both BZ_1 and BZ_2 receptors, an intriguing possibility is that CGS 9896 may be an agonist at BZ_2 receptors (mediating anxiolytic action -and thus cross-generalization may occur between it and chlordizepoxide-and an antagonist at BZ_1 receptors (mediating ataxic-sedative activities) and thus may antagonize the sedative stimulus of zolpidem.

PYRIMIDINYLPIPERAZINES

Prototypical of this novel series of second-generation anxiolytics (SGAs) are busiprone, gepirone, and ipsapirone. Buspirone, the most extensively studied member of this series, is a clinically effective antianxiety agent (e.g., Riblet et al. 1982) All three of these drugs form a common metabolite, 1-(2-pyrimidinyl) piperazine (1-PP).

In animal studies these drugs, unlike benzodiazepines, lack anticonvulsant, sedative, and muscle-relaxant effects (Eison et al. 1986; Riblet et al. 1982). They also appear to lack abuse and physical dependence properties (e.g., Riblet et al. 1982). Behaviorally, the SGAs can (but do not always) exhibit anxiolytic activity in animals, but such effects are not attenuated by benzodiazepine receptor antagonists (Traber et al. 1984; Young and Glennon 1988). Neurochemical studies have demonstrated that these agents do not bind to benzodiazepine receptors. They do, however, interact with serotonin 5-HT1A receptors (Eison et al. 1986; Glaser and Traber 1983).

DD studies have been used to distinguish SGAs from benzodiazepines. For example, animals have been trained to discriminate either buspirone, ipsapirone, or a benzodiazepine from vehicle (table 3). In tests of stimulus generalization, the SGAs and 1-PP are not recognized by animals trained to discriminate a benzodiazepine from vehicle, and benzodiazepines are not

TABLE 3. *Results of generalization studies in animals trained to discriminate buspirone, isaprone, 8-OH DPAT, diazepam, lorazepam, midazolam, and oxazepam from vehicle*

	Training Drug						
Test Agent	Buspirone	Ipsapirona	8-OH DPAT	Diazepam	Lorazepam	Midazolam	Oxazepam
Buspirone	Gen	Gen	Gen	NGen	NGen	NGen	NGen
Ipsapirone	-	Gen	Gen	NGen	-	-	-
Gepirone	Gen	Gen	Gen	NGen	-	-	-
1-PP	-	-	NGen	NGen	-	-	-
8-OH DPAT	Gen	Gen	Gen	NGen	-	-	-
Diazepam	-	NGen	NGen	Gen	Gen	Gen	-
Midazolam	NGen	-	-	Gen	Gen	Gen	-

NOTE: See text for references; Gen = Generalization; NGan = No Generalization; - = Not Tested

recognized by animals trained to discriminate an SGA from vehicle (Hendry et al. 1983; Mansbach and Barrett 1987; Pierson et al. 1987; Spealman 1985; Spencer and Traber 1987; Young et al. 1987). Finally, animals trained to discriminate buspirone or ipsapirone from vehicle generalize to gepirone, buspirone, and ipsapirone (Cunningham 1989; Pierson et al. 1987; Spencer and Traber 1987; Young and Glennon 1988). Taken together, these data indicate that the SGAs do not share stimulus properties with the benzodiazepines.

To further differentiate SGAs and benzodiazepines, DD studies have emphasized their different mechanisms of action. For example, stimulus generalization occurs between an SGA and the 5-HT1A agonist 8-OH DPAT when either drug is used as the training stimulus (Mansbach and Barrett 1987; Spencer and Traber 1987; Young and Glennon 1988). In contrast, stimulus generalization does not occur between diazepam and 8-OH DPAT when either agent is used as the training stimulus. An 8-OH DPAT stimulus also fails to generalize to 1-PP (Young and Glennon 1988). These results indicate that the SGAs (but not 1-PP) produce stimulus effects similar to those of the 5-HT1A agent 8-OH DPAT. Lastly, a benzodiazepine stimulus but not an 8-OH DPAT stimulus, can be blocked by Ro 15-1788 (flumazenil), whereas an 8-OH DPAT-stimulus can be antagonized by the purported 5-HT1A antagonist (-)pindolol (Tricklebank et al. 1987; Young and Glennon 1988). Thus, the

stimulus effects of SGAs appear to be independent of the effects of the common metabolite 1-PP and, unlike those of benzodiazepines, appear to be mediated by a 5-HT1A mechanism.

CONCLUSION

Although benzodiazepines still constitute one of the most widely prescribed classes of drugs in the world, a number of new drugs have been investigated for their anxiolytic potential. Some are related to the benzodiazepines by their interaction at benzodiazepine receptors, while others interact at 5-HT1A systems.

The ability of various benzodiazepines to serve as discriminative stimuli has been well documented. Benzodiazepine stimuli have been demonstrated to generalize to other benzodiazepines (including certain metabolites), and a significant correlation exists between their relative potency and both their affinity for benzodiazepine receptors in the brain and their human anxiolytic potency. Moreover, the benzodiazepine stimulus is stereoselective and can be attenuated by benzodiazepine receptor antagonists. Taken together, these data provide evidence that benzodiazepines exert their stimulus effects through an interaction with benzodiazepine receptors.

Newer compounds have been developed that are chemically unrelated to the benzodiazepines but exert their effects by interacting with benzodiazepine receptors, and they appear to be anxioselective (CL 218,872, CGS 9896). An interesting byproduct of this research is that some of these compounds may be sedative selective (zopiclone, zolpidem). In DD studies, CL 218,872 and zopiclone appear to produce stimulus effects that are typical of the benzodiazepines, although neither drug itself has been used as a training stimulus. In comparison, CGS 9896 and zolpidem appear to produce stimulus effects that might indicate an anxioselective profile for the former and a sedative-selective profile for the latter.

Finally, although both the benzodiazepines and the SGAs, such as busiprone and ipsapirone, can be used to establish stimulus control, their stimulus properties are clearly dissimilar. By extrapolation, this suggests that the SGAs probably produce qualitatively different subjective effects in human patients, as compared to those produced by benzodiazepines. The significant interaction of the SGAs with 5-HT1A systems qualify these drugs as a novel mechanistic class of antianxiety agents.

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Distinctive Discriminative Effects of Ethanol

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Experiments on discriminative effects of ethanol in laboratory animals have contributed important information on the comparison of ethanol with other drugs. This paper summarizes and interprets that research and also proposes a model to explain differential discriminative effects of ethanol from the discriminative effects of barbiturates and benzodiazepines. Stolerman et al. (1989), a bibliography of publications on discriminative drug effects through 1988, is a useful resource that includes ethanol and alcohol in an excellent index of drugs and topics.

COMPARISONS WITH BARBITURATES AND BENZODIAZEPINES

After animals have been trained to discriminate a drug effect from the control condition, they can be tested with other drugs to determine whether these novel drugs resemble the training drug condition or the control (i.e., nondrug) condition. In studies with animals trained to discriminate ethanol from the nondrug condition, the other drugs that have been tested most often are barbiturates and benzodiazepines. These studies consistently show that the drug response is induced when sufficiently high doses of barbiturates or benzodiazepines are tested. In animals trained to discriminate a barbiturate or benzodiazepine from the nondrug condition, however, the drug response is not consistently induced during tests with ethanol.

An early report by Overton (1966) reported similar discriminative stimulus effects of several sedative or hypnotic drugs, including ethanol, chloral hydrate, pentobarbital, phenobarbital, and chlordiazepoxide. The author pointed out that in rats trained to discriminate the benzodiazepine chlordiazepoxide from nondrug, ethanol did not dependably induce the drug choice. Subsequently, Barry (1974) and Barry and Krimmer (1977, 1978) summarized several studies showing generalization of the ethanol response to barbiturates and benzodiazepines but not consistent generalization of the barbiturate or benzodiazepine response to ethanol.

These differential effects were tested systematically and demonstrated clearly by De Vry and Slangen (1986). Discrimination of three drugs (ethanol, pentobarbital, and chlordiazepoxide) from the nondrug vehicle was trained by using six groups of rats, including separate groups trained with lower and higher doses of each of the three drugs. In tests with other drugs, the response trained with ethanol generalized to the other two drugs. The response trained with pentobarbital generalized to chlordiazepoxide but not to ethanol. The response trained with chlordiazepoxide did not generalize to ethanol, and a much higher dose of pentobarbital was needed to induce the chlordiazepoxide response than to induce the drug response in rats trained with pentobarbital or ethanol.

These findings indicate asymmetrical generalization. The ethanol response generalizes to barbiturates and benzodiazepines, but neither the barbiturate nor the benzodiazepine response generalizes equivalently to ethanol. The ethanol effect, therefore, has the greatest generality, whereas the benzodiazepine effect has the greatest specificity. Pentobarbital is intermediate in these attributes of generality and specificity.

MODEL FOR ASYMMETRICAL GENERALIZATION

Barry and Krimmer (1979) suggested that the strength or distinctiveness of the drug stimulus depends partly on the dose and partly on the characteristics of the drug. Two drugs at a dose with equivalent potency might differ in distinctiveness of difference from the nondrug condition. This conception implies that the difference from the nondrug condition is more distinctive for pentobarbital than for ethanol and more distinctive for chlordiazepoxide or diazepam than for pentobarbital.

A model for the differences among these three types of drugs attributes the most diverse effects to ethanol and the most specific effects to benzodiazepines; barbiturates are intermediate. These differential drug effects are consistent with known pharmacological actions of these drugs.

Ethanol is a small molecule with pervasive effects on the central nervous system (CNS). Impairment of neurotransmission is seen in the anesthetic effect at doses only moderately higher than the doses required for behavioral effects. The mechanism for the ethanol effect appears to be primarily an increase in fluidity of the membranes of the cell. This uniform increase in membrane fluidity occurs at many sites in the CNS and hence induces a wide variety of responses because of the diversity of functions in the CNS. The effects on

neurotransmission are secondary to these generalized effects on the functioning of the cells rather than due primarily to binding of the ethanol molecule to a particular neurotransmitter receptor.

The principal behavioral effects of benzodiazepines appear to result from the drug binding to a specific endogenous receptor. Drugs that block the receptor therefore antagonize the effects of benzodiazepines, indicating a specific rather than a diverse effect on the CNS. Benzodiazepines impair neurotransmission and cause anesthesia only at doses that are far above the range used for clinical treatment and behavioral experiments.

The specificity of the barbiturate effect is intermediate. In common with benzodiazepines, an important effect of barbiturates is binding to the endogenous benzodiazepine receptor complex. Like ethanol, barbiturates increase membrane fluidity. Anesthesia is a prominent effect of barbiturates, as of ethanol, at doses only moderately higher than the doses required to induce behavioral changes.

An analogy for this difference among the three types of drugs is the distinctiveness of an auditory stimulus. The nondrug condition corresponds to a generalized noise, low in volume and constituting a mixture of all the 12 pitches (7 natural tones, 5 sharps). Waterfalls and electric fans produce this type of generalized noise. The ethanol stimulus corresponds to five tones with adjacent pitches, such as F, F sharp, G, G sharp, and A. The magnitude of the dose is determined by the loudness of the group of five tones. Pentobarbital corresponds to two tones with adjacent pitches, such as F and F sharp. Chloridazepoxide or diazepam corresponds to a single tone, such as F. These sounds can be reproduced with an organ, pitch pipe, or piano.

According to this model, any of the three types of drugs can easily be discriminated from the nondrug condition if the dose is high enough. Differences between the drugs are indicated by tests that substitute one of the other two drugs for the training drug. Generalized noise is more similar to the five adjacent pitches (ethanol) than to the single tone (benzodiazepine). In animals trained to discriminate a group of five pitches (ethanol) from generalized noise, two pitches or a single tone is more similar to the group of five pitches than to the generalized noise. In animals trained to discriminate the single tone (benzodiazepine) from generalized noise, the two pitches and especially the group of five pitches might be perceived as more similar to the generalized noise than to the single tone. In animals trained to discriminate two pitches

(barbiturate) from generalized noise, the single tone is more similar to the two pitches, but the group of five pitches might be perceived as more similar to the generalized noise.

This model of differences between these three types of sedative drugs indicates a progression of increasing specificity from the nondrug condition to ethanol to barbiturates to benzodiazepines. This progression is consistent with the results of experiments in which animals are trained to discriminate one of these types of drugs from the nondrug condition and then tested with the other types of drugs. Other experiments provide further information on the validity and usefulness of this model.

ETHANOL COMPARED WITH OTHER DRUGS

Some drugs in addition to barbiturates and benzodiazepines have been reported to induce the ethanol response. Overton (1966) found that the ethanol response was chosen in tests with volatile anesthetic agents, such as chloral hydrate and ether, and also with meprobamate. Rees et al. (1987) found that the ethanol response trained in mice was chosen in tests with toluene, halothane, and 1,1,1-trichloroethane. Signs and Schechter (1988) found that the ethanol response trained in rats was chosen in tests with a serotonin agonist, 1-(3-trifluoromethylphenyl) piperazine.

The ethanol response has been reported to be chosen to a partial degree in tests with 3-carboxysalsolinol, which is generated during ethanol metabolism (Chipkin et al. 1979; Schechter 1980) and in tests with acetaldehyde, another metabolite of ethanol (York 1981). A partial ethanol response has also been reported in tests with the opioid antagonist naloxone (Altshuler et al. 1981). These partial similarities suggest that the discriminative ethanol effect has diverse attributes.

Several drugs have shown evidence of sharing discriminative effects of ethanol because the ethanol effect was augmented when ethanol was administered together with the other drug. This interaction with the effect of ethanol was found for apomorphine (Schechter 1985), chlordiazepoxide (Schechter and Lovano 1985), and nicotine (Signs and Schechter 1966); it may be useful as a sensitive measure of the existence of common attributes of the discriminative effect. In animals trained to discriminate ethanol from the nondrug condition, tests with apomorphine or nicotine would probably induce the nondrug response rather than the drug response.

Most types of drugs do not seem to show any similarity to ethanol. These include opioid analgesics, neuroleptics, antimuscarinics, delta-9-tetrahydrocannabinol, hallucinogens, and stimulants. Animals trained to discriminate ethanol from the nondrug condition predominantly choose the nondrug response in tests with these drugs (Barry 1974). Also, animals trained to discriminate any of these drugs from the nondrug condition predominantly choose the nondrug response in tests with ethanol. The discriminative ethanol effect therefore is differentiated from most other pharmacological conditions.

ANTAGONISTS OF ETHANOL

Specificity of a drug effect is indicated if this effect can be antagonized when the drug is combined with a single other drug. An example is the antagonism of morphine by naloxone. After animals have been trained to discriminate morphine from the nondrug condition, a sufficiently high dose of naloxone together with the training dose of morphine shifts the choice from the morphine to the nondrug response.

Experiments on rats have failed to identify a single consistent antagonist of the discriminative ethanol response. The ethanol response in rats has been antagonized by bemegride (Krimmer 1974) by amphetamine (Schechter 1974) and by p-chlorophenylalanine, which depletes brain serotonin (Schechter 1973). The ethanol response was not antagonized by the benzodiazepine inverse agonist Ro 15-4513 in rats (Hiltunen and Järbe 1988) or in mice (Middaugh et al. 1989). Contrary to these findings, however, Rees and Balster (1988) reported that the discriminative ethanol response in mice was antagonized by Ro 15-4513.

In contrast to the failure to find a consistent, specific antagonist of the discriminative ethanol effect in rats, barbiturates and benzodiazepines have specific antagonists. The stimulant drug bemegride antagonizes the discriminative barbiturate response in rats (Barry and Krimmer 1979; Krimmer 1974; Overton 1966). The benzodiazepine inverse agonist Ro 15-4513 antagonizes the discriminative benzodiazepine response but not ethanol response in rats (Hiltunen and Järbe 1989*a*, 1989*b*).

When rats are trained to discriminate Ro 15-4513 from the nondrug control condition, the Ro 15-4513 response is induced by the stimulant drug pentylenetetrazol. This response to pentylenetetrazol is antagonized by

diazepam or pentobarbital but not by ethanol (Harris et al. 1987; Hiltunen and Järbe 1989*b*; Järbe and Hiltunen 1988).

DISCRIMINATION BETWEEN TWO DRUGS

If animals are trained to discriminate a drug from the nondrug condition, tests with other drugs provide only indirect comparisons between the stimulus attributes of the two drugs. A more direct comparison is to train the animals to discriminate between the two drugs. Successful training of this discrimination demonstrates that the drugs have differential effects.

Krimmer and Barry (1973) reported on successful training of rats to discriminate ethanol (1,000 mg/kg) from pentobarbital(10 mg/kg); both drugs were injected in a volume of 10 mL/kg. Tests with higher doses of either drug reliably induced the response for that drug. Tests with lower doses of pentobarbiial, and tests with saline, shifted some animals to the ethanol response.

Overton (1977) reported that a group of rats learned to discriminate ethanol from pentobarbital and another group learned to discriminate ethanol from phenobarbital. A third group failed to discriminate pentobarbital from phenobarbital. These findings give evidence for close similarity of the discriminative effects of the two barbiturates.

Barry et al. (1981) trained rats to discriminate either of two ethanol doses from either of two chlordiazepoxide doses. This procedure required the animals to discriminate the qualitative differences between the two drugs rather than a possible quantitative difference between a stronger effect of a single dose of one drug and a weaker effect of a single dose of the other drug.

Other studies have tested effects of ethanol in rats trained to discriminate pentobamital from chlordiazepoxide. Barry and Krimmer (1979) summarized tests with conflicting results on whether tests with ethanol induced the chlordiazepoxide or the pentobarbital response. In a subsequent experiment (Barry et al. 1982) rats were trained to discriminate pentobarbiial from chlordiazepoxide with two doses of both drugs in order to establish discrimination between the qualitatively different effects of the two drugs. After this training, tests with ethanol induced the pentobarbital response rather than the chlordiazepoxide response.

Another type of discrimination between two drugs is to train discrimination between drugs that have contrasting effects, such as a depressant and a stimulant. Gauvin et al. (1989) trained rats to discriminate chlordiazepoxide from pentylenetetrazol. After this training, ethanol was found to resemble the chlordiazepoxide rather than the pentylenetetrazol condition. The similarity of ethanol to the benzodiazepine as a depressant drug, therefore, is more obvious when the benzodiazepine is discriminated from a stimulant than from the nondrug condition.

A further test by Gauvin et al. (1989) gave evidence that the withdrawal illness after a high ethanol dose resembles a stimulant drug, pentylenetelrazol, more than a depressant drug, chlordiazepoxide. A saline injection normally induced nearly equal choice of the diazepam and pentylenetetrazol responses. At 12 hr after a high ethanol dose of 3 or 4 g/kg, the saline injection induced a preponderance of pentylenetetrazol choices. This finding is consistent with a report by Lal et al. (1988) that the ethanol withdrawal illness resembles the effect of pentylenetetrazol. Rats trained to discriminate pentylenetetrazol from the saline control injection chose the saline response in tests after ethanol. Ethanol withdrawal illness was subsequently demonstrated at 24 hr following 4 days of gastric intubation of a high ethanol dose. During this ethanol withdrawal illness, the saline injection induced a preponderant choice of the pentylenetetrazol response.

DIFFERENT ETHANOL DOSES AND TIME INTERVALS

Studies of discrimination between ethanol and the nondrug condition have used a wide variety of doses. The highest were 2.4 g/kg intraperitoneally in rats (Overton 1966) and 3 g/kg orally in pigeons (Järbe and McMillan 1983). These doses have incapacitating effects in some animals. The lowest doses were 0.2 g/kg (Schechter 1980) and 0.3 g/kg (York 1978) intraperitoneally in rats and 0.1 g/kg intravenously in rats (Ando 1975). These doses have no observable: behavioral or physiological disruptive effects.

A low dose and a high dose of a drug differ quantitatively, but they might also produce qualitatively different stimulus conditions. Ethanol appears to have behaviorally stimulant effects at low doses and depressant effects at high doses. Accordingly, the drug stimulus might be a different type at different doses (York 1978). A finding by Barry et al. (1981) might indicate qualitatively different effects of different doses. Rats failed to learn a discriminative response, when one response was reinforced after injection with either of two doses of

ethanol (1.0 or 1.5 g/kg) and the alternative response was reinforced in the saline control condition. Another group of rats learned to discriminate either of the same two ethanol doses from either of two chlordiazepoxide doses. The two ethanol doses thus were more differentiated from each other and from chlordiazepoxide than from the saline control condition.

Tests for generalization to other drugs indicate greater specificity with high training doses, resulting in less generalization of the drug response to other drugs. For example, York (1978) trained three groups of rats to discriminate one of three ethanol doses (0.33, 0.66, 0.99 g/kg) from saline. Barbital induced the ethanol choice in the animals trained with 0.33 g/kg but not in those trained with 0.99 g/kg. This finding suggests that the high dose has a more specific effect, generalizing to a lesser degree to other drugs. Different doses of ethanol therefore are not merely different magnitudes of the same stimulus.

Different time intervals after administration also might induce different stimuli. Shippenberg and Altshuler (1985) trained a discrimination of 1.0 g/kg ethanol from the nondrug condition at an interval of 6 min for one group of rats and 30 min for another group. Both groups showed only a partial drug response when tested with ethanol after the alternative interval. A sedative rather than excitatory effect of ethanol at the longer interval was inferred from the finding that naloxone partially antagonized the ethanol response trained at the interval of 6 but not 30 min. Schechter (1989) trained a discrimination of a lower ethanol dose (0.6 g/kg) from the nondrug condition in two groups of rats at the same intervals of 6 and 30 min. The drug response learned at 30 min was induced at 6 min, but the drug response learned at 6 min was only partially induced at 30 min. This difference suggests that the effect of 0.6 g/kg ethanol is a more specific stimulus at the shorter time interval, thus generalizing only partially to the more diverse stimulus of ethanol at the longer interval. The more diverse ethanol stimulus at the longer interval may include actions of metabolites, such as acetaldehyde, and the beginnings of the withdrawal symptoms in addition to the same effects as at the shorter interval.

EXPERIMENTAL PROCEDURES FOR ETHANOL DISCRIMINATION

The discriminative ethanol response has been trained with a wide variety of procedures. The ethanol stimulus thus differs from the nondrug condition and is trainable and measurable under many different conditions.

The most prevalent procedure is to train the animals to select equivalent but different responses, such as levers on opposite sides of a food cup. The incentive for the discrimination learning is usually a pellet of food in a hungry rat. The lever pressing is usually on a fixed ratio schedule of 10 or more responses to obtain a pellet of food.

The earliest studies used the locomotor response of running in a straight alley (Conger 1951) or choosing opposite directions in a T-shaped maze (Barry et al. 1965; Overton 1966). The operant lever-pressing response was introduced by Barry (1968) and Kubena and Barry (1969*a*, 1969*b*) as a technique for training and testing discriminative effects of ethanol and other drugs, The discrimination was learned with lower drug doses with this procedure, perhaps because distracting stimuli were minimized by the less active physical response of pressing a lever in a chamber or by the use of food reward instead of escape from painful shock as the incentive for the discriminative response.

A different type of choice is between responding and refraining from responding instead of between two equivalent, alternative responses. Some studies have associated the ethanol or nondrug condition with delivery of a food pellet and the alternative condition with no food (Winter 1975; York 1978), with a painful shock (Kubena and Barry 1969*b*), or with both food and shock (Conger 1951). Animals learn to respond or to refrain from responding, depending on which consequence is associated with their ethanol or nondrug condition. Conger (1951) also demonstrated more rapid learning of the discrimination by rats that received food after ethanol and food plus shock after the nondrug treatment than by another group of rats that received food after the nondrug treatment and shock after ethanol. This finding is in accordance with a tendency for ethanol to diminish avoidance of an aversive event.

A differential response is not necessary for discriminative learning. Barry (1968) demonstrated that rats learned the discriminative response when pressing a lever that controlled the environmental condition (lighted or dark chamber). Food was delivered in one illumination condition after ethanol injection and in the alternative illumination condition after saline injection.

CHARACTERISTICS OF THE ANIMALS

Most of the experiments on discriminative ethanol effects have been in rats. Other species tested include gerbils (Järbe 1977), pigeons (Järbe and McMillan 1983), mice (Rees and Balster 1988; Rees et al. 1987), and squirrel monkeys

(York and Bush 1982). Most of the findings do not appear to differ among species.

York (1981) compared two lines of albino rats that had been bred for 33 generations for difference in consumption of a 10 percent ethanol solution in tests with simultaneous availability of water and the ethanol solution. The discrimination between ethanol and the nondrug condition was learned more rapidly by the line of rats with low ethanol consumption. This difference gave evidence that in this ethanol-nonpreferring line of rats, ethanol provided a more salient cue that was probably aversive. This study also reported a greater contribution of acetaldehyde to the ethanol stimulus in the ethanol-nonpreferring line of rats.

Krimmer (1990) compared two lines of rats, differing in the duration of loss of righting reflex after injection of a high ethanol dose. After training to discriminate 0.6 g/kg ethanol from the saline control condition, the initial tests with 0.15 and 0.3 g/kg induced higher percentages of ethanol choice by the line of rats with longer duration of loss of righting reflex. This finding indicates that greater susceptibility to the effect of a high ethanol dose on righting reflex was associated with greater sensitivity to the discriminative effect of low ethanol doses.

CONCLUSIONS

Pharmacological classifications view ethyl alcohol as a hypnotic sedative, closely similar to general depressants such as barbiturates, ether, and chloral hydrate and also related to antianxiety agents such as benzodiazepines. Studies of discriminative effects of ethanol in laboratory animals agree with the pharmacological classifications. Several studies indicate discriminative effects of ethanol in rats at lower doses than the minimum sufficient to cause observable changes in behavior.

Ethanol has distinctive discriminative effects, demonstrated by discriminability of this drug from barbiturates or benzodiazepines. A model of the comparison among these three types of drugs indicates that the discriminative effects are most diverse for ethanol and most specific for benzodiazepines. Barbiturates are intermediate, sharing characteristics of both ethanol and benzodiazepines.

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Training Dose: Influences in Opioid Drug Discrimination

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INTRODUCTION

Drug discrimination procedures have been used in a number of species and under a variety of conditions to investigate the effects of drugs from many different pharmacological classes. Typically animals are trained to respond on one manipulandum after administration of a drug and to respond on another manipulandum after administration of drug vehicle. After repeated presentations, selective discriminative stimulus control is established: animals reliably respond on the drug-associated lever following drug administration and on the vehicle-associated lever following vehicle administration. Although drugs from various pharmacological classes have been used to establish stimulus control, the discriminative stimulus effects of opioids will be the topic of this paper. Drugs are classified as opioid based on well-characterized sets of pharmacological effects, on the susceptibility of these effects to antagonism by opioid antagonists such as naloxone or naltrexone, and on the ability of these compounds to induce tolerance and selectively exhibit cross-tolerance to other opioid agonists.

One interesting feature that has been investigated using the opioid drug discrimination paradigm is the effect of changes in training dose on the slopes of generalization gradients, on profiles of substitution, and on antagonism by opioid antagonists. A potentially useful way to interpret data collected in opioid drug discrimination experiments is based on receptor theory involving the concepts of pharmacological selectivity and efficacy. A behavioral context from which to interpret results obtained when training dose is manipulated involves the issue of discriminative stimulus control. The changes in slopes of generalization gradients, profiles of substitution, and antagonism by opioid antagonists that occur when training dose is manipulated will be the topic of this paper. This analysis includes both behavioral and pharmacological perspectives.

CHANGES IN TRAINING DOSE

Changes in Generalization Gradients

A number of investigators have shown that training dose is an important variable in the drug discrimination paradigm (Colpaert et al. 1980*a,* 1980*b;* Hottzman 1982; Koek and Woods 1989; Shannon and Holtzman 1979). For example, in a study examining the effects of progressively tower training doses in rats discriminating 0.04 mg/kg fentanyl from saline, Colpaert et al. (1980*b)* showed that stimulus control could be maintained at a training dose as low as 0.0025 mg/kg. In this study, the slope of the fentanyl generalization gradient decreased as the training dose was decreased (figure 1). Similarly, the slope of morphine generalization gradients was shallower as training dose was reduced. Although the ED_{50} for stimulus generalization also decreased with a reduction in training dose, the concomitant decrease in slope may indicate that the apparent change in potency might be due simply to a loss of stimulus control.

Different Patterns of Substitution

Another effect of varying training dose is to alter the pattern of stimulus generalization to other drugs, both opioid and nonopioid (Colpaert et al. 1980*a*; Koek and Woods 1989; Shannon and Holtzman 1979). For some test compounds, increasing the dose of the training compound decreases the percentage of drug-appropriate responding produced by those test compounds, while decreasing the training dose increases the percentage of drug-appropriate responding. Shannon and Holtzman (1979), for example, showed that nalbuphine and cyclazocine produced more drug-appropriate responding in rats discriminating between saline and 1.75 mg/kg morphine than in rats discriminating between saline and 5.6 mg/kg morphine, while profadol and pentazocine completely substituted for the morphine discriminative stimulus in rats discriminating either dose of morphine (figure 2). Similarly, Koek and Woods (1989) found that pigeons discriminating morphine from saline partially generalized cyclazocine, (-)-N-allylnormetazocine ((-)-NANM) and ketamine, but not U50488 when the training dose was 5.6 mg/kg. However, decreasing the training dose of morphine to 1.8 mg/kg resulted in a shift to the left in the morphine generalization gradient and increased the percentage of drug-appropriate responding after administration of cyclazocine but not (-)-NANM or ketamine. Increasing the training dose of morphine to 18.0 mg/kg resulted in a rightward shift in the morphine generalization gradient and a

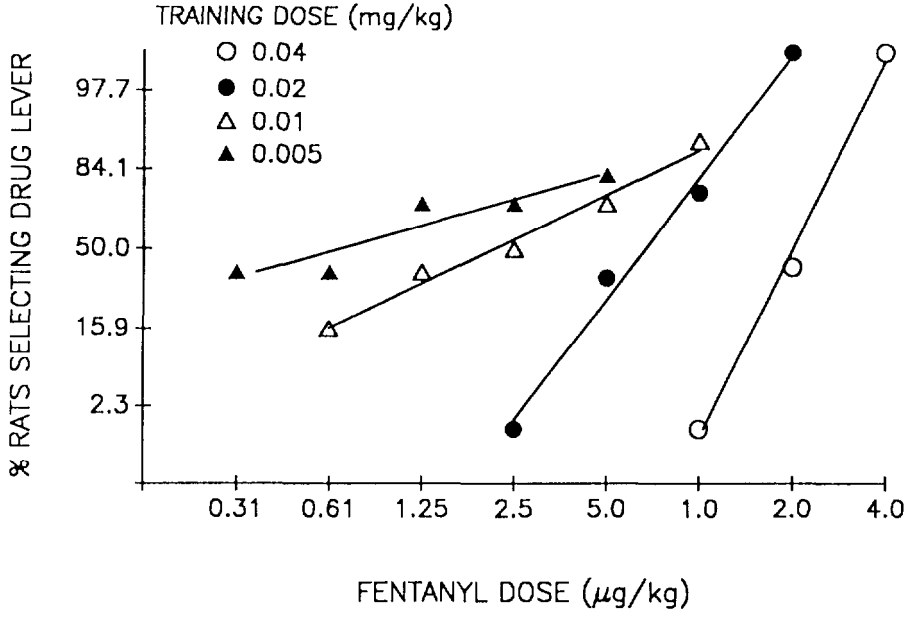

FIGURE 1. *Log-probit plot comparing fentanyl dose-response functions in rats trained to discriminate 0.04, 0.02, 0.01, or 0.005 mg/kg fentanyl from saline (n = 8 per group). Each point represents the percentage of rats making the first 10 responses on the drug lever. (Replotted from Colpaert et al. 1980*b.*)*

decrease in the percentage of drug-appropriate responding after administration of cyclazocine or (-)-NANM, but not ketamine.

Using a different training drug, Colpaert et al. (1980*b*) examined the ability of morphine, cyclazocine, apomorphine, and damphetamine to induce stimulus generalization with the fentanyl discriminative stimulus in rats discriminating either 0.04, 0.02, 0.01, or 0.005 mg/kg fentanyl from saline (figure 3). When the fentanyl training dose was 0.04 mg/kg, neither apomorphine, d-amphetamine, nor cyclazocine produced any fentanyl-appropriate responding. However, cyclazocine produced an increasing amount of fentanyl-appropriate responding as the fentanyl training dose was decreased from 0.02 (29 percent) to 0.01 (50 percent) to 0.005 (83 percent) mg/kg. Damphetamine also produced an increasing amount of fentanyl-appropriate responding when the training dose was progressively lowered, although the maximum percentage of

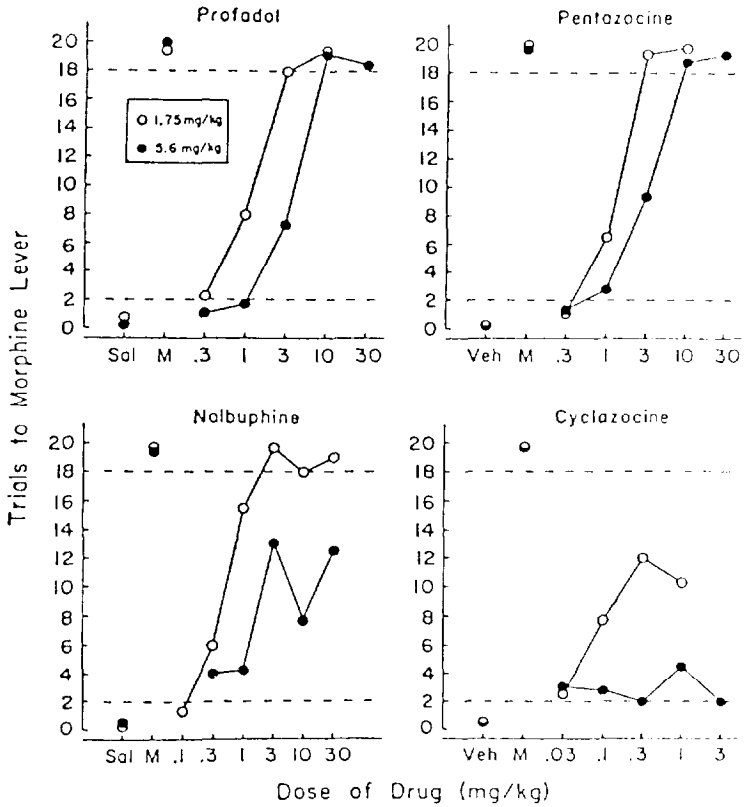

FIGURE 2. *Comparison of the dose-related effects of four analgesics with agonist and antagonist properties in rats trained to discriminate between saline and 1.75 or 5.6 mg/kg morphine. Each point is the mean number of trials completed on the morphine-appropriate choice lever in a 20-trial session; the remaining trials were completed on the saline-appropriate lever. Means are based on one observation in each of 4-5 rats. The mean number of trials completed on the morphine-appropriate fever after an injection of saline (Sal). lactic acid vehicle (Veh), or the morphine (M) training dose are represented by the points above Sal, Veh, and M, respectively. (From Shannon and Holtzman 1979.)*

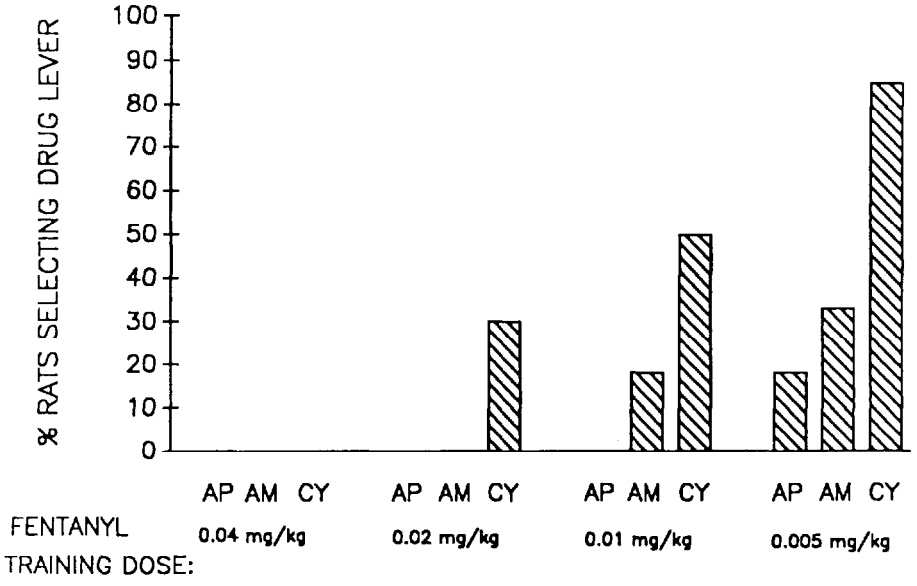

FIGURE 3. *Stimulus generalization of 0.31 mg/kg apomorphine (AP), 0.63 mg/kg d-amphetamine (AM), and 0.04 mg/kg cyclazocine (CY) in rats trained to discriminate 0.04, 0.02, 0.01, or 0.005 mg/kg fentanyl from saline (n = 8 per group). Each bar represents the percentage of rats making the first 10 responses on the drug lever. Where there is an apparent absence of data for the conditions such as for all drugs at 0.04 mg/kg fentanyl Paining dose, no rats selected the drug lever. (Replotted from Colpaert et al. 1980*b.*)*

fentanyl-appropriate responding with amphetamine was only 35 percent, regardless of fentanyl dose. Apomotphine produced a maximum of only 17 percent fentanyl-appropriate responding at all training doses of fentanyl. In contrast, morphine produced greater than 90 percent fentanyl-appropriate responding at each of the fentanyl training doses, although the potency of morphine decreased as training dose decreased. The results of these studies thus show that varying the dose of the training drug can produce differences in the pattern of stimulus generalization using both opioid and nonopioid test drugs. While some drugs such as morphine completely substitute for the fentanyl discriminative stimulus regardless of fentanyl training dose, other

drugs, both opioid (e.g., cyclazocine) and nonopioid (e.g., *d*-amphetamine), partially substitute for fentanyl only when the dose of training drug is small.

Agonist, Antagonist Effects

As described above, under some conditions, some opioids and nonopioids produce only partial substitution. In an attempt to more fully characterize the partial substitution that occurs with various test compounds, Koek and Woods (1989) evaluated the effects of the opioid antagonist naltrexone on the discriminative stimulus effects produced by morphine, cyclazocine, (-)-NANM, U50488, and ketamine in pigeons discriminating between 5.6 mg/kg morphine and saline. Morphine produced greater than 90 percent and U50488 produced less than 10 percent drug-appropriate responding, while cyclazocine, (-)-NANM, and ketamine produced intermediate levels of drug-appropriate responding. Naltrexone completely antagonized the discriminative effects of morphine, cyclazocine, and (-)-NANM but not ketamine, indicating that morphine, cyclazocine, and (-)-NANM most likely produce their discriminative stimulus effects through opioid mechanisms whereas, under these conditions, ketamine probably produces its discriminative effects through a nonopioid mechanism.

Drug Combinations

Another method of evaluating the effects produced by drugs that either partially or fully substitute for a training drug is to examine the effects of these compounds administered in combination. For example, (-)-NANM, cyclazocine, and ketamine were evaluated in combination with morphine in pigeons discriminating between saline and one of several doses of morphine (Koek and Woods 1989). When the training dose of morphine was 1.8 mg/kg, cyclazocine completely substituted for the morphine discriminative stimulus while (-)-NANM and ketamine partially substituted for morphine; (-)-NANM, but not ketamine, antagonized the ability of cyclazocine to substitute for the morphine discriminative stimulus. When the training dose of morphine was increased to 18.0 mg/kg, the morphine-like discriminative stimulus effects of cyclazocine and (-)-NANM decreased. Under these conditions where cyclazocine and (-)-NANM failed to substitute for morphine, both compounds antagonized the discriminative stimulus effects of morphine. In contrast, the percentage of morphine-appropriate responding produced by ketamine was not changed by increasing the training dose of morphine to 18.0 mg/kg; moreover, ketamine failed to antagonize morphine at either training dose. Together with the results

showing no antagonism by naltrexone of the apparent morphine-like discriminative stimulus effects of ketamine, these results further support the notion that the effects of ketamine under these conditions are nonopioid. The potency of naltrexone in antagonizing the discriminative stimulus effects produced by different agonists provides strong evidence that these agonists produce their discriminative stimulus effects through a similar mechanism (e.g., Bertalmio and Woods 1987). However, changes in the percentage of morphine-appropriate responding and the ability of some compounds to antagonize morphine when training dose is varied can also be used to evaluate whether or not drugs are producing discriminative stimulus effects through a similar mechanism.

Colpaert and Janssen (1984) evaluated a larger range of drugs in rats trained to discriminate 0.04 mg/kg fentanyl from saline and found that cyclazocine, nalorphine, ketocyclazocine, and ±NANM partially substituted for the fentanyl discriminative stimulus. These compounds also partially antagonized the fentanyl discriminative stimulus, which is consistent with, but does not prove that the discriminative stimulus effects of these compounds were similar to each other. These investigators also found that analysis of data from individual animals was important in evaluating the discriminative stimulus effects of agonists because not all of the animals produced the same pattern of responding: some rats responded completely on the fentanyl-associated lever while others never responded on the fentanyl-associated lever. In animals in which cyclazocine did not substitute for fentanyl, cyclazocine antagonized fentanyl. In animals in which cyclazocine did substitute for fentanyl, doses of cyclazocine with no apparent discriminative stimulus effects failed to antagonize the discriminative stimulus effects of fentanyl. Finally, nalorphine and ketocyclazocine substituted for fentanyl in the same animals in which cyclazocine substituted for fentanyl. Thus, different patterns of responding occur in groups of subjects when training dose is manipulated; different patterns of responding also occur among individual subjects with training dose.

WHY?

In summary, changes in the dose of a training drug in a drug discrimination study can produce very dramatic effects including the following: changes in the potency of different agonists; changes in slopes of generalization gradients to the training drug as well as to other drugs; different patterns of substitution for drugs from the same and different pharmacological classes (e.g., opioids and nonopioids); and changes in the patterns of discriminative stimulus effects when

drugs are studied in combination. These effects that occur as training dose is manipulated could be a result of a variety of factors including changes in levels of discriminative stimulus control, changes in the pharmacological selectivity of a training drug, or differences in efficacy among drugs and efficacy requirements across experimental conditions. One or more of these factors may contribute to the results obtained as training dose is varied.

Discriminative Stimulus Control

The notion that changes in levels of discriminative stimulus control account for some of the effects observed when training dose is varied is supported by experiments (Colpaert et al. 1980*a*, 1980*b*) in which rats discriminating 0.04 mg/kg fentanyl from saline were trained to progressively smaller doses of fentanyl. In these animals, the two measures of performance were the number of sessions required to reach a predetermined criterion level of performance and the accuracy in responding after criterion had been met. The number of sessions to criterion increased as training dose was decreased, possibly indicating that the stimulus produced by the training drug was more difficult to differentiate from the nondrug condition as training dose was reduced. Two types of errors occur in a simple two-choice (drug, vehicle) discrimination: (1) responding on the drug lever in the absence of the training stimulus or (2) responding on the saline lever in the presence of the training stimulus. Colpaert et al. (1980*a*) found that the total number of errors increased both on drug training days and on saline training days as the training dose was decreased; however, the accuracy of responding during drug training sessions was better than during saline training sessions as training dose was decreased, indicating the possibility of a drug-lever bias and not simply a loss of stimulus control.

In describing effects that are produced by different agonists under conditions of different training doses, loss of discriminative stimulus control, as evidenced by errors in lever selection, could account for decreases in the slope of generalization gradients as training dose is decreased. Furthermore, to the extent that a drug-lever bias occurs with small training doses, animals will have a higher probability of showing drug-appropriate responding in the absence of the training stimulus (e.g., pharmacologically unrelated drugs). Thus, while stimulus control might contribute to the changes in slopes of generalization gradients and different patterns of substitution that result from manipulations of training dose, it does not provide a satisfactory explanation for why the compounds that produce less drug-appropriate responding antagonize the

compounds that produce more drug-appropriate responding. Other factors must also contribute to the effects that occur when training dose is altered.

Pharmacological Selectivity

Differences in the pharmacological selectivity of a training drug at different doses also might contribute to the changes in potencies and slopes of the generalization gradients and the patterns of substitution that occur as training dose is altered. Kuhn et al. (1976) showed that in rats trained to discriminate 10.0 mg/kg pentazocine from saline, morphine completely substituted for the pentazocine stimulus. Moreover, naloxone antagonized the discriminative stimulus effects of morphine and of pentazocine, indicating that these two drugs produced their discriminative stimulus effects through similar mechanisms. White and Holtzman (1982) trained squirrel monkeys to discriminate 3.0 mg/kg pentazocine from saline and showed that the opioid mu agonist levorphanol, as well as the nonopioids phencyclidine and dextrorphan, substituted for the pentazocine discriminative stimulus. The opioid kappa agonists ketocyclazocine and ethylketocyclazocine and the mixed-action opioid nalbuphine produced less than 50 percent pentazocine-appropriate responding. The nonopioids apomorphine, *d*-amphetamine, secobarbital, and mescaline produced no pentazocine-appropriate responding. In these animals, naltrexone antagonized the discriminative effects of L-pentazocine and levorphanol, but not dextrorphan, indicating that the discriminative stimulus effects of pentazocine could have both an opioid and a nonopioid component.

This hypothesis could explain the different patterns of responding sometimes observed when the training dose is varied; that is, at some training doses, drugs like pentazocine might be selective for a particular receptor type and as the training dose is increased pentazocine might also act at other receptors. Under the condition in which the training dose is such that more than one receptor type is activated, drugs that act at either receptor type may substitute, while at lower training doses, drugs that activate only a single receptor type may substitute. The notion that selectivity differences occur with different training doses can also account for why naltrexone antagonizes the discriminative effects of some, but not all, of the compounds that substitute for the discriminative stimulus effects of pentazocine. At larger training doses, when pentazocine might have discriminative stimulus effects that are nonopioid, some nonopioid test drugs might substitute and these discriminative stimulus effects would not be antagonized by naltrexone.

Efficacy

Because for any agonist small doses presumably occupy fewer receptors than large doses, different amounts of receptor stimulation probably occur when different doses are administered. If receptor occupancy was the only determinant of the magnitude of a response, all agonists would be equally effective in producing a maximal response and simply increasing the dose of agonist would be sufficient to produce that level of response. However, the dose of drug administered is not the sole determinant of the effectiveness of drugs in producing biological responses. Agonists also vary in a property, independent of potency, that enables them to activate receptors and thereby produce responses. Because different agonists vary in their abilities to produce a response once the receptor is activated, they are said to have different efficacies. For a given level of effect, high-efficacy agonists occupy fewer receptors while low-efficacy agonists occupy more receptors. Conversely, at equal levels of receptor occupancy, an agonist with higher efficacy will produce a larger effect than an agonist with lower efficacy (e.g., Kenakin 1987).

Regarding results with drug discrimination procedures then, a third explanation that could account for the changes in potency, slopes of the generalization gradients, and patterns of substitution produced by varying the training dose of a compound is that the efficacy requirements of the drug discrimination paradigm change with training dose. A lower efficacy agonist will not substitute for an agonist with higher efficacy when the training dose of that high-efficacy agonist is large, but the low-efficacy agonist will substitute under small training dose conditions. Many drugs substitute for a small training dose of high-efficacy agonist, even drugs that need to occupy all of the receptors to produce this small stimulus. However, when a large dose of a high-efficacy agonist is used as the training stimulus, agonists with low efficacy do not substitute because the stimulus cannot be produced, even though in principle all of the receptors are occupied.

Under conditions in which a low-efficacy agonist fails to substitute for a high-efficacy agonist, it is important to confirm that these drugs produce their effects through similar receptor types. One method of confirming that agonists with different efficacies are producing their effects through similar receptor types is to antagonize the discriminative stimulus effects of both low- and high-efficacy agonists with an antagonist such as naloxone, which has no efficacy. If these drugs are producing their discriminative stimulus effects through similar receptor types, the potency of naloxone in antagonizing the

discriminative stimulus effects of these agonists should be the same. Furthermore, according to receptor theory, low-efficacy agonists that fail to substitute for the discriminative stimulus effects produced by a high-efficacy agonist should antagonize the discriminative stimulus effects of agonists that do substitute. Once it has been established that similar receptor types mediate the discriminative stimulus effects of a set of drugs, a number of different methods can be used to determine whether or not these drugs vary in efficacy.

Irreversible Antagonists. One way of evaluating efficacy differences among agonists is to study drugs under conditions in which the number of available receptors is systematically decreased. Irreversible antagonists are tools that can be used to inactivate a portion of the receptor population (Furchgott and Bursztyn 1967). Although, like irreversible antagonists, competitive antagonists also produce rightward shifts in agonist dose-effect curves, they cannot be used to differentiate agonists with varying efficacies. Since the agonist-competitive antagonist interaction is reversible and governed by the law of mass action, the maximum level of effect produced by an agonist is not altered. In contrast, once bound, irreversible antagonists do not dissociate from receptors; therefore, increasing amounts of irreversible antagonist result in decreasing numbers of available receptors. When a proportion of the receptor population is inactivated by a particular dose of an irreversible antagonist, dose-effect curves for high-efficacy agonists may be shifted to the right without a decrease in the maximum effect. Dose-effect curves for low-efficacy agonists, on the other hand, will also be shifted to the right in the presence of the same dose of irreversible antagonist, but a decrease in the maximum effect also occurs. A larger dose of irreversible antagonist would be required to produce a decrease in the maximum effect produced by a higher efficacy agonist.

Since low-efficacy agonists must occupy a larger number of receptors than high-efficacy agonists to produce a given level of effect, inactivating a proportion of receptors with an irreversible antagonist results in a decrease in the maximum effect produced by low-efficacy agonists before a decrease occurs in the maximum effect produced by a high-efficacy agonist. Thus, administration of different doses of an irreversible antagonist can be used to differentiate agonists with different efficacies. While several investigators have indeed shown that irreversible antagonists can reduce the maximum effect produced by certain agonists in rodent analgesia assays (Adams et al. 1990; Porreca et al. 1982; Tallarida and Cowan 1982), very little is known about the effects of the interaction between irreversible antagonists and agonists that vary in efficacy in the drug discrimination paradigm.

Tolerance. Another pharmacological tool that can produce agonist dose-effect curves that are qualitatively the same as those obtained with irreversible antagonists is chronic treatment with doses of agonist sufficient to induce tolerance. While chronic treatment with an agonist does not necessarily produce a reduction in receptor number, this method appears to produce the same result as treatment with an irreversible antagonist and therefore might be a useful technique to evaluate and confirm efficacy differences among agonists.

One experiment demonstrating that agonist efficacies can be differentiated in tolerant animals was conducted by Young et al. (1991). Rats discriminating 3.2 mg/kg morphine from saline were chronically treated with 10.0 mg/kg morphine either once or twice daily for at least 2 weeks, during which time training was suspended. After twice daily treatments with morphine, the dose-effect curves for morphine, etorphine, methadone, and buprenorphine were shifted to the right with no decrease in maximum levels of drug-appropriate responding, as compared to dose-effect curves for these compounds prior to chronic treatment. Furthermore, the morphine dose-effect curve was shifted further to the right than etorphine, buprenorphine, or methadone, suggesting that morphine is less efficacious than these compounds. While the dose-effect curve for nalbuphine was also shifted to the right after chronic morphine treatment (10 mg/kg every 12 hr) as compared to pretreatment dose-effect curves, the maximum level of drug-appropriate responding was also decreased (figure 4). In contrast, in animals chronically treated with only a single dose of 10.0 mg/kg morphine per day, the nalbuphine dose-effect curve was shifted to the right but the maximum was not decreased. To the extent that etorphine, buprenorphine, methadone, and nalbuphine are producing their discriminative stimulus effects through the same receptor type, these data are consistent with the notion that nalbuphine has lower efficacy than the other agonists tested because the dose-effect curve for nalbuphine was shifted down and to the right rather than simply to the right as were the other agonists. Because tolerance and treatment with an irreversible antagonist appear to produce similar results, they are both useful as tools to differentiate agonists with different efficacies.

Results from yet another study (France and Woods 1990) demonstrated that efficacy differences could be detected among various agonists in animals chronically treated with morphine. Pigeons received 10.0 mg/kg/day morphine 6 hr prior to sessions and discriminated among injections of 0.032 mg/kg naltrexone, saline, and 10.0 mg/kg morphine. The discriminative stimulus effects of a number of mu-receptor-selective agonists, including etonitazene, nalbuphine, meperidine, butorphanol, and buprenorphine, were evaluated in

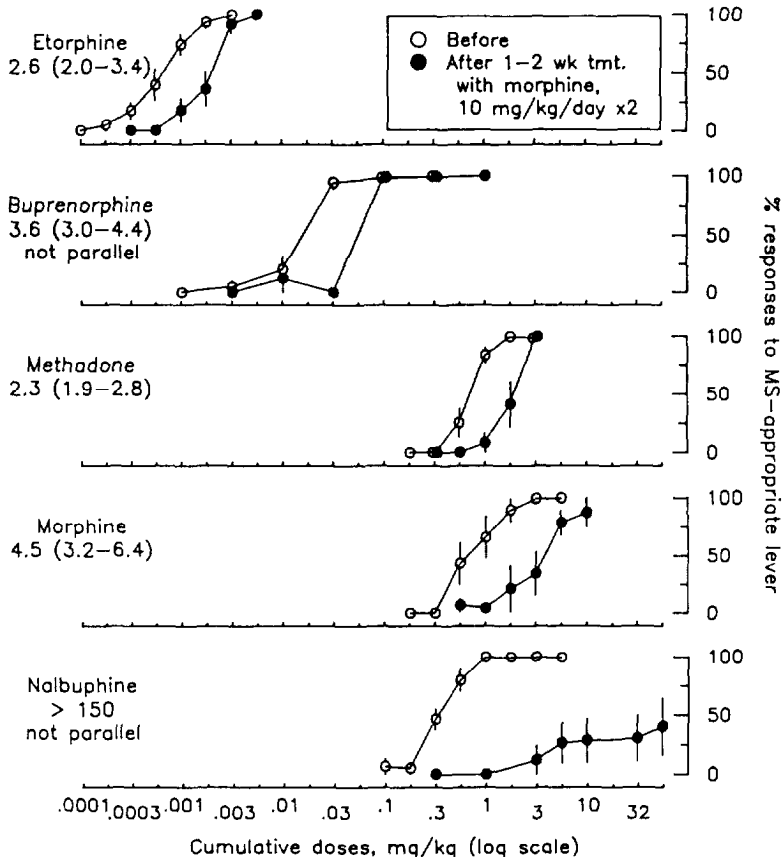

FIGURE 4. *Comparison of dose-response functions for morphine-like stimulus (MS) effects before and during repeated treatment with morphine, arranged by potency before treatment, Open circles represent pooled generalization tests conducted before repeated treatment. Closed circles represent pooled generalization tests conducted after 1 and 2 weeks of repeated treatment with twice daily injections of 10.0 mg/kg morphine. Tolerance ratios and 95 percent confidence levels were calculated by parallel line assay. Dose-response functions for etorphine, methadone, and morphine were parallel across treatments (n = 5-7 per group). (from Young et al. 1991.)*

pigeons that had received either morphine (the dependent condition) or saline (the abstinent or withdrawal condition). In the dependent condition, while etonitazene completely substituted for morphine, buprenorphine only partially substituted for the morphine discriminative stimulus. Butorphanol, meperidine, and nalbuphine, on the other hand, partially substituted for naltrexone. In the abstinent condition, pigeons responded primarily on the naftrexone-associated key when saline was administered under test conditions. Under this condition, increasing doses of etonitazene produced a switch in responding from the naltrexone to the saline to the morphine key. In contrast, buprenorphine and butorphanol produced a switch from the naltrexone to the saline key without a further switch to the morphine key. Nalbuphine and meperidine produced only a partial switch from responding on the naltrexone to the saline key.

Both the antagonism of morphine and naltrexone produced by buprenorphine, butorphanol, and nalbuphine and the patterns of substitution that occur with these drugs under these conditions are generally consistent with results obtained using the standard two-key discrimination. The efficacy requirements of switching responding from the naltrexone to the saline key in the abstinent condition might be analogous to a low training dose condition, while a further switch in responding from the saline to the morphine key is analogous to the high training dose condition. Thus, only agonists with high efficacy (e.g., etonitazene) will produce a switch in responding from the naltrexone to the saline to the morphine key, while tower efficacy agonists (e.g., buprenorphine) will only produce a switch from the naltrexone to the saline key. One inconsistency between the data collected in the two-key procedure (Young et al. 1991) and the three-key procedure (France and Woods 1990) is that buprenorphine is apparently more efficacious than morphine in the two-key procedure and is less efficacious than morphine in the three-key procedure. Further experimentation is required to resolve this issue.

Also consistent with predictions based on results using two-key procedures is that drugs with low efficacy antagonize drugs with high efficacy. In the dependent condition, doses of buprenorphine, butorphanol, and nalbuphine that produced saline-appropriate responding antagonized the discriminative stimulus effects of both morphine and naltrexone. While nalbuphine does not have enough efficacy to mimic the discriminative stimulus effects of morphine, nalbuphine antagonizes the effects of morphine, a higher efficacy agonist. These results confirm then that efficacy differences do indeed exist among agonists and may be detected using both the two-choice and the three-choice drug discrimination paradigm.

CONCLUSION

While toss of discriminative stimulus control or changes in pharmacological selectivity might play a role in the effects that are produced when training dose is varied in opioid discrimination experiments, differences in efficacy and changes in efficacy requirements are also probably important factors contributing to the results obtained. The notion that efficacy differences exist among agonists that activate the same receptors has been supported experimentally in animals chronically treated with an agonist. The fact that some opioids, such as nalbuphine, do not produce a full effect under conditions in which the chronic treatment conditions induce a large amount of tolerance suggests that these agonists have tower efficacies than agonists that do produce a full effect under similar conditions. Thus, when the efficacy requirement of the assay is changed, such as when the training dose is varied, low-efficacy agonists show different patterns of substitution.

Other drugs, for example, morphine, etonitazene, or fentanyl, that presumably have higher efficacies will produce a full effect even when the efficacy requirement is high, such as when the training dose is increased. In addition, the notion of efficacy differences might also explain why drugs such as cyclazocine antagonize the discriminative stimulus effects of morphine when the training dose of morphine is increased. While the efficacy requirements in the high training dose condition are such that cyclazocine cannot produce a full effect, cyclazocine still occupies the same receptors that morphine activates to produce its discriminative stimulus effects; cyclazocine effectively acts as an antagonist under these conditions. Thus, the notion of efficacy differences among agonists appears to explain a number of results obtained when training dose is varied in the drug discrimination paradigm. Other variables, however, such as loss of discriminative stimulus control and changes in pharmacological selectivity of agonists may also contribute to this profile of effects. Hence, both a behavioral and pharmacological analysis of these outcomes will be necessary to account for the varied results of changes in training dose in opioid discrimination studies.

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Discriminative Stimulus Properties of Phencyclidine and Other NMDA Antagonists

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INTRODUCTION

Although phencyclidine (PCP) was initially developed as an injectable anesthetic, most of the scientific interest in it is based on its abuse. It should be noted that PCP is a problem almost exclusively in North America, with little evidence of abuse in other areas of the world. Worldwide interest in PCP research has been sparked by the discovery that PCP and other PCP-like drugs, such as ketamine and dizocilpine (MK-801), are antagonists of glutamatergic neurotransmission (Lodge et al. 1987) and thus could offer some protection against excitotoxic neural injury (Foster et al. 1987; Olney 1990). The possibility that excitatory amino acid antagonists may have therapeutic potential as neuroprotectants and/or anticonvulsants has led to concern about their potential to produce PCP-like psychotomimetic side effects and, more generally, to an interest in understanding more fully the neural basis for PCP abuse.

PCP produces an intoxication in humans characterized by altered perceptual experiences and feelings of dissociation from the environment (Luby et al. 1962; Pollard et al. 1965). PCP abusers report a dreamy, carefree state with impaired judgment and heightened enjoyment of various activities (Siegel 1978). Occasionally, PCP intoxication results in panic states and highly disordered thinking that can be dangerous to both the user and to others (McCarron 1986). The fact that this pattern of effects is not produced by other common drugs of abuse suggests that PCP might produce a unique profile of behavioral and pharmacological effects relevant to its abuse.

Considerable research has been carried out in animals using drug discrimination procedures to further study the perception of intoxication with PCP-like drugs. However, since the late 1950s no experimental laboratory studies of PCP intoxication in humans have been carried out, presumably

because of risks to human subjects. Thus, drug discrimination studies with PCP-like drugs have been the only source of experimental information on the nature and underlying neural basis of PCP intoxication. This situation is somewhat unique among drugs of abuse in that no reference data are available on the subjective effects of PCP using standard assessments such as the Addiction Research Center Inventory or the Profile of Mood States. Often, this has led to considerable confusion concerning the nature of PCP-like effects.

There is no need here to provide an extensive historical review of drug discrimination studies with PCP-like drugs because this has been done recently in other publications (Balster 1987; Balster and Willetts 1988). However, before proceeding to a more thorough discussion of recent studies, it will be helpful to summarize some of the main findings of earlier work.

PHARMACOLOGICAL SPECIFICITY OF PCP DISCRIMINATION

Substitution studies in subjects trained to discriminate PCP or another PCP-like drug, such as ketamine, generally demonstrate considerable pharmacological specificity for the PCP stimulus (Browne 1986; Poling et al. 1979; Shannon 1983). Opioids, sympathomimetic stimulants, anticholinergics, and other classes of hallucinogens do not generalize from PCP. Perhaps the only exceptions are central nervous system (CNS) depressants such as ethanol and the barbiturates, in which cross-substitution with PCP appears to depend somewhat on the test conditions. Barbiturates can often result in partial substitution for PCP, although this is often accompanied by response-rate suppression (McMillan 1982; McMillan and Wenger 1983; Overton 1975; Willetts and Balster 1988*b*). Recent evidence (Mansbach and Balster 1991) suggests that the level of generalization from PCP to pentobarbiial is affected by training dose of PCP, with lower training doses associated with more PCP-lever selection. This is consistent with the results of other studies showing less pharmacological specificit at lower training doses of other drugs (Colpaert et al. 1980; Shannon and Holtzman 1979).

Further evidence for cross-generalization among PCP and CNS depressants comes from recent studies with ethanol discrimination. In pigeons trained to discriminate ethanol from vehicle, both PCP and ketamine fully substituted for the ethanol stimulus (Grant et al. 1991). In ethanol-trained mice, PCP and ketamine also substituted for ethanol, but unlike the results in pigeons, PCP-lever responding produced by ethanol was accompanied by response-rate disruption (Grant et al. 1991). This emerging evidence for a degree of overlap in

the discriminative stimulus properties of PCP and classical CNS depressants may reflect the ability of PCP-like drugs to produce other depressant behavioral and pharmacological effects (Balster and Wessinger 1983).

Although the discriminative stimulus properties of PCP are not generally shared by other drug classes, certain chemical analogs of PCP produce complete cross-generalization with PCP or ketamine, and the structural requirements for the production of PCP-like discriminative stimulus effects of arylcyclohexylamines are quite well established (Balster and Willetts 1988; McMillan et al. 1988; Overton et al. 1989).

A number of other drugs are capable of producing PCP-like discriminative stimulus effects (figure 1). They do not bear an obvious structural relationship to PCP and themselves comprise diverse chemical groups.

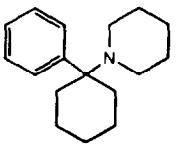
Studies with these classes of PCP-like drugs have made important contributions to our understanding of the neural basis of PCP discrimination.

PCP and Sigma

Working independently, two laboratories used receptor binding technology with ^{3}H-PCP to identify a specific binding site in the mammalian nervous system for PCP-like drugs (Vincent et al. 1979; Zukin and Zukin 1979). The functional significance of this site for the discriminative stimulus effects of PCP was established by Zukin and Zukin (1979), who found a good structure-activity correlation between binding affinity and potency for PCP-like discriminative stimulus effects as reported by Shannon (1981). Of particular importance was the finding that (±)-N-allylnormetazocine (NANM) both displaced radioligands at the PCP site and substituted for PCP in drug discrimination studies (Brady et al. 1982; Holtzman 1982; Shannon 1981). This drug had been widely studied as the putative sigma (σ)-opioid agonist SKF-10,047 as defined by Martin and his colleagues (1976) on the basis of a selective pharmacological profile in the dog. This led to the identification of the PCP binding site as the elusive o-receptor (Quirion et al. 1981; Zukin and Zukin 1983).

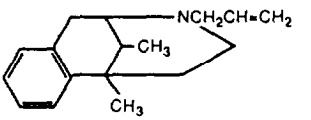
This association of PCP with a-receptors has now led to considerable confusion, which some historical perspective will help to unravel. The problem developed when biochemical pharmacologists using radioligand binding techniques discovered that some σ-agonist benzomorphans, including (+)-NANM, had potent actions on a central nervous system site that was clearly

ARYCYCLOALKYLAMINES



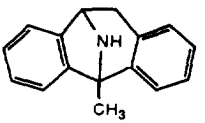
Phencyclidine
(PCP)

BENZOMORPHANS



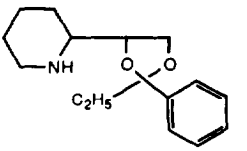
N-Allylnormetazocine
(SKF-10,047)

DIBENZO CYCLOALKENIMINES



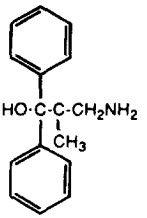
Dizocilpine
(MK-801)

1,3 - SUBSTITUTED DIOXOLANES



Etoxadrol

DIPHENYLPROPANOLAMINES



2-Methyl-3,3-Diphenyl-3-Propanolamine
(2-MDP)

BENZ(f)ISOQUINOLINES

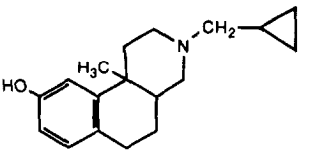


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FIGURE 1. Structures of drugs with PCP (phencyclidine)-like discriminative stimulus effects.

not the PCP site (Su 1982; Tam 1983). Indeed, (+)-NANM had an even higher affinity for this site than for the PCP receptor. Among the most potent ligands for this site was haloperidol, a drug with little affinity for PCP receptors. Another drug that became widely used in binding studies for this site was the dopamine analog (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine[(+)-3-PPP] (Largent et al. 1984). Thus, this site could properly, if not redundantly, be referred to as the haloperidol-sensitive, high-affinity (+)-NANM/(+)-3-PPP binding site. Various combinations of these terms have been widely used. The result of the discovery of this site is that we now have two σ sites, the PCP/σ site and the high-affinity σ site.

To address the nomenclature problem, a group convened at a PCP conference in Ann Arbor, Michigan, to propose some consensus. The proposed solution (Quirion et al. 1987) was to name the haloperldol-sensitive high-affinity site the σ receptor and the historical PCP/σ site the PCP receptor, a proposal that has done little to resolve the confusion, particularly among new entrants to the field who are not familiar with the PCP/σ legacy. This proposal was doubly unfortunate since this binding site had not as yet met criteria established to label it a receptor, and there was little evidence that this site mediated the profile of *a*-agonist effects produced by PCP, (+)-NANM, or other o-agonists.

Which site is responsible for mediating the discriminative stimulus properties of PCP and o-agonist drugs? Most evidence currently points to the PCP receptor as an important neural substrate for PCP discrimination and generalization to arylcyclohexylamines and benzomorphan a-agonists (Balster and Willetts 1988; Kozlowski et al. 1986). There is also considerable evidence that the discriminative stimulus properties of (+)-NANM are mediated through its interaction with the PCP site (Balster 1989; Steinfels et al. 1987*b*). On the other hand, reports of haloperidol antagonism of (+)-NANM discrimination (Steinfels et al. 1987*a*) and substitution by (+)3-PPP (Steinfels et al. 1986) are inconsistent with PCP-receptor mediation.

Studies of the discriminative stimulus properties of more selective drugs for the σ site have produced results that have done little to clarify the role of this site in acute drug effects. In rats trained on a 1,3-di-ortho-tolyl-guanidine (DTG) discrimination (Holtzman 1989), PCP, (+)-NANM, and (+)-pentazocine all substituted for DTG, consistent with a role for the σ site in DTG discrimination. On the other hand, substitution was also obtained with drugs having little or no affinity for the σ site. Indeed, there were negative correlations between o-binding affinity and potency for producing DTG-like effects. Steinfels et al.

(1988) trained a discrimination based on a relatively low dose of the potent σ ligand (+)-pentazocine. In these animals, (+)-NANM substituted for (+)-pentazocine, and it was more potent-the reverse of the affinity of these compounds for σ sites. PCP only partially substituted for (+)-pentazocine.

In summary, it is clear that the PCP receptor is involved in the discriminative stimulus properties of PCP and many σ-agonist benzomorphans. The role, if any, of the high-affinity a-binding site in the discriminative stimulus effects of drugs needs to be determined.

Dioxolanes

Drug discrimination studies with the 1,3-substituted dioxolanes such as etoxadrol and dexoxadrol and their isomers have provided important information about the steric requirements for producing PCP-like effects. PCP itself lacks a chiral center, and steroisomers of other arylcyclohexylamines such as ketamine provide evidence for only relatively small potency differences (Brady and Balster 1982). On the other hand, 1,3-substituted dioxolanes show pronounced stereospecificity. For example, dexoxadrol, but not levoxadrol, substitutes fully for PCP and binds to the PCP receptor (Mendelsohn et al. 1984; Slifer and Balster 1984). Further assessment of the enantiomeric and diastereomeric selectivity of dioxolanes for PCP-like effects has led to an advanced understanding of the active conformations of PCP receptor agonists and the development of molecular models of the PCP pharmacophore (Jacobson et al. 1987; Thurkauf et al. 1988).

Dizoclipine

Dizocilpine (MK-801) has recently played a very important role in furthering our understanding of the function of the PCP receptor in drug discrimination. Dizocilpine is a potent and selective ligand for the PCP site (Wong et al. 1986). It substitutes completely in PCP- or ketamine-trained animals with a potency consistent with its 5- to 10-fold greater affinity than PCP for the PCP receptor (Beardsley et al. 1990; Jackson and Sanger, 1988; Koek et al. 1988; Tricklebank et al. 1987; Willetts and Balster 1988*a*). Furthermore, PCP-like drugs substitute completely in dizocilpine-trained animals (Tricklebank et al. 1989). Thus, dizocilpine, because of its potency and selectivity for PCP receptor activation, has become a widely used tool for probing the function of this cellular site. Of particular importance is its identification as an excitatory amino acid antagonist (Wong et al. 1986).

PHENCYCLIDINE AS AN N-METHYL-D-ASPARTATE ANTAGONIST

Certainly the most important recent development in the neurobehavioral pharmacology of PCP was the discovery that PCP and ketamine were able to selectively block the effects of activation of the N-methyl-D-aspartate (NMDA) subtype of excitatory glutamate receptor (Lodge et al. 1987). It was also found that all drugs which bound to the PCP receptor and had PCP-like discriminative stimulus effects, including dizocilpine, were able to antagonize NMDA (see Lodge and Johnson 1990 for review). Biochemical and electrophysiological studies have led to the development of a model for the NMDA receptor complex, which is represented in figure 2.

In this model, glutamate, or possibly other endogenous ligands, bind to the NMDA-preferring site, resulting in the opening of a cationic channel. The resultant influx of Ca^{++} produces excitatory postsynaptic potentials and, in cases of excessive stimulation, excitotoxicity. Excessive release of glutamate may occur with ischemic stroke or head injury and thus excitatory amino acid antagonists may offer a means of providing neuroprotection. The NMDA receptor may also be involved in epileptogenesis, anxiety, and/or learning and memory, providing additional therapeutic possibilities for drugs that may modify activity at this receptor complex.

The PCP site is thought to lie within the cationic channel. Binding of ligands to the site may physically block the passage of ions, functionally antagonizing NMDA receptor activation. PCP can thus be referred to as a noncompetitive NMDA antagonist. PCP-like drugs are effective antagonists of NMDA-produced convulsions (Church and Lodge 1990; Koek and Colpaert 1990) and other NMDA-produced toxicity (Leander et al. 1988; McDonald et al. 1989). PCP and dizocilpine also have been shown to provide protection against traumatic (Hayes et al. 1988) and ischemic neural injury (Olney et al. 1989).

The emergence of NMDA antagonists as possible therapeutic agents has sparked interest in whether all NMDA antagonists will produce PCP-like intoxication and abuse liability. As reviewed above, there is good evidence that binding to a PCP site is predictive of PCP-like discriminative stimulus effects, and this is certainly true for the potent NMDA antagonist dizocilpine. This fact, combined with the excellent correlation between the potency of PCP-site ligands for antagonism of NMDA and their PCP-like discriminative stimulus effects (Koek et al. 1990; Koek and Woods 1988; Martin and Lodge 1988), provides support for the prediction that all noncompetitive NMDA antagonists

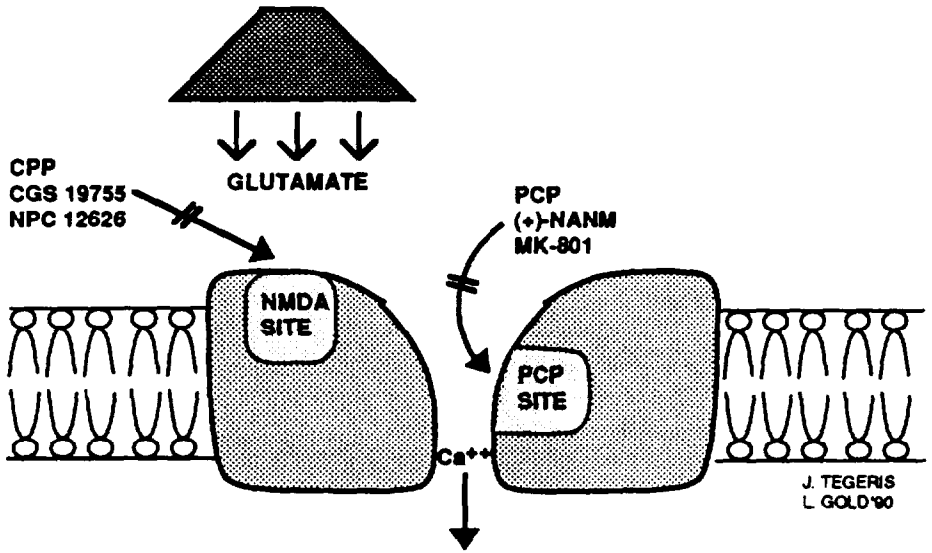

FIGURE 2. *Model of the NMDA (N-methyl-D-aspartate)/PCP (phencyclidine) receptor complex.*

acting on the PCP site are likely to produce PCP-like subjective effects and abuse liability. Results of human testing with dizocilpine and/or other of these drugs will be important in providing validation of the drug discrimination model of subjective effects.

Another class of NMDA antagonist has been developed that does not have affinity for the PCP site. These drugs are competitive blockers of NMDA receptor site activation (see figure 2) and include APH, CPP, CGS 19755, and NPC 12626 (see Watkins et al. 1990 for review). Although competitive NMDA antagonists can produce certain observable PCP-like effects at high doses (Koek and Colpaert 1990; Woods 1989), evidence of differences in their discriminative stimulus effects is beginning to emerge (Willetts et al. 1990). Although studies in pigeons generally show cross-substitution between PCP and competitive NMDA antagonists (Koek et al. 1986; Leander 1989), studies in rats and rhesus monkeys reveal important differences. Competitive NMDA antagonists produce at most only partial PCP-like or dizocilpine-like discriminative stimulus effects, and drug-lever selection when it does occur is usually associated with marked response-rate reductions (Ferkany et al. 1989;

Jackson and Sanger 1988; Koek and Colpaert 1990; Tricklebank et al. 1989; Willetts and Balster 1988*b*). In ketamine-trained rhesus monkeys, CGS 19755 completely fails to substitute (France et al. 1989). In addition, in rats trained with NPC 12626, PCP and dizocilpine produce only partial substitution accompanied by response-rate decreasing effects (Willetts et al. 1989). Of particular importance is the evidence that competitive NMDA antagonists can have behavioral effects, as evidenced by response-rate reductions and antagonism of NMDA, at doses lower than those that produce any evidence of PCP or ketamine-like discriminative stimulus effects (France et al. 1989; Willetts and Balster 1989).

These differences in the profiles of discriminative stimulus effects of noncompetitive and competitive NMDA antagonists are summarized in figures 3 and 4. Figure 3 shows the results of tests with PCP as an antagonist of NMDA discrimination and as a substitute for PCP and NPC 12626. It can be seen that PCP-like effects can be produced at doses of PCP that do not antagonize NMDA, and that NPC 12626-like effects, to the extent that they occur, are produced only at high doses. This is in contrast to the results with CPP. a typical competitive NMDA antagonist (figure 4). It can be seen that CPP is a potent and effective antagonist of NMDA at doses considerably lower than those that produce even partial PCP-like effects. Unlike PCP, CPP substitutes fully for NPC 12626.

There are a number of possible implications of the differences in the discriminative stimulus effects of competitive and noncompetitive PCP-like NMDA antagonists. Of practical importance is the possibility that competitive antagonists may not have PCP-like subjective effects or at least may have good dosage separation between therapeutically useful actions and PCP-like effects. Studies in humans will be needed to confirm this hypothesis, and the results will help in the future interpretation of drug discrimination results in animals.

These results also show that antagonism of NMDA per se is not sufficient to produce PCP-like discriminative stimulus effects. Does this mean that NMDA antagonism does not play a role in PCP discrimination? This seems unlikely because of the strong structure-activity relationship between NMDA antagonism and PCP-like discriminative stimulus effects. I am not aware of any drug that substitutes fully for PCP that does not also antagonize NMDA. One possibility is that the functional consequences for the cell of competitive and noncompetitive antagonism are different enough to result in different profiles of behavioral effects. The analogy with the GABA/benzodiazepine receptor may be worth

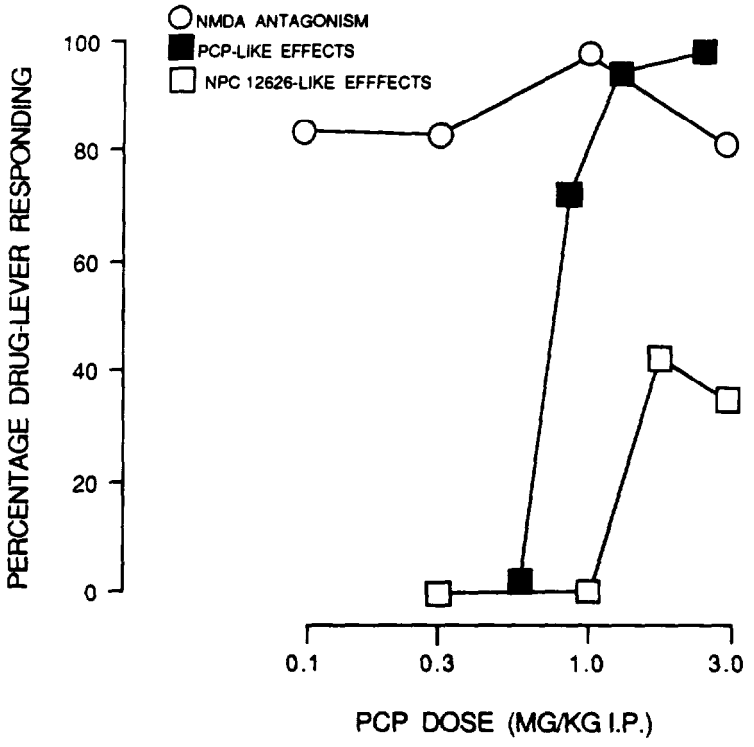

FIGURE 3. *Effects of various doses of PCP (phencyclidine) in three drug discrimination procedures, antagonism of NMDA (N-methyl-D-aspartate) discrimination (Willetts and Balster 1989), substitution for PCP (Willetts and Balster 1988*b*), and substitution for NPC 12626 (Willetts et al. 1989).*

considering, in which direct GABA receptor agonists such as muscimol have a somewhat different profile of discriminative stimulus and other behavioral effects than do indirect acting GABA agonists such as the benzodiazepines and barbiturates (Grech and Balster 1990; Sanger 1985). Differences can also be shown between benzodiazepines and barbiturates (Ator and Griffiths, 1989).

It is also possible that NMDA and PCP receptors are not always functionally coupled to each other (Rao et al. 1990) or perhaps subtypes of NMDA

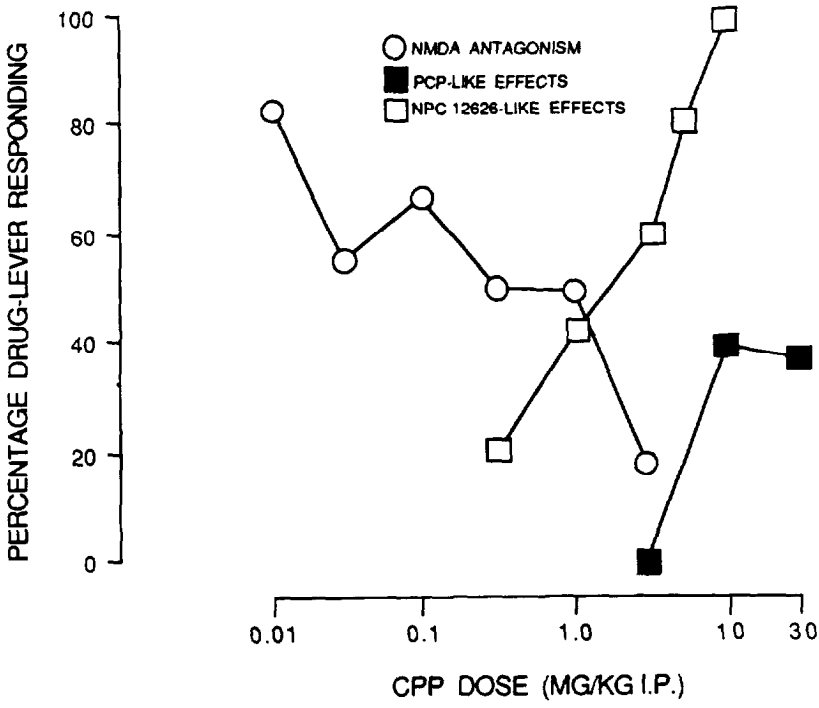

FIGURE 4. *The effects of various doses of CPP in three drug discrimination procedures; antagonism of NMDA (N-methyl-D-aspartate) discrimination (Willetts and Balster 1989), substitution for PCP (phencyclidine) (Willetts and Balster 1988*b*), and substitution for NPC 72626 (Willetts et al. 1989).*

receptors exist with differing propensity for PCP-like channel blockade (Monaghan et al. 1988). In any case, the results of drug discrimination studies with NMDA antagonists have shown once again the power of this methodology to dissect subtle differences in the behavioral effects of psychoactive drugs.

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Discriminative Stimulus Effects of Psychomotor Stimulants and Benzodiazepines in Humans*

Chris-Ellyn Johanson

This chapter will review a series of studies on the discriminative stimulus (DS) effects of psychomotor stimulants and benzodiazepines in humans. Although there has been an enormous increase in studies of the DS effects of drugs in animals over the last two decades (Stolerman and Shine 1985; Stolerman et al. 1989), relatively few studies have been conducted with humans. Human studies are necessary for several reasons. First, as is always the case, our acceptance of results obtained with other species is increased if under comparable conditions, similar results are generated in humans. Second, drug discrimination is presumed to be an animal model of subjective drug effects in humans (Schuster and Johanson 1988). However, to validate this model, it is necessary to measure DS and subjective effects simultaneously and that is possible only in studies that use human subjects.

The present series of studies was designed to determine the similarity between drug discrimination results obtained in animals and results obtained with humans using drugs from two different pharmacological classes, amphetamine and diazepam, as DS. In addition, the subjective effects of these drugs as well as of test compounds were measured simultaneously in order to compare the DS and subjective effects of drugs directly. Because almost no studies on the DS effects of drugs had been conducted with humans when these studies were initiated, it was also necessary to demonstrate the feasibility of conducting such studies in humans despite not being able to expose subjects to large doses of drugs over tong periods of time. Thus, it was necessary to develop a procedure that used relatively low doses and minimized the duration of the training and

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testing period. At the same time, it was considered essential to try to mimic as closely as possible the procedural characteristics used in animal studies. When a determination had been made that humans could learn an AMP or DZ discrimination under these conditions, further evaluations were conducted to verify that the discrimination was pharmacologically sensitive (dose responsitivity) and specific.

METHODS

Subjects

Two series of studies were conducted using either 10 mg *d*-amphetamine (AMP) or 10 mg diazepam (DZ) as the DS. Unless noted, the procedure used in these two series of studies was identical. However, the AMP studies were conducted at the University of Chicago and the results have been published previously (Chait and Johanson 1988; Chait et al. 1984; 1985; 1986*a*; 1986*b*; 1989). The DZ studies were conducted at the Uniformed Services University of the Health Sciences. Subjects from both series were recruited using advertisements posted in their respective university areas and published in local newspapers. In addition, the DZ studies were listed by the normal volunteer recruiting office of the National Institutes of Health.

Potential candidates underwent a physical and a psychiatric examination and received a blood screen prior to participation. Candidates were not accepted if they had a previous history of drug abuse or dependence other than tobacco dependence, a history of psychiatric illness, or any significant present or past medical or psychiatric problem that would place them at risk if they participated in the experiment. Females were not allowed to participate if they were pregnant as determined by a urine test or if they planned to become pregnant in the near future. The protocol was reviewed and approved by the local institutional human use committees and informed consent was obtained. Subjects were paid for their participation.

General Procedure

Subjects were instructed that their job was to learn to discriminate between two different drugs, drug A and drug B. They were told that they could receive either stimulants, sedatives, or placebo, and that drugs A and B would differ. Subjects were not informed that they would be learning to discriminate between an active drug and placebo. Subjects reported to the laboratory three to four times each

week between 8:15 a.m. and 10:00 a.m. over a 6- to 10-week period. Upon arrival, subjects filled out three subjective effects questionnaires as described below. After completion, subjects received a capsule but were then free to leave the laboratory. They took three additional sets of questionnaires with them to fill out 1, 3, and 6 hr later.

Phase 1: Sampling/Training (Sessions 1-4). The purpose of phase 1 was to familiarize subjects with the effects of drug A and drug B and to begin training the discrimination. Subjects received drug A on the first and third sessions, and drug B on the second and fourth sessions. The order of presentation of drugs A and B on the third and fourth sessions was reversed for half the subjects in the DZ experiments. On each of these four sessions, the letter code of the capsule was revealed to the subject prior to ingestion. For half of the subjects in each individual experiment, drug A was placebo and drug B was 10 mg &amphetamine (AMP) or 10 mg diazepam (DZ). The assignments were reversed for the other subjects.

Phase 2: Training/Selection (Sessions 5-11). The purpose of phase 2 was to provide additional training and then select subjects that reliably learned the discrimination. In these seven sessions, subjects received drug A three times and drug B four times or vice versa. They were administered in a mixed order with the restriction that the same drug was not scheduled more than two sessions in succession. The order was different for different subjects. On these sessions, subjects were not told which drug they received when they ingested the capsule. At 1, 3, and 6 hr after capsule ingestion, in addition to the questionnaires described below, subjects filled out a form on which they identified the drug they believed they had received using the A and B letter codes, and indicated on a 100-mm visual analog scale (VAS) how certain they were that their identification was correct. This VAS was labeled ‘Unsure (Just Guessing)” at the left end and “Positive (Absolutely Sure)” on the right end. Subjects were told that they were free to change their identification from hour to hour, based on what they believed at the time. There were no consequences attached to the 1- and 3-hr identifications. However, at the 6-hr period, subjects telephoned the experimenter, identified themselves, and reported their 6-hr letter code identification. If their response was correct, they were told so and received a monetary bonus when they returned to the laboratory for the next session. Those whose responses were incorrect were also informed and received no money at the next session.

The discrimination was considered reliable if at least five of the seven identifications were correct.' Subjects were not informed of this criterion at the beginning of the experiment. Those subjects that failed to reach this level of performance were asked to terminate participation after session 11.

Phase 3: Testing (Sessions 12-end): Subjects that were reliable discriminators as defined above entered phase 3 but were not told that a new phase had started. The purpose of phase 3 was to determine whether subjects would identiiy lower doses of the training drug (DZ or AMP) and other drugs as placebo or the training drug (DZ or AMP). The test phase consisted of six test sessions intermixed with additional training sessions. Test sessions were identical to training sessions except that subjects were not informed when they telephoned whether their response was correct-they were told only that it was a test session and that they would receive money at the next session. Subjects were not told the purpose of test sessions, nor did they know when test sessions were scheduled until after they had reported their 6-hr drug identification.

The additional training sessions were interspersed with test sessions in order to determine whether subjects maintained the discrimination. These training sessions were exactly like the training sessions during phase 2; that is, subjects received either drug A or drug B, were told whether their response was correct when they telephoned, and were reinforced appropriately. Subjects received placebo and the training drug an equal number of times in mixed order on these training sessions. The training sessions were interspersed among the test sessions in an unsystematic fashion with the restriction that no more than two test sessions or two training sessions occurred in succession. The order varied across subjects.

Subjective Effects Questionnaires

Profile of Mood States (POMS). An experimental version of the 65-item POMS (McNair et al. 1971) consisting of 72 adjectives commonly used to describe momentary mood states was used. Subjects indicated how they felt at the moment in relation to each of the 72 adjectives on a 5-point scale from “not at all” (0) to “extremely” (4). There are eight clusters (scales) of items that have been separated using factor analysis. The names of these scales (Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, and Elation)

*The criteria for the series of AMP studies was six of seven correct or five correct in a row. For the DZ experiments the criterion was five of seven coned.

describe the adjectives in the cluster. Two additional (unvalidated) scales (Arousal and Positive Mood) were derived from the other scales as follows:

Arousal=(Anxiety+Vigor)-(Fatigue+Confusion)

Positive Mood=Elation-Depression.

Addiction Research Center Inventory (ARCI). The ARCI is a hue-false questionnaire with empirically derived scales that are sensitive to the effects of a variety of classes of abused drugs. A short form of the inventory consisting of five scales with a total of 49 items was used (Marlin et al. 1971). The five scales were the Morphine-Benzedrine Group (MBG), a general measure of drug-induced euphoria; the Amphetamine (A) and Benzedrine General (BG), which measure amphetamine-like effects; the Pentobarbital-Chlorpromazine-Alcohol Group (PCAG), a measure of sedation; and the LSD, a measure of dysphoria and somatic symptoms.

Visual Analog Scales (VAS). This form has six horizontal 100-mm lines, each labeled with an adjective. These adjectives are “stimulated,” “high,” “anxious,” “sedated,” “down,” and “hungry.” The left ends of the lines were labeled “not at all” and the right ends, “extremely.” Subjects were instructed to place a mark on each line indicating how they felt at that moment.

RESULTS AND DISCUSSION

Amphetamine Discrimination

Across four studies (Chait and Johanson 1988; Chait et al. 1985; 1986*a*; 1986*b*; 1989), 53 of the 100 participants were considered reliable discriminators, having identified the training compounds correctly five times in a row or on six of the seven sessions during phase 2. In subjects that learned the discrimination, the percent of correct identifications and the certainty of identification increased from hour 1 to hour 6, and there were no differences between AMP and placebo. Post hoc analyses revealed that all participants experienced subjective effects typical of those for stimulant drugs, although the discriminators had a tendency to be more sensitive (Chait et al. 1989).

The 53 participants who learned the discrimination entered the test phase. During each of the four studies, different drugs were evaluated during the test phase as follows:

Study 1 (N = 17): Placebo, AMP, DZ, and 2 and 5 mg *d*-amphetamine.

Study 2 (N = 27): Placebo, AMP, 25 and 50 mg phenmetrazine, and 20 and 40 mg fenfluramine.

Study 3 (N = 20): Placebo, AMP, 25 and 75 mg phenylpropanolamine (PPA), and 0.5 and 2 mg mazindol.

Study 4 (N = 36): Placebo, AMP, 100 and 300 mg caffeine, and 25 and 50 mg benzphetamine.

In study 1, seven participants learned the discrimination between placebo and AMP. When tested with these training drugs, they responded correctly. Participants consistently identified 2 mg amphetamine as placebo; at the 5 mg dose, the capsule was identified as placebo 50% of the time across participants. DZ was identified as placebo by five of the seven participants and produced a profile of subjective effects typical for a benzodiazepine. In study 2, 14 participants learned the discrimination and identified phenmetrazine at both doses as AMP. The low dose of fenfluramine was identified as placebo, whereas the high dose produced intermediate levels of AMP-appropriate responding, reflecting both within- and between-subject variability. In study 3, 12 participants learned the discrimination and identified the high doses of both mazindol and PPA as drug. The lower doses were identified as placebo or the identifications were variable. In study 4, 20 participants learned the discrimination and did not reliably identify either caffeine or benzphetamine as AMP.

The profile of subjective effects of drugs (i.e., fenfluramine and DZ) that did not substitute for AMP differed from AMP. On the other hand, phenmetrazine and the high dose of PPA that substituted for AMP produced subjective effects that were similar to AMP. However, mazindol, despite the fact that the high dose substituted for AMP as a DS, produced subjective effects that differed somewhat from those of AMP. More specifically, mazindol's subjective effects were restricted to increases in anxiety, an effect also produced by AMP (Schuster and Johanson 1988). Since these anxiety-increasing effects (as well as the more positive effects such as increased scores on the Arousal scale) were also seen with phenmetrazine, if is possible that they were the basis of the discrimination.

In participants who learned the discrimination, the results with test drugs were similar to those seen in animals. That is, phenmetrazine, mazindol, and PPA-but not fenfluramine or benzphetamine-have been shown to substitute for amphetamine as a DS in rhesus monkeys (de la Garza and Johanson 1987). Furthermore, it may be concluded that the discrimination was dose related and was also pharmacologically specific because DZ was identified as placebo.

DZ Discrimination

One study using DZ as the DS has been completed and a second study is presently under way. In the first study, 16 of 18 subjects learned the discrimination reliably, and 14 of them entered phase 3. The drugs that were tested during phase 3 were 2 and 5 tng DZ, 1 and 2 mg brazepam, 50 mg pentobarbital, and 10 mg AMP. Figure 1 shows the percent of subjects identifying each of the test compounds as DZ across hours 1 to 6. As can be seen, the lowest doses of diazepam and lorazepam were identified as placebo

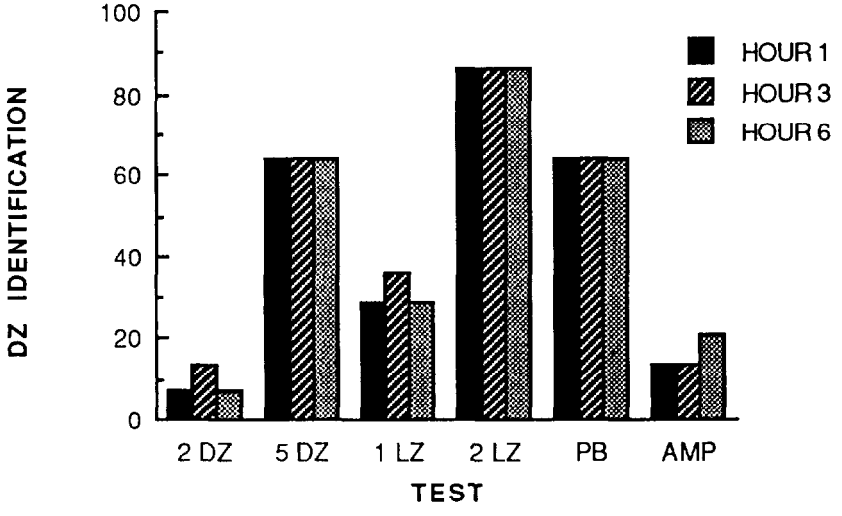

FIGURE 1. *The percent of subjects (N = 14) that identified each of the test drugs as DZ across hours 1, 3, and 6. Only the identification at hour 6 resulted in monetary bonus and subjects were aware that they could change their drug identification at each hour. Abbreviations: DZ=diazepam; LZ=lorazepam; PB=50 mg pentobarbital; AMP= 10 mg* d*-amphetamine. The numbers next to the drug name indicate dose in mg units.*

by the majority of subjects, whereas the 5 mg dose of diazepam was identified as DZ by 64 percent of the 14 subjects. The highest dose of lorazepam was identified as DZ by the majority of subjects. Pentobarbital was similar to the 5 mg dose of diazepam in terms of hour 6 identifications whereas AMP was identified as placebo. Identifications did not change across hours as was also true for identifications of DZ and placebo during training sessions. This indicates that the effects of DZ and drugs identified as DZ had a rapid onset of action. Certainty ratings increased across hours but were already high at hour 1.

In the second ongoing study, eight subjects have completed phase 3. The drugs that were tested during this phase included 25 and 50 mg ttipelennamine, 0.125 and 0.25 mg triazolam, and 5 and 10 mg buspirone. Neither dose of tripelennamine was identified as DZ, nor was either of the lower doses of the other two compounds. However, 0.25 mg triazolam was identified as DZ by seven of the eight subjects. The subject that dii not identify this dose of triazolam as DZ was tested with 0.5 mg triazolam and identified it as DZ. Finally, the 10 mg dose of buspirone was identified as DZ by five of the eight subjects.

In general the results of these DZ discrimination studies are similar to those found with animals. Diazepam and related benzodiazepines have been shown to function as DS in a variety of species, including rats (Colpaert et al. 1976; Haug and Gotestam 1982), baboons (Ator and Griffiths 1983), squirrel monkeys (Spealman 1985), and pigeons (Evans and Johanson 1989; Garza et al. 1987; Järbe and McMillan 1983). In animals trained to discriminate diazepam, other benzodiazepines substitute as DS and the discrimination is dose dependent. For instance, Shannon and Herling (1983) trained rats to discriminate between 1 or 3 mg/kg DZ and found that lorazepam in the same dose range substituted completely for the DZ cue. Triazolam also substituted but was approximately 10 times more potent. Pentobatbital at doses 10 times higher also substituted for the diazepam DS. Drugs from other pharmacological classes, including stimulants and antihistamines have not substituted for benzodiazepines (Evans 1987; Evans and Johanson 1989; Spealman 1985). However, in animals trained to discriminate benzodiazepines, buspirone has consistently failed to be discriminated as drug-like (Evans and Johanson 1989).

GENERAL DISCUSSION

In summary, the results of the studies described above indicate that humans can be trained to discriminate 10 mg *d*-amphetamine or 10 mg diazepam from placebo using a procedure that (1) does not require laboratory space, (2) limits

exposure to drugs by using low doses and a relatively short experimental protocol, and (3) in many ways is comparable to procedures used in animal studies. In the AMP series, it is interesting to note that only half of the participants learned the discrimination. While this finding may indicate that additional methodological studies would be desirable to improve training, the results with the DZ experiment suggest that DZ and AMP differ in discriminability. However, because only a small number of subjects have been tested with DZ and because the differences may have been due only to dose of the training drug (Chait et al. 1989), additional studies are needed to verify this hypothesis. Nevertheless, the procedure appears feasible, the results indicate that the discriminations are pharmacologically sensitive and specific, and the similarities between the findings in humans and in other animals are promising.

One of the goals of the studies that have been described was to evaluate the relationship between DS and subjective effects. Many strategies can be used to determine the correlation between these two measures of drug effect. Because the results of the AMP experiments have been extensively presented in previous publications, these strategies will be illustrated for the most part using results generated from the initial DZ discrimination study.

First, it must be determined whether drugs used as training stimuli produce reliable time-related changes on measures of subjective effects relative to placebo that are similar to those that have been previously reported. In the present experiment, DZ produced effects on several scales of the POMS, ARCI, and VAS that were similar to those previously reported for DZ. For instance, as shown in figure 2, DZ significantly increased scores on the Confusion scale and decreased scores on the Arousal scale of the POMS. Differences from placebo were greatest at hour 1 and had largely disappeared by hour 6. DZ also produced significant increases on Fatigue (POMS), PCAG (ARCI), Down (VAS), and Sedated (VAS) and decreases on Vigor (POMS), A (ARCI), BG (ARCI), MBG (ARCI), and Stimulated (VAS) relative to placebo while following a generally similar time course to that seen in figure 2. This time course was similar to that found in previous studies using acute administration of diazepam.

A second strategy that can be used to evaluate the degree of relationship between subjective and DS effects is to compare subjective effects in subjects that do and do not learn the discrimination. If subjects who do not learn the discrimination also do not report changes in subjective state relative to placebo, there is indirect evidence that these effects form a basis for the discrimination. Because only a few subjects in the DZ experiment failed to learn the

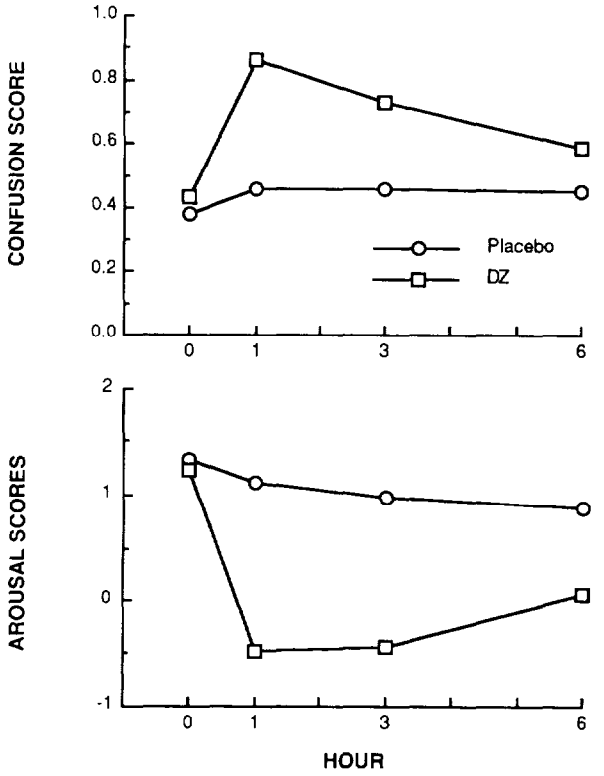

FIGURE 2. *Effects of 10 mg diazepam (DZ) compared to placebo on two scales of the Profile of Mood States (POMS) in subjects (N = 16) that reliably learned the discrimination.*

discrimination, it is necessary to illustrate this strategy using results from the AMP experiment. As shown in figure 3, AMP produced increases in ratings of High on the VAS relative to placebo in subjects that learned the discrimination, whereas the minimal effects in nondiscriminators on this scale were minimal. That was true for other subjective effects as well and is presumptive evidence of the importance of subjective effects in acquiring the discrimination (Chait et al. 1989).

A third strategy is to compare the subjective effects of drugs that are identified as the training drug and those identified as placebo in relationship to the subjective effects of the two training compounds. For instance, as shown in figure 4, most subjects in the DZ experiment identified 2 mg diazepam as

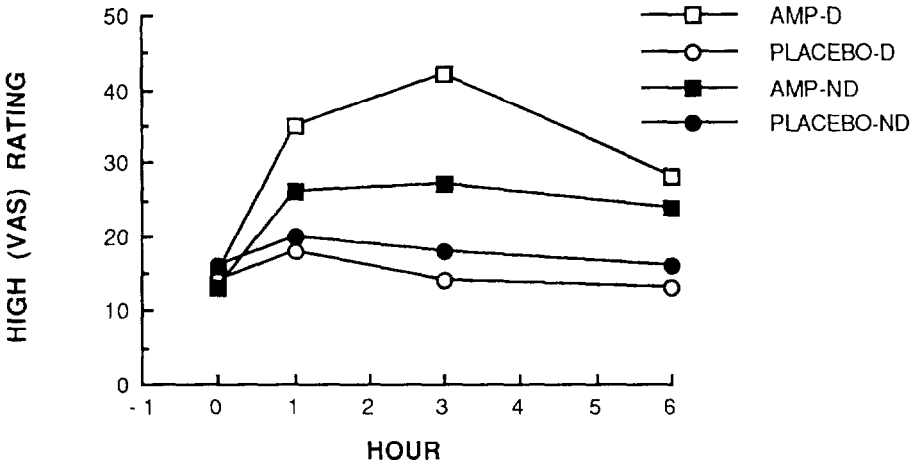

FIGURE 3. *Differences in the effects of amphetamine (squares) and placebo (circles) on one of the scales of the VAS in subjects that learned (N = 53) and failed to learn (N = 47) the amphetamine discrimination. (Redrawn from Chait et al. 1989.)*

placebo, whereas they identified 2 mg lorazepam as DZ. Likewise, the subjective effects of 2 mg diazepam were virtually identical to those of placebo, as illustrated in figure 4 for the POMS Confusion scale. In contrast, the subjective effects of 2 mg lorazepam were similar to the subjective effects of DZ. Again, this is illustrated in figure 4 for the PCAG scale of the ARCI. Thus, drugs that were identified as placebo had placebo-like effects, whereas drugs that were identified as DZ had DZ-like subjective effects. This convergence of effects is particularly striking in the case of AMP, which was tested in DZ-trained subjects and identified as placebo. As shown in figure 5, under these conditions, the subjective effects of AMP were similar to those of placebo. In contrast, in previous experiments using AMP as the training drug, the subjective effects of AMP were different from placebo and typical of stimulant drugs (e.g., increases in the High rating; see figure 3). These contextual effects on alterations in mood clearly need to be evaluated in future studies.

A fourth strategy that can be used to evaluate the relationship between DS and subjective effects is to compare the subjective effects of the same test compound that is identified as placebo by some subjects but identified as the training drug by other subjects. For instance, in the DZ experiment, 64 percent of the subjects identified 50 mg pentobarbital as DZ, whereas the remaining

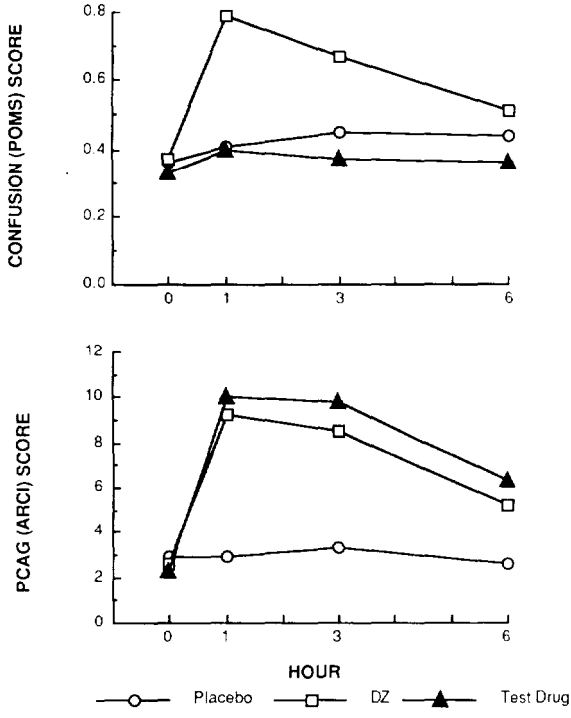

FIGURE 4. *Representative subjective effects of a drug that was identified as placebo (2 mg diazepam) and a drug that was identified as drug (2 mg lorazepam) in subjects trained to discriminate placebo from 10 mg diazepam.*

subjects identified it as placebo. The subjective effects of this test drug might be expected to differ in these two groups of subjects. To test this assumption, a three-way ANOVA was performed, with an added between-group factor. As shown in figure 6, the subjective effects of pentobarbital differed significantly in these two groups of subjects. In subjects that identified PB as DZ, its effects were more similar to DZ than to placebo. Likewise, in the other group of subjects, the subjective effects of PB were more like those of placebo. Similar group differences were reported in a study by Chait et al. (1986*a*). Approximately half of the subjects in that study identified 40 mg fenfluramine as AMP, whereas the remaining subjects identified it as placebo. Correspondingly, in the former group, fenfluramine produced significant increases on the BG

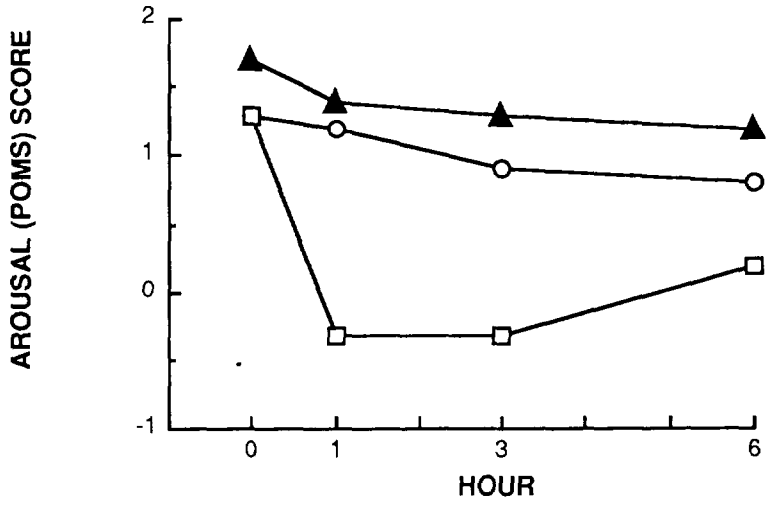

FIGURE 5. *The subjective effects of 10 mg d-amphetamine on the Arousal scale of the POMS in contrast to 10 mg diazepam (DZ) and placebo in subjects trained to discriminate between DZ and placebo.*

scale of the ARCI, the Vigor scale of the POMS, and the Stimulated and High rating on the VAS relative to placebo; in the other group, there were no increases.

In summary, the four strategies used to evaluate the relationship between DS and subjective effects reveal that the correspondence between these two measures is remarkably robust. Reliable differences were found between the subjective effects of the training drug and placebo, and the effects of drug were similar to those that have been reported previously following acute administration of diazepam and amphetamine. Likewise, in the AMP studies in which an appreciable number of subjects did not learn the discrimination, the nondiscriminators failed to report reliable differences in the subjective effects of AMP and placebo. Test compounds that were identified as drug in the DZ experiment produced subjective effects that were similar to those reported following DZ. Likewise, the subjective effects of the drugs that were identified as placebo showed placebo-like changes in mood. Finally, the subjective effects reported by subjects that identified a drug as DZ were similar to those of DZ, whereas among other subjects that identified the same drug as placebo, the subjective effects were like those of placebo.

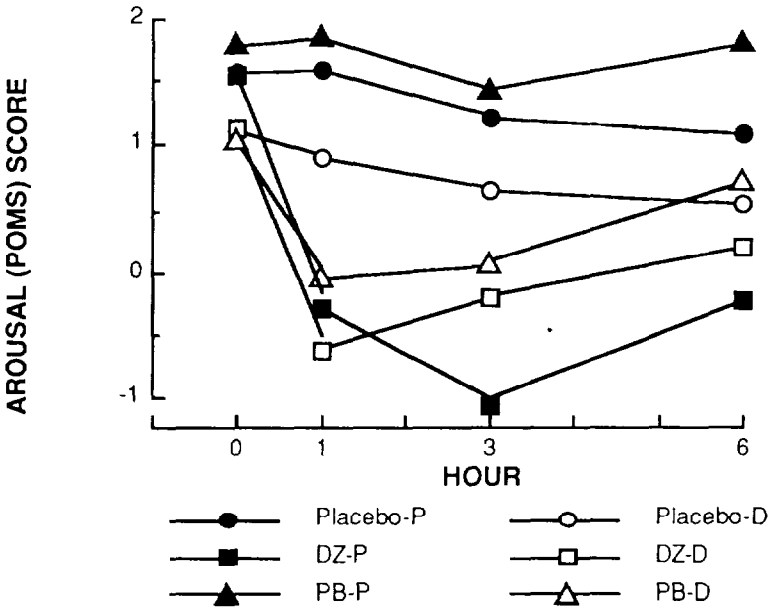

FIGURE 6. *Representative changes in subjective effects (Arousal scale of the POMS) produced by 10 mg diazepam (squares), placebo (circles), and 50 mg pentobarbital (triangles) in subjects that identified 50 mg pentobarbital as placebo (closed symbols) or DZ (open circles).*

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Tolerance to Drugs Acting as Discriminative Stimuli

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The initial effects of many drugs diminish during repeated administration of an initially effective dose, so that larger doses become necessary to produce the original effect, an outcome termed tolerance (Johnson and Fleming 1989). In contrast, sensitivity to drugs acting as discriminative stimuli remains strikingly consistent over extended training periods during which subjects receive regular doses of their training drug. Whether or not this long-lasting sensitivity implies that discriminative stimulus effects of drugs are insensitive to tolerance processes is a topic of debate (reviewed by Colpaert 1978*a;* Young and Sannerud 1989). The present paper reviews studies of whether tolerance develops to the discriminative stimulus effects of drugs, focusing on studies of whether the doses required for maintenance or generalization of stimulus control can be altered by changes in the dose, frequency, duration, or type of repeated drug treatment.

An individual's initial sensitivity to the stimulus effects of a training drug is a product of both the potency of the drug and the behavioral conditions under which it is presented. The doses required for generalization of stimulus control are determined jointly by the dose employed for training and the conditions of differential reinforcement. Generally, subjects trained with higher doses of a drug require higher doses for stimulus control than do subjects trained with lower doses (Beardsley et al. 1987; Colpaert et al. 1980; Shannon and Holtzman 1979; White and Appel 1982). For individual subjects, increasing or decreasing the training dose produces a corresponding increase or decrease in the dose required for stimulus control (Gauvin and Young 1989). Sensitivity to a training drug is also modulated by prevailing reinforcement contingencies. For example, the doses of a drug required for stimulus control can be changed by arranging conditions so that the relative frequency of reinforcement following injections of a drug differs from the frequency of reinforcement following injections of saline (De Vry et al. 1984; Koek and Slangen 1982; McMillan and

Wenger 1984). Changes in sensitivity to a drug stimulus that result from such changes in training dose or reinforcement contingencies are not viewed as instances of tolerance.

Once established, stimulus control by a particular drug is generally maintained over extended periods of training without increases in training dose. Additionally, the range of doses required for generalization can be remarkably consistent over repeated observations. For example, Colpaert and colleagues (1978*a,* 1978*b)* reported that the range of doses of fentanyl or cocaine required for discriminative control does not systematically increase or decrease over extended training periods. In experiments with fentanyl, 2 weeks of daily training with 0.04 mg/kg fentanyl and saline separated each of five test sequences in which selected doses of fentanyl were tested on successive days. In individual subjects, the doses of fentanyl or morphine required for fentanyl-like stimulus effects oscillated by twofold to fourfold over successive tests but did not progressively increase or decrease. Dose-response functions were similar throughout the 17-week test sequence (Colpaert et al. 1976). These results mirror the general observation that sensitivity to the stimulus effects of drugs is maintained over extended periods of repeated drug exposure without evidence of tolerance.

It may be, of course, that tolerance does develop during extended training periods but is obscured as continued differential reinforcement acts to transfer stimulus control to progressively lower doses (Hirschhorn and Rosecrans 1974). If so, the doses required for stimulus control would be expected to decrease following a drug-free interval. However, experiments in which training and its correlated drug exposure are halted for a time have shown few changes in the range of doses required for stimulus control (McMillan 1987; Sannerud and Young 1987). Although few drugs have been examined systematically after drug holidays, the available data support the suggestion (Colpaert et al. 1976) that the regular drug administration required by most training protocols does not confer tolerance to drug stimulus control.

Tolerance can develop to the discriminative stimulus effects of drugs, however, if the discriminative relationship between a drug stimulus and reinforced behavior is suspended during repeated treatment with appropriate doses of the training drug itself or related compounds. Under such procedures, stimulus control by drug dose is first established with conventional training procedures, initial dose-effect functions are obtained, and then training is discontinued while a high dose of drug is administered repeatedly. Finally, during continued

absence of training, dose-effect functions are redetermined in order to assess changes in the doses required for stimulus control. Under such procedures, tolerance, defined by an increase in the dose required for stimulus control, has been reported for a wide variety of training stimuli, including *d*-amphetamine, caffeine, cocaine, fentanyl, morphine, and nicotine, suggesting that susceptibility to tolerance may be a common characteristic of drug-discriminative stimuli. Such tolerance to drug-discriminative stimuli is determined jointly by the conditions of repeated drug treatment, the training dose, and the behavioral conditions imposed during repeated treatment (Young 1990; Young and Sannerud 1989). The following sections use our recent studies of tolerance to the discriminative stimulus effects of morphine to illustrate some of these characteristics of tolerance to drugs acting as discriminative stimuli.

GENERAL METHODS

In all experiments, saline and a dose of 3.2 mg/kg morphine were established as discriminative stimuli for food-reinforced behavior of rats, as described by Young et al. (1990). Briefly, training sessions were divided into several discrete trials, each consisting of a 15-min timeout (TO) component followed by a 5-min ratio component. At the start of each trial, the rat was administered an injection of saline or morphine and placed in a darkened chamber. At the end of the TO component, stimulus lamps were illuminated and lever presses were reinforced under a fixed ratio (FR) 15 schedule of food delivery. Following saline administration, responses on the right lever produced food; following morphine administration, responses on the left lever produced food. Each response on the incorrect lever reset the ratio counter. A trial ended, and the chamber was darkened, at the end of 5 min or after delivery of 50 pellets, whichever occurred first. At the end of 5 min, the subject was removed and administered an injection, and the next TO component was initiated. Training sessions varied in the sequence of trials conducted, so that a similar number of drug and saline training trials were conducted each week.

Training sessions were conducted daily until discriminative control was established. Then, several tests of generalization to cumulative doses of morphine were conducted in each subject in order to determine initial sensitivity. During test sessions, saline or an increasing dose of morphine was administered at the start of successive TO components, and completion of 15 consecutive responses on either lever produced food during each ratio

component. At least three training sessions were conducted between successive tests.

To examine development of tolerance, training sessions were halted, and each rat was administered daily injections of morphine and returned to the home cage. Several groups of subjects were tested, each exposed to a different dose or frequency of morphine treatment. In order to study the development and loss of tolerance, cumulative dose-response tests were conducted at various times during repeated treatment and after treatment ended. Training was not resumed until sensitivity to the stimulus and rate-aftering effects of morphine returned to initial levels.

RESULTS

Development and Loss of Tolerance to Stimulus Effects of Morphine

As shown in figure 1 (left panels), repeated treatment with morphine produced a dose-dependent tolerance to the stimulus effects of morphine, and magnitude of tolerance increased with maintenance dose. Repeated treatment with daily injections of 10 mg/kg or twice daily injections of 10 or 17.8 mg/kg increased the dose of morphine required for stimulus control by twofold, fourfold or fivefold, respectively (Sannerud and Young 1987; Young et al. 1990; cf. Shannon and Holtzman 1976). Daily treatment with saline or the training dose did not change the dose of morphine required for stimulus control, demonstrating that the loss of sensitivity to the stimulus effects of morphine was not the result of suspended training. Other experiments have shown that treatment with daily doses of 20 or 110 mg/kg increases the dose required for stimulus control by only twofold or fourfold in rats trained with higher doses of 5.6 or 10 mg/kg morphine (Miksic and Lal 1977; Young et al. 1990), suggesting that the proportional relation between maintenance dose and training dose may control the magnitude of tolerance. A similar proportional relation has been reported for caffeine. Repeated treatment with 60 mg/kg/day increases the dose of caffeine required for stimulus control by fourfold in rats trained with a dose of 10 mg/kg caffeine, but only by threefold in rats trained with a higher dose of 30 mg/kg (Holtzman 1987).

As shown in the right panel of figure 1, sensitivity to the stimulus effects of morphine recovered within 3 to 5 days after termination of repeated treatment. Because discrimination training did not resume until these tests were completed, it appears that development of tolerance to the stimulus effects of

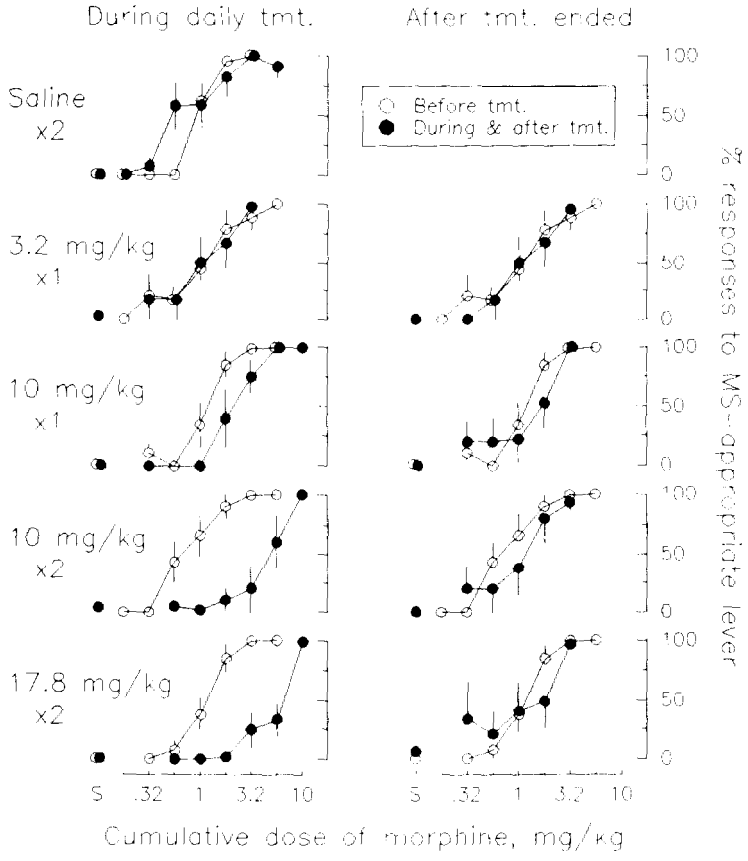

FIGURE 1. *Dose-response functions for stimulus control by morphine in rats before, during, and after repeated treatment with saline or selected doses of morphine. Morphine (3.2 mg/kg) and saline were established as discriminative stimuli for food-reinforced performances in rats. Open circles represent the mean ± 1 SEM for two to four tests conducted in each of five to nine subjects before repeated treatment, and are replotted in both panels. Closed circles represent the mean ± SEM for one observation in each subject during the second week of treatment (left panel) and 3 to 5 days after treatment ended (right panel). Abscissae: dose of morphine, fog scale. Ordinates: responses to morphine-appropriate lever, as percentage of total session responses. (Data replotted from Sannerud and Young 1987 and Young et al. 1990.)*

morphine reflected a pharmawdynamic process rather than establishment of control by a higher training dose (cf. Colpaert 1978*b),* inasmuch as the effects of establishing a higher training dose would be expected to persist after the end of treatment.

A direct relationship between maintenance dose and magnitude of tolerance also has been reported for cocaine (Wood and Emmett-Oglesby 1986, 1987; Wood et al. 1984, 1987). In one series of experiments, rats were trained to discriminate saline and a dose of 10 mg/kg cocaine. Generalization functions were determined before and after repeated treatment with doses of 5, 10, or 20 mg/kg cocaine administered three times a day for approximately 1 to 2 weeks. The magnitude of tolerance to the stimulus effects of cocaine varied with maintenance dose. Repeated treatment with a daily dose of 15 mg/kg did not change the dose of cocaine required for generalization, whereas treatment with daily doses of 30 and 60 mg/kg increased the dose required twofold. In other subjects treated with 60 mg/kg, stimulus control by the original training dose reappeared within 18 days after treatment ended without resumption of training, again suggesting that the loss of sensitivity to the stimulus effects of cocaine was not the result of shifting control to a higher training dose. The longer recovery time for cocaine, as compared to morphine (figure 1), may reflect differences in the physiological processes underlying altered sensitivity to the drugs.

Time Course of Tolerance Development

The time wurse of tolerance development was examined by comparing sensitivity to the stimulus effects of morphine at various times after initiation of treatment with twice daily doses of 10 mg/kg morphine (figure 2, left panels). Consistent with the findings of Shannon and Holtzman (1976), an approximately twofold tolerance to the stimulus effects of morphine developed within 3 days, The magnitude of tolerance increased when the duration of treatment was extended to 1 week, but did not increase markedly after a second week of treatment. Other studies showed that one or two acute treatments with 10 mg/kg morphine do not alter sensitivity to the stimulus effects of morphine 12 hr later (Sannerud and Young 1987; Young et al. 1991 *b).* Thus, it appears that tolerance to the stimulus effects of morphine develops slowly, over a period of days. The gradual onset of tolerance with repeated injections parallels that reported for continuously infused opioids. Emmett-Oglesby et al. (1989) have shown that continuously infused fentanyl

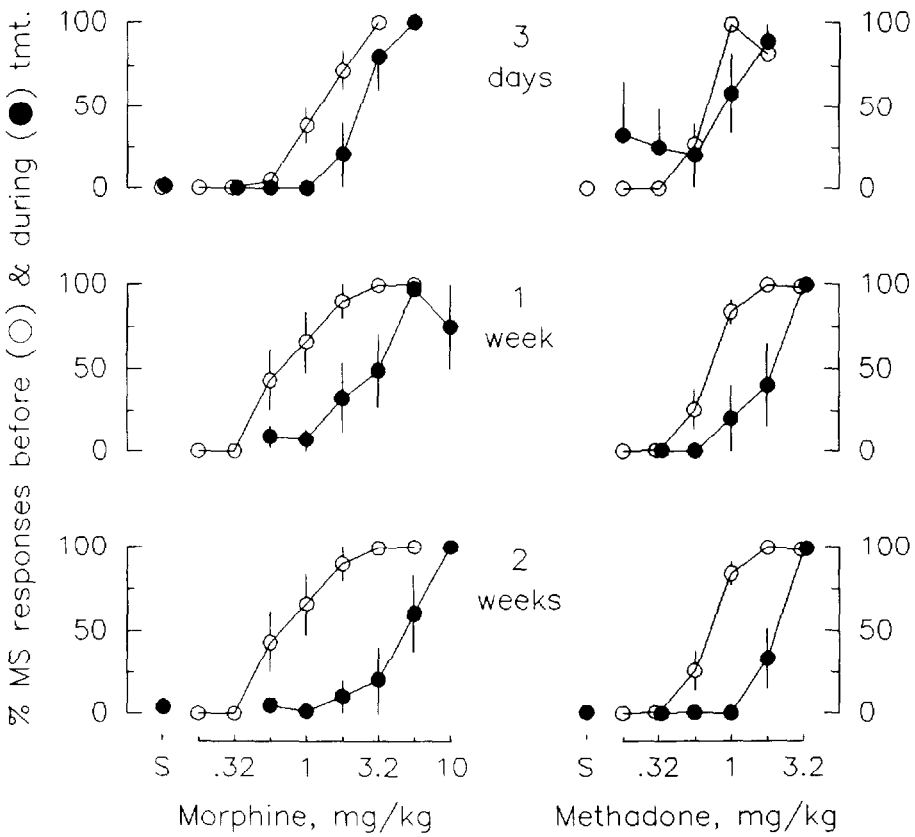

FIGURE 2. *Time course of development of tolerance to stimulus effects of morphine (left panel) or morphine-like stimulus effects of methadone (right panel). Rats trained to discriminate 3.2 mg/kg morphine and saline were treated for various times with twice daily doses of 10 mg/kg morphine. Abscissae: dose of drug, log scale. Ordinates: responses to morphine-appropriate lever as percentage of total session responses. Open circles represent the mean ± 1 SEM for three or four tests conducted in each of five or six subjects before repeated treatment. Closed circles represent the mean ± SEM for one observation in each subject during repeated treatment. (Data replotted from Young et al. 1990, Young et al. 1991a, and unpublished observations, 1990.)*

evokes stimulus control within 8 hr of the onset of a continuous infusion, and that tolerance to these effects begins to develop within 12 to 48 hr.

Cross-Tolerance

The tight panel of figure 2 illustrates cross-tolerance to the morphinelike stimulus effects of a second *mu* agonist, *dl*-methadone. Repeated treatment for 3 days with twice daily injections of 10 mg/kg morphine did not markedly alter stimulus control by methadone, but treatment for 1 or 2 weeks increased the dose of methadone required for stimulus control by approximately twofold to threefold. In agreement with an earlier report by Shannon and Holtzman (1976) repeated morphine treatment evoked less tolerance to methadone than to morphine itself. These differences in magnitude of tolerance may reflect differences in agonist activity. The lesser tolerance to methadone is consistent with reports that under certain conditions, repeated treatment with morphine produces less tolerance to the rate-altering and stimulus effects of both *l*- and *dl*-methadone than to similar effects of morphine (e.g., Craft et al. 1989; McMillan et al. 1980; Shannon and Holtzman 1976), although similar tolerance has also been reported (e.g., Leander et al. 1975). Such differences may result from differences in agonist efficacy or drug disposition (Lange et al. 1983; Werling et al. 1988; Young et al. 1991*a).*

Tolerance and cross-tolerance to the stimulus effects of opioids appear pharmacologically specific. Tolerance develops during repeated treatment with appropriate doses of morphine or fentanyl but not during treatment with doses of pentobarbital sufficient to induce tolerance to the hypnotic effects of pentobarbital itself (Emmett-Oglesby et al. 1988; Miksic and Lal 1977; Shannon and Holtzman 1976; Young et al. 1990). Moreover, repeated treatment with doses of morphine sufficient to induce tolerance to the stimulus effects of morphine itself does not induce cross-tolerance to the stimulus effects of cocaine (Wood and Emmett-Oglesby 1986).

Tolerance to the stimulus effects of cocaine is also pharmacologically specific, developing during repeated treatment with appropriate doses of amphetamine, apomorphine, or cocaine, but not during treatment with morphine (Wood and Emmett-Oglesby 1986, 1987). Repeated treatment with *d*-amphetamine also produces cross-tolerance to the cocaine-like stimulus effects of amphetamine, and treatment with cocaine produces cross-tolerance to the cocaine-like stimulus effects of methylphenidate, phenmetrazine, and phentermine (Wood and Emmett-Oglesby 1986, 1988). Interestingly, cocaine treatment produces an

insurmountable tolerance to the cocaine-like stimulus effects of apomorphine and diethylpropion (Wood and Emmett-Oglesby 1987, 1988). Further characterization of such cross-tolerance should provide useful information about suggested commonalities in the neuronal systems mediating the cocaine-like stimulus effects of these drugs. The patterns of cross-tolerance between caffeine and methylphenidate also suggest a common neuronal system (Holtzman 1987). Methylphenidate evokes generalization to a caffeine training stimulus, and repeated treatment with either drug confers tolerance to the caffeine-like stimulus effects of the other, supporting suggestions of catecholaminergic involvement in the stimulus effects of caffeine.

Modulation of Tolerance by Behavioral Processes

The magnitude of tolerance to the stimulus effects of a drug can be diminished by continuing discrimination training during the period of repeated drug treatment. This effect was demonstrated in experiments that assessed whether continuing training during repeated drug treatment would modulate the development of tolerance to the stimulus effects of morphine (Sannerud and Young 1987). Rats were trained as described above, and sensitivity to morphine was assessed before and after repeated treatment with twice daily injections of 17.8 mg/kg morphine. In one condition, training was suspended during repeated treatment. In a second condition, training was continued during treatment. When training was suspended, repeated treatment increased the dose of morphine required for stimulus control to 10 mg/kg in seven of the nine subjects (figure 3, upper right panel). However, in agreement with earlier studies (Colpaert et al. 1978*c*; Hirschhorn and Rosecrans 1974), tolerance was diminished when discrimination training was continued throughout repeated treatment (lower right panel). When training was continued, the training dose of 3.2 mg/kg continued to evoke stimulus control in three subjects, and 5.6 mg/kg evoked control in the remaining six.

A similar pattern was observed when the experiment was repeated using a lower treatment dose of morphine (figure 3, left panels; Young et al. 1990; A.M. Young et al. unpublished observations 1990). Repeated treatment with twice daily injections of 10 mg/kg morphine produced a fourfold tolerance to the stimulus effects of morphine when training was suspended during treatment (upper left panel) but no tolerance when discrimination training sessions were continued throughout treatment (lower left panel). Although they have yet to be replicated with drugs from other pharmacological classes, these results suggest that conditioning and pharmacodynamic processes interact to regulate

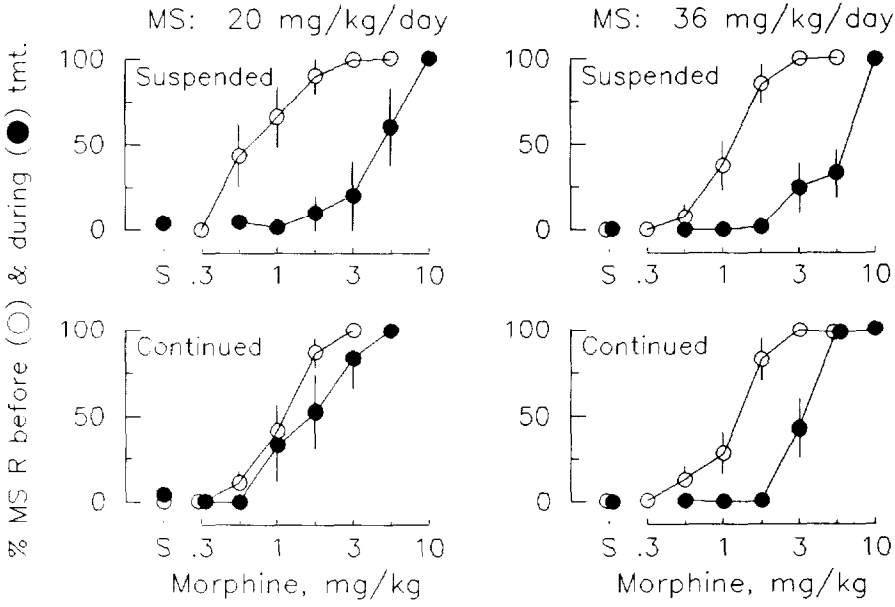

FIGURE 3. *Modulation of tolerance to the stimulus effects of morphine by exposure to discrimination training sessions. Morphine (3.2 mg/kg) and saline were established as discriminative stimuli for food-reinforced performances in rats. Open circles represent the mean ± 1 SEM for two to four tests conducted in each of five to nine subjects before repeated treatment. Closed circles represent the mean ± SEM for one observation in each subject after 2 weeks of repeated treatment with morphine. Training was suspended during treatment in the experiments summarized in the upper panels. Training with saline and 3.2 mg/kg morphine was continued during treatment in the experiments summarized in the tower panels. See figure 1 for other details. (Data reploted from Sannerud and Young 1987, and Young et al. 1990 and unpublished observations, 1990.)*

sensitivity to drug stimulus control. When an individual regularly encounters a discriminative relation between a drug and opportunities for reinforcement, development of tolerance to the drug stimulus is minimized. Tolerance does develop, however, if the individual simultaneously encounters frequent high doses of a drug and behavioral contingencies that limit opportunities for discriminative learning.

SUMMARY AND CONCLUSIONS

The experiments described above highlight the ways behavioral and pharmacodynamic processes interact to modulate the development of tolerance to the discriminative stimulus effects of drugs. These studies suggest that frequent drug exposure does not lead inevitably to the development of tolerance to a drug's discriminative effects. Rather, the interplay between a drug stimulus and reinforcement opportunities shapes the sensitivity of discriminative performances over successive episodes of drug exposure.

Maintaining a discriminative relation between a drug and behavior strengthens the likelihood that an initially effective dose will maintain discriminative control. Development of tolerance requires exposure to both treatment regimens appropriate to the agent under study and behavioral contingencies that limit an individual's ability to learn a new discrimination. When both requirements are met, tolerance does develop to drugs acting as discriminative stimuli.

When training is suspended during a period of chronic drug treatment, the dose of drug required to evoke stimulus control can be increased by treatment with appropriate maintenance doses of the training drug or a closely related drug. Tolerance is proportional to maintenance dose, develops relatively slowly, and disappears after termination of repeated drug treatment. Tolerance appears pharmacologically specific and can be accompanied by cross-tolerance to other drugs that evoke cross-generalization with the training drug. Finally, tolerance can be diminished markedly by continuing training with the original training dose. Taken together, these patterns suggest that development of tolerance to drugs acting as discriminative stimuli is the result of joint actions of conditioning and pharmacodynamic processes.

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Tolerance: Role of Conditioning Processes

Shepard Siegel

The display of tolerance is greatly affected by cues present at the time of drug administration, and a complete account of tolerance must acknowledge the role of such cues. Demonstrations of the importance of such predrug cues have inspired accounts of tolerance that emphasize learning principles. We will first review the evidence that predrug cues modulate tolerance, and then discuss analyses of tolerance that have been presented to account for these findings.

ENVIRONMENTAL CUES AND TOLERANCE

Tolerance to a variety of effects of many drugs is environmentally specific. That is, tolerance to the last of a series of drug administrations is more pronounced if this final administration occurs in the same environment as the prior drug administrations.

Demonstrations of Environmental Specificity of Tolerance

The details of the designs of experiments demonstrating environmental specificity of tolerance differ, but all incorporate two groups of subjects, both receiving the drug a sufficient number of times for tolerance to develop during the initial, tolerance-development phase of the experiment. The effect of the drug is evaluated in a subsequent tolerance-test phase. For one of the two groups, this test is conducted following the same cues that signaled the drug during the tolerance-development phase (same-tested). For the second group, the tolerance test is conducted following cues other than those that signaled the drug during the tolerance-development phase (different-tested).

In most experiments concerned with environmental specificity of tolerance, subjects have equal experience with both environments prior to the tolerance test; thus, it is not the case that one of the environments is more novel or stressful than the other. One frequently used design has been termed “discriminative control of tolerance” (Siegel 1979). During the tolerance-

development phase of such an experiment, all subjects receive a number of injections of the drug and a number of injections of physiological saline. Different environmental cues are associated with each substance, such that all drug injections take place in one environment (E_D), and all saline injections take place in a different environment (E_S). Thus, on some days the subject is injected with the drug in E_D, and on other days it is injected with saline in Es. Finally, the effect of the drug is tested for all subjects, with same-tested subjects receiving this test in E_D, and different-tested subjects receiving this test in E_S.

In addition to including same- and different-tested groups, the design of tolerance environmental-specificity studies typically includes a third group. This control group permits evaluation of the drug-elicited response in subjects receiving the drug for the first time. Results obtained during the tolerance test in a number of experiments using this procedure are summarized in figure 1.

Figure 1A summarizes results reported by Crowell et al. (1981) in their study of tolerance to the hypothermic effect of ethanol. The figure depicts the change in colonic temperature (postinjection minus preinjection) following a test-session injection of 1.3 g/kg ethanol. Same-tested rats (“SAME”) received 20 pretest injections of ethanol, each in the same environment as that accompanying the test injection. Different-tested rats (“DIFF”) also received 20 pretest injections of the drug, but in a distinctively different environment. Control rats (“CONTL”) received their first injections of ethanol on the test session. As can be seen by comparing control rats with same-tested rats, tolerance to the hypothermic effect of the drug was apparent: control rats were hypothermic, but same-tested rats were not. However, results obtained from different-tested rats indicate that hypothermic tolerance is not the inevitable result of repeated ethanol administrations. Different-tested rats had the same pharmacological history as same-tested rats (i.e., they received the same dose of ethanol, equally often, and at the same intervals), but different-tested rats were as hypothermic as control rats. There are other reports that tolerance to the thermic effects of ethanol in rats exhibits environmental specificity. In addition, environmental specificity of tolerance has also been demonstrated with respect to both the narcotizing effect of ethanol in mice and the cardiac and cognitive-performance effects of ethanol in humans (see summary by Siegel 1987).

The environmental specificity of tolerance has been demonstrated with many other drugs. Figures 1B through 1E summarize the results of other experiments that have demonstrated that the display of tolerance is more pronounced in

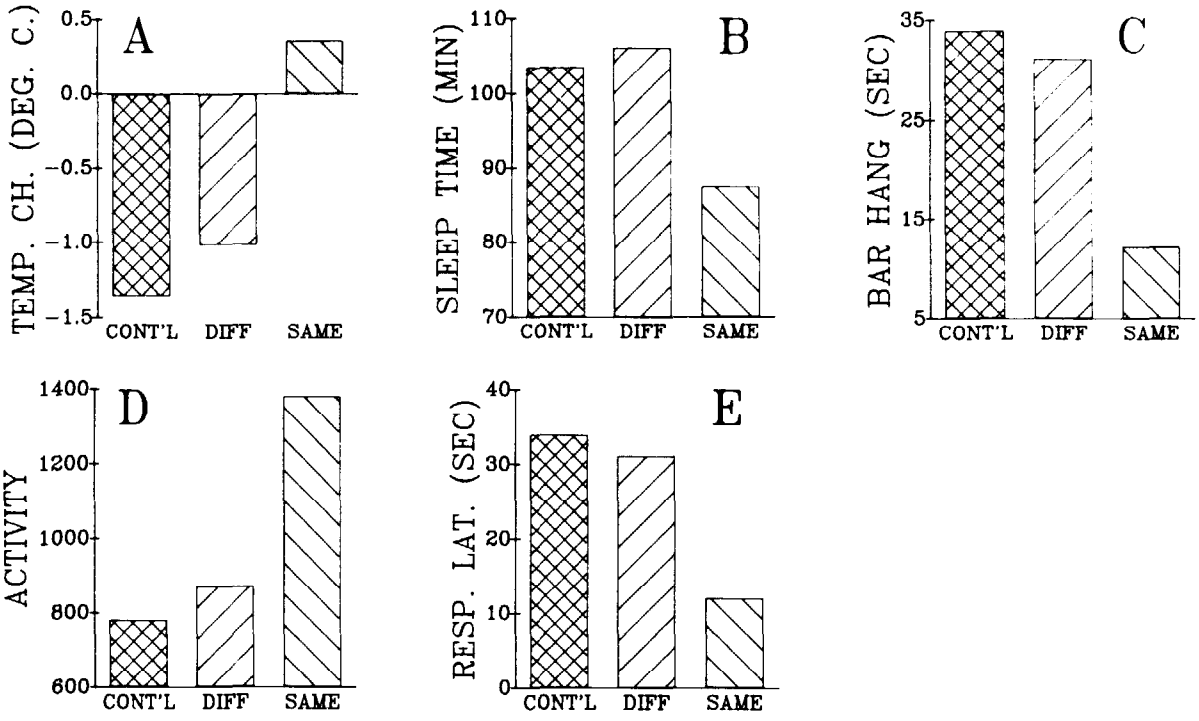

FIGURE 1. *Summary of results of experiments demonstrating environmental specificity of tolerance to: (A) the hypothermic effect of ethanol in rats (Crowell et al. 1981), (B) the sedative effect of pentobarbital in rats (Hinson et al. 1982), (C) the cataleptic effect of haloperidol in rats (Poulos and Hinson 1982), (D) the ambulatory activity-suppressive effects of midazolam in rats (King et al. 1987), and (E) the analgesic effect of morphine in snails (Kavaliers and Hirst 1986).*

same-tested than in different-tested subjects. Figure 1B summarizes results reported by Hinson et al. (1982) concerning tolerance to the sedative effect of pentobarbital. During the tolerance-acquisition phase of this experiment, rats were injected with gradually increasing doses of the barbiturate (30-45 mg/kg). On the tolerance-test session, following injection of 45 mg/kg of the drug, each subject's "sleeping time" was scored from videotape records. As can be seen, tolerance to the sedative effect of pentobarbital (figure 1B), like tolerance to the hypothermic effect of ethanol (figure 1A), displays environmental specificity. Same-tested rats were less sedated by the drug (displayed shorter sleeping times) than equally drug-experienced different-tested rats. Indeed, different-tested rats were as behaviorally narcotized as control rats that received the drug for the first time on the tolerance-test session. This experiment by Hinson et al. (1982) confirmed a previous report of environmental specificity of pentobarbital tolerance (Cappell et al. 1981).

Environmental specificity of tolerance is found not only with sedatives, such as ethanol and pentobarbital, but also with many other types of drugs. Figure 1C summarizes results reported by Poulos and Hinson (1982) concerning tolerance to the cataleptic effect of the neuroleptic drug haloperidol. Catalepsy was measured with a standard bar-hanging procedure; both the rat's paws were placed on a bar, and the amount of time that the animal remained hanging was scored. During both the tolerance-acquisition and tolerance-test phases of the experiment, the dose of haloperidol was 3 mg/kg. Tolerance to the cataleptic effect of the drug was seen in same-tested rats but not in different-tested rats. As can be seen in figure 1C, these different-tested rats were about as cataleptic as control animals.

Environmental specificity of tolerance has also been demonstrated with respect to a variety of benzodiazepines (Siegel 1986). Figure 1D summarizes data reported by King et al. (1987) concerning tolerance to the ambulatory activity-suppressive effects of the short-acting benzodiazepine midazolam. Same-tested rats were significantly more active on the tolerance-test session than were different-tested or control rats.

Although environmental specificity of tolerance has been demonstrated with many effects of a variety of drugs, most experiments concerning this phenomenon have evaluated tolerance to the analgesic effect of morphine. Environmental specificity of such tolerance has been demonstrated with many analgesia-assessment procedures, and in a variety of species, including humans (Siegel and MacRae 1984). The generality of the phenomenon is

illustrated in figure 1E, which summarizes data reported by Kavaliers and Hirst (1986) concerning morphine tolerance in the terrestrial gastropod snail *Capaea nemoralis.* During each tolerance-development session, snails were injected with 1 µg/kg morphine. Analgesia was assessed by measuring the latency of the "foot"-lifting response when the snails were placed on a 38.5° surface. As apparent in figure 1 E, same-tested snails responded more quickly to the thermal stimulation (i.e., were more tolerant to the analgesic effect of morphine) than were different-tested snails. The fact that environmental specificity of tolerance can be seen even in invertebrates suggests that such specificity "may be a general phenomenon having an early evolutionary development and broad phylogenetic continuity" (Kavaliers and Hirst 1986, p. 1201).

Environmental Specificity of Tolerance to the Lethal Effect of Drugs

Perhaps the most dramatic evidence for environmental control of tolerance comes from demonstrations that tolerance to the lethal effect of some drugs demonstrates environmental specificity. The results of experiments demonstrating such environmental modulation of drug-induced mortality are summarized in figure 2.

The data summarized in figure 2A demonstrate that environmental cues modulate tolerance to the lethal effect of ethanol (Melchior and Tabakoff 1982). In this experiment, mice were injected with 3.5 g/kg ethanol twice daily for 4 days. On the 5th day different subgroups were injected with 4.5-7.0 g/kg ethanol in either the drug-associated or a novel environment. The LD_{50} dose of the drug was determined. As can be seen in figure 2A, the LD_{50} dose is smallest for control mice, who had no pretest exposure to ethanol, but the effects of environmental cues are still evident: The LD_{50} dose is significantly lower for different-tested than for same-tested mice.

Figure 2B summarizes data reported by Siegel et al. (1982) indicating environmental control of tolerance to the lethal effect of heroin. In this experiment, rats prepared with chronically implanted intravenous cannulae received a series of heroin infusions (with the dose gradually increased from 1 to 8 mg/kg). Finally, on the tolerance-test session, these rats were administered 15 mg/kg of the opiate in either the same environment as that previously associated with the drug or an alternative environment. As can be seen in figure 2B, both these groups with pretest experience with sublethal doses of heroin were more likely to survive than control animals, which received heroin for the first time on the tolerance-test session. These results indicate that tolerance

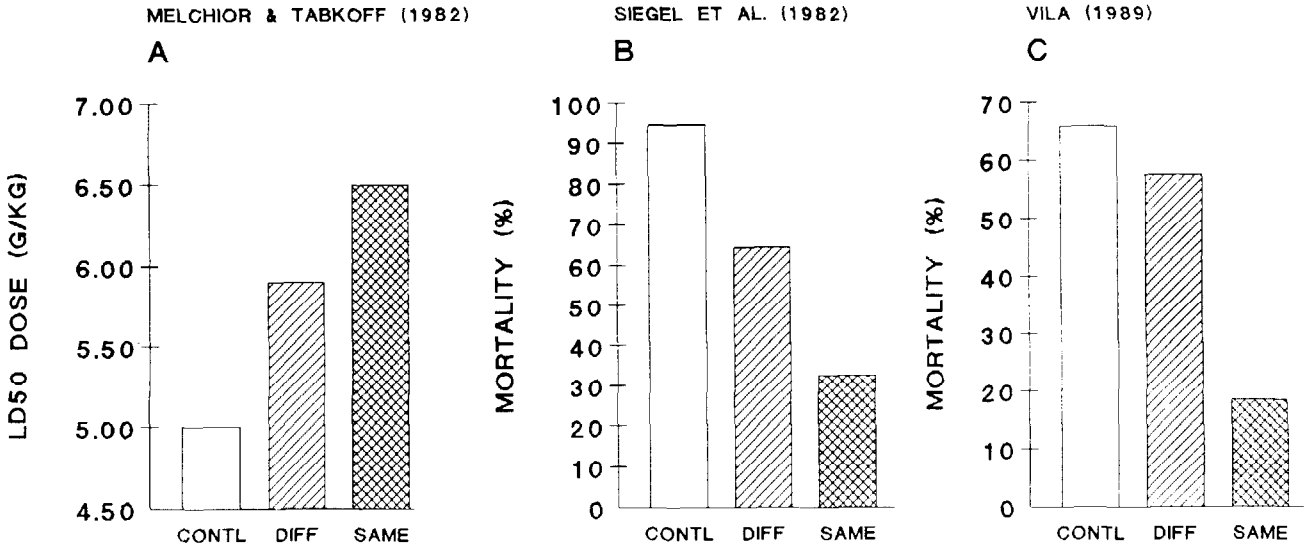

FIGURE 2. *Summary of results of experiments demonstrating environmental specificity of tolerance to the lethal effect of: (A) ethanol (Melchior and Tabakoff 1982). (B) heroin (Siegel et al. 1982). and (C) pentobarbital (Vila 1989).*

resulted from pretest injections independently of the environment associated with these injections. However, mortality was significantly higher in different-tested than same-tested rats, indicating, once again, that identical pretest pharmacological histories do not necessarily result in equivalent tolerance to the lethal effect of heroin. Case report data suggest that environmental cues similarly modulate tolerance to the lethal effect of heroin in human heroin addicts (Siegel 1984) and in patients receiving medically prescribed morphine (Siegel and Ellsworth 1986).

Figure 2C depicts the results of an experiment by Vila (1989). The design of this experiment concerning pentobarbital overdose was similar to that of Siegel et al. (1982) concerning heroin overdose. Experimental group rats were intraperitoneally injected with pentobarbital (30 mg/kg) in one environment and saline in another. After 20 administrations of each substance, the rats were injected with a high dose of the barbiturate (95 mg/kg) in either the drug-associated (same-tested) or saline-associated (different-tested) environment. Control rats had no prior exposure to pentobarbiial priir to injection of the large dose on the test session. As is apparent in figure 2C, environmental control of tolerance to the lethal effect of the drug was complete; drug-experienced rats challenged with the drug in the context of the usual drug cues displayed lower mortality than drug-experienced rats challenged with the drug in the context of alternative cues. Indeed, in the latter case there was *no* evidence of tolerance-mortality among different-tested rats was near that of control rats who received pentobarbital for the first time on the final test session.

Summary of Research Demonstrating Environmental Specificity of Tolerance

Many studies have demonstrated environmental specificity of tolerance. Such environmental specificity occurs with respect to tolerance to a variety of effects (including the lethal effect) of many drugs. It should be noted that often such environmental specificity is not absolute, i.e., some tolerance is noted in different-tested subjects compared to control subjects receiving the drug for the first time. Furthermore, although environmental specificity of tolerance is a very general observation, there are occasional reports to the contrary. For example, as summarized above (figure 1D), King et al. (1987) reported clear environmental specificity of tolerance to the sedative effect of midazolam; however, Griffiths and Goudie (1986) reported no environmental specificity of tolerance to this drug's hypothermic effect (see also Siegel 1989). However, the general finding, obtained with many drugs, dosages, species, and procedural variations, is that tolerance is more pronounced when the drug is given in the

usual drug administration environment than when it is given elsewhere. Speaking casually, tolerance is greater when a drug is expected than when it is unexpected.

PAVLOVIAN CONDITIONING AND TOLERANCE

To incorporate the contribution of drug expectation to drug tolerance, several investigators have suggested that learning contributes to tolerance. That is, tolerance is due, in part, to an association between drug-predictive cues and the systemic effect of the drug. The study of such associations is the study of Pavlovian conditioning.

The Pavlovian Conditioning Situation

In the Pavlovian conditioning situation, a contingency is arranged between two stimuli; typically, one stimulus reliably predicts the occurrence of the second stimulus. The second of these paired stimuli is usually termed the unconditional stimulus (UCS). The UCS, as the name implies, is selected because it elicits relevant activities from the outset (i.e., unconditionally), prior to any pairings. Responses elicited by the UCS are termed unconditional responses (UCRs). The stimulus signaling the presentation of the UCS is "neutral," (i.e., it elicits little relevant activity prior to its pairing with the UCS), and is termed the conditional stimulus (CS). The CS, as the name implies, becomes capable of eliciting new responses as a function of (i.e., conditional upon) its pairing with the unconditional stimulus.

Drugs as Unconditional Stimuli

A wide range of exteroceptive and interoceptive stimuli have been used in Pavlovian conditioning experiments. Drugs constitute a particularly interesting class of UCSs. After some number of drug administrations, each administration reliably signaled by a CS, pharmacological conditional responses (CRs) can be observed in response to the CS.

The Pharmacological Conditional Response. Most pharmacological conditioning research has been greatly influenced by Pavlov's theory of CR formation. According to this theory, the CR is a replica of the UCR, and, indeed, much drug conditioning work has demonstrated CRs that mimic the drug effect (Stewart and Eikelboom 1987). In contrast, in 1937 Subkov and Zilov reported that dogs with a history of epinephrine administration (each injection eliciting a

tachycardiac response) displayed a conditional bradycardiac response. The authors cautioned against "the widely accepted view that the external modifications of the conditional reflex must always be identical with the response of the organism to the unconditional stimulus" (Subkov and Zilov 1937, p. 296). Subsequent research has suggested that the characteristics of the pharmacological CR depend very much on the nature and mechanism of the drug effect (Eikelboom and Stewart 1982; Siegel 1989). For many effects of many drugs, the CR is an anticipatory compensation for the drug effect. For example, the subject with a history of morphine administration (and its analgesic consequence) often displays a CR of hyperalgesia (Krank 1987; Krank et al. 1981; Siegel 1975). Similar compensatory CRs have been reported with respect to the thermic, locomotor, behaviorally sedating, and gastrointestinal effects of morphine. The CR seen with many nonopiate drugs is similarly opposite to the drug effect, e.g., atropine, chlorpromazine, amphetamine, methyldopa, lithium chloride, haloperidol, ethanol, caffeine, and several immunostimulatory and immunosuppressive drugs (MacQueen et al. in press; Siegel 1989).

The Pharmacological Conditional Response and Drug Tolerance. Drug-compensatory CRs would be expected to be a feature of normal drug administration procedures. In those cases in which the same drug is repeatedly administered, with discrete environmental stimuli signaling each drug administration, drug-compensatory CRs should function to increasingly attenuate the drug effect. A decreasing response to a drug over the wurse of successive administrations defines tolerance.

Pharmacological Conditioning and Tolerance's Environmental Specificity. The observation that there often is pronounced environmental specificity to the display of tolerance is readily interpretable by an analysis of tolerance that incorporates Pavlovian conditioning principles. If the repeatedly drugged organism is administered the drug in the context of normal predrug cues, the compensatory CR partially cancels the drug effect; thus, tolerance is apparent. However, if this organism is administered the drug in the context of cues not previously associated with the drug, there would be no CR attenuating the drug effect, and tolerance attributable to such a CR would not be observed.

Other Conditlonlng Accounts of Tolerance. A substantial amount of data have been presented supporting the compensatory-CR analysis of tolerance. There are also data that have been interpreted as contrary to this analysis (Siegel 1989), and some investigators have attempted to develop theories that

will accommodate evidence that tolerance is clearly affected by drug-associated cues, yet not necessarily through a compensatory-CR mechanism. These explanations are known as “habituation” theories of tolerance. One such alternative associative framework for tolerance, based on Wagner’s information processing theory of memory (e.g., Wagner 1976), has been suggested by Siegel (1977) and elaborated by Baker and Tiffany (1985). According to this view, drug-associated environmental cues “prime” the drug effect in short-term memory, causing a decrease in the effectiveness of processing of the pharmacological stimulation.

Although there are some important theoretical distinctions between the two associative accounts of tolerance (Siegel 1989), both make many similar predictions. For example, both predict that an alteration in predrug cues should attenuate the display of tolerance in drug-experienced subjects.

OTHER SIGNALS FOR DRUGS

There are potentially many cues, in addition to the environment of drug administration, that can signal the effect of a drug, and there is evidence that a variety of drug-associated stimuli can influence the display of tolerance.

Magnetic Fields as Signals for Drugs

Results of an experiment by Kavaliers and Ossenkopp (1985) suggest that quite subtle cues, present at the time of drug administration, may contribute to the development of tolerance. In this experiment, mice received 10 daily injections of morphine, either in the presence or absence of rotating magnetic fields (2-35 gausses). Analgesia was assessed following each of these pretest drug administrations. Subjects were then tested for analgesic tolerance in both the presence and the absence of magnetic stimuli.

The results of this experiment indicated that magnetic fields per se affect the development of tolerance. During the pretest phase of the experiment, mice repeatedly administered morphine in conjunction with magnetic field exposure were relatively retarded in the acquisition of tolerance to the analgesic effect of morphine. In addition, the field also functioned as an effective cue for the drug. In the test session, tolerance was more pronounced following drug administration in the presence of the same magnetic stimuli (either presence or absence of field) that prevailed during the pretest drug administrations than following drug administration in the presence of the alternative magnetic stimuli.

According to by Kavaliers and Ossenkopp (1985), their resub extend the conditioning analysis of tolerance. The finding that magnetic field exposure during pretest sessions attenuated the acquisition of tolerance is congenial with suggestions that such exposure detrimentally affects the acquisition of learned responses (Kavaliers and Ossenkopp 1985). The further finding that magnetic fields can become associated with morphine and influence the display of tolerance suggests that magnetic stimuli may play a heretofore unappreciated role in the development of tolerance. The authors suggest that their results may be relevant to reported circadian differences in morphine effects in mice.

Thermal Signals for a Drug

As discussed previously, Kavaliers and Hirst (1986) demonstrated environmental specificity of tolerance in the terrestrial snail *Capaea nemoralis.* These investigators also demonstrated that thermal stimuli can serve as drug-associated cues that control the display of tolerance in this species. Snails were repeatedly injected with morphine in the presence of one of two thermal cues: either ambient temperature (22%) or a higher temperature (35°C). In a test session, tolerance to the analgesic effect of morphine was seen when snails were injected in the presence of the thermal cue previously associated with the drug, but not when they were injected in the presence of the alternative thermal cue.

According to Kavaliers and Hirst (1986) the higher temperature used in their experiment is normally avoided by morphs of Capaea; thus, it is unclear whether the effective morphine-associated signal was the temperature per se, or the stress induced by this thermal stimulation. in any event, the results of this experiment further suggest that even in the snail, a variety of stimuli can become associated with a drug and contribute to the development of tolerance.

Pharmacological Signals for Drugs

Another category of predrug signals that contribute to tolerance are pharmacological cues. That is, if a given drug (D_1) is repeatedly administered before a second drug (D_2), a pharmacological CR is elicited by D_1. Such drug-drug associations may contribute importantly to the development of tolerance (Krank and Bennett 1987; Taukulis 1986). In fact, because the early effect of a drug almost invariably signals a later effect, responses made to pharmacological cues may be a very common feature of tolerance.

Pharmacological Signaling of One Drug by Another Drug. Taukulis (1986) demonstrated that a drug can serve as a signal for another drug and control the expression of tolerance to the signaled drug. In his experiment, atropine sulfate was routinely injected prior to pentobarbital injection. Tolerance to the hypothermic effect of the barbiturate was much more pronounced when it was preceded by atropine than when it was presented without the signal provided by atropine. That is, same-tested rats (receiving the barbiturate subsequent to the usual signal provided by the antichoiinergic) were more tolerant than different-tested rats (receiving the barbiturate in the absence of the usual signal provided by the anticholinergic).

Results of recent research concerning the effects of pentobarbital on morphine tolerance are readily interpretable as a result of an association between a pharmacological signal (generated by the interoceptive effects of the barbiturate) and morphine. Terman and colleagues (1983, 1985) reported that rats with a history of morphine administration, administered pentobarbital prior to a final injection of morphine, do not display the analgesic tolerance seen in nonanesthetized rats. That is, the barbiturate apparently blocks morphine tolerance. Some interpretations of pentobarbital blockage of morphine tolerance have postulated direct pharmawdynamic interaction between the barbiturate and the opiate (Pontani et al. 1985). Results of a recent experiment, however, indicate that “state-dependent learning,” rather than pharmacodynamic interaction, best accounts for such barbiturate-opiate effects (Siegel 1988).

There is a considerable amount of evidence that drug states in general, and the state generated by barbiturates in particular, can serve as salient stimuli (see Järbe 1986). That is, learned responses acquired when the subject is not under the influence of a centrally acting drug, such as pentobarbital, may fail to be displayed subsequently when the subject is tested while under the influence of this drug. To the extent that tolerance to the analgesic effect of morphine is mediated by learning, it might be expected that tolerance will display such drug-state dependency-pharmacological cues, such as those generated by pentobarbital, may function very much like environmental cues in affecting the display of tolerance. in other words, just as there is environmental specificity of morphine tolerance (because of associations between morphine-signaling environmental cues and the opiate), there might also be state specificity of morphine tolerance (because of associations between morphine-signaling pharmacological cues and the opiate).

Siegel (1988) confirmed the finding that pentobarbital interferes with the expression of morphine tolerance in rats that had not previously received barbiturate-opiate pairings. Additionally, the results supported the state-dependency interpretation of this interference.

Pharmacological Signaling of a Drug by itself. A drug may serve not only as a cue for another drug but also as a cue for itself, and this association may contribute to tolerance (Greeley et al. 1984). In this Greeiey et al. (1984) study, rats in one group (paired) consistently received a low dose of ethanol (0.8 g/kg) 60 minutes prior to receiving a high dose (2.5 g/kg). Another group of rats (unpaired) received the low and high doses on an unpaired basis. When tested for the tolerance to the hypothermic effect of ethanol, paired subjects, but not unpaired subjects, displayed tolerance. Moreover, if the high dose of ethanol was not preceded by the low dose, paired rats failed to display their usual tolerance. This tolerance, dependent on an ethanol-ethanol pairing, was apparently mediated by an ethanol-compensatory thermic CR; paired rats, but not unpaired rats, demonstrated a hyperthermic CR (opposite to the hypothermic effect of the drug) in response to the low dose of ethanol.

Results of this study provide convincing evidence that a small dose of a drug can serve as a signal for a larger dose of the same drug. Because a gradual increase in systemic concentration is an inevitable consequence of most drug administration procedures, such drug-drug associations may play a hitherto unappreciated role in the effects of repeated drug administrations.

Cues Accompanying Self-Administration

The Pavlovian conditioning analysis emphasizes the contribution of predrug cues to the development of drug tolerance and dependence. In the discussion thus far, these cues have been conceptualized as environmental (i.e., the physical location of drug administration), pharmacological (i.e., one drug signaling another, or the early effect of a drug signaling the later effect), or other detectable stimuli (magnetic fields). Often, of course, drugs are self-administered. It might be expected that interoceptive cues accompanying self-administration (e.g., cognitive-volitional or proprioceptive signals for the systemic effect of the drug) similarly contribute to the effects of repeated pharmacological stimulation.

Self-administration cues have been evaluated in experiments that compare the effects of drugs in animals that self-administer the drug with effects in animals

yoked to these self-administering animals. Typically, the self-administering subject is prepared with a chronic jugular cannula, allowing for repeated intravenous injection. The subject can self-inject an opiate by pressing a lever in an experimental chamber. Yoked animals are similarly cannulated, and placed in a similar chamber, but lever presses have no consequence. Rather, the yoked subject receives the drug at the same time as the self-administering subject. Thus, yoked animals have no control over drug delivery, but rather receive the same doses of the drug, equally often, and est session, tolerance was more pronounced following drug administration in the presence of the same magnetic stimuli (either presence or absence of field) that prevailed during the pretest drug administrations than following drug administration in the presence of the alternative magnetic stimuli.

According to by Kavaliers and Ossenkopp (1985), their results extend the conditioning analysis of tolerance. The finding that magnetic field exposure during pretest sessions attenuated the acquisition of tolerance administration are the most obvious type of predrug signals, there are many other potential cues for the effect of a drug. Some of these are quite subtle yet are important contributors to the display of tolerance. Thus, the CR that mediates tolerance may be elicited by any of a variety of exteroceptive or interoceptive stimuli routinely present at the time of drug administration.

OTHER EVIDENCE FOR THE CONDITIONING ANALYSIS OF TOLERANCE

The fact that any of a variety of drug-signaling stimuli contribute to the display of tolerance to many effects of many different drugs provides the strongest evidence for a conditioning account of tolerance. There are, in addition, many other findings that implicate conditioning in tolerance. Generally, these additional findings demonstrate that nonpharmacological manipulations of drug-predictive cues have similar effects on tolerance and CRs. Thus, tolerance, like other learned responses, is subject to extinction, partial reinforcement effects, inhibiiory learning, higher order conditioning, stimulus generalization, disruption by novel stimuli (“external inhibition”), and compound conditioning effects (“overshadowing” and “blocking”) (Siegel 1989).

In summary, although an appreciation of pharmacodynamic and pharmacokinetic principles is of great importance in understanding drug tolerance, a complete account of the phenomenon must acknowledge the important contribution of conditioning principles.

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Responding to Drug-Related Stimuli in Humans as a Function of Drug-Use History

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INTRODUCTION

In the last 30 years there has been much interest in the role played by classical conditioning in the elicitation of physiological and subjective responses by drug-related stimuli. It has been suggested that environmental events that signal drug use elicit conditioned responses that influence the drug-seeking and self-administration behavior of human drug abusers. Clinically, these conditioned responses are thought to play an important role in the relapse process.

Figure 1 shows how a conditioning model applies to substance abuse.

The conditioned stimulus, a drug-related event such as the sight of a "shooting gallery," elicits a drug-related conditioned response. Different theoretical models (e.g., Siegel 1979; Stewart et al. 1984; Wikler 1965) hypothesize that this response can be either drug-like or drug-opposite in nature. Regardless of its form, this conditioned response is thought to motivate the individual to perform instrumental responses such as seeking out and self-administering the drug. The reinforcer or reward in this paradigm is the effect of the drug.

The purpose of this paper is to examine whether the responsivity brought about by drug-related stimuli in human drug abusers is the product of classical conditioning. The focus will be on data collected by our group at the Philadelphia Veterans Affairs Medical Center (VAMC)/University of

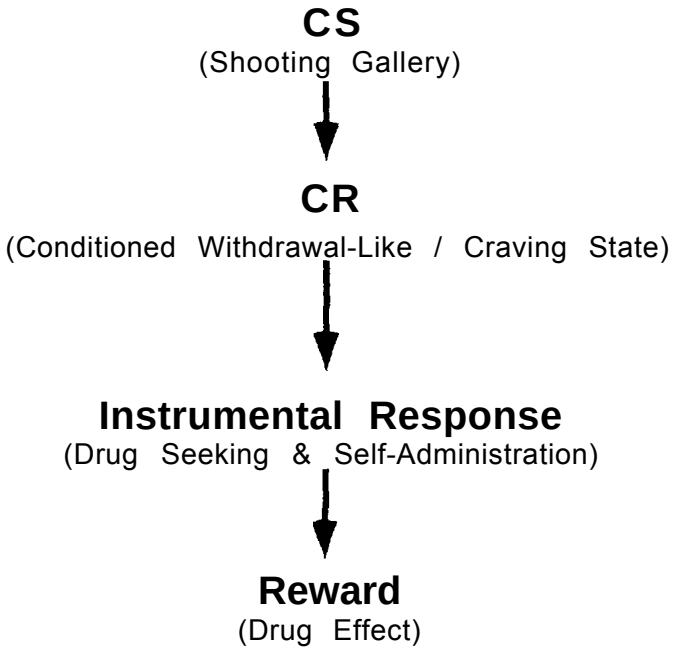

FIGURE 1. *Hypothesized role of conditioning processes in human drug use.*

Pennsylvania Addiction Research Center in studies with patients who abuse opiates and cocaine.

We have used two approaches to study conditioning in human drug abusers. Cur first experiments attempted to establish conditioned responses in the laboratory using stimuli such as tones and flavors that bear no intrinsic relation to drug use (e.g., O'Brien et al. 1977; Ternes et al. 1982). We quickly realized, however, that such studies do not shed much light on conditioning outside the laboratory. Although robust conditioning might occur under the carefully controlled conditions used in the lab, addicts are probably not exposed to ideal conditioning parameters in their natural environments.

Because of the artificial nature of laboratory conditioning studies, our recent work has looked at responding to cues that are assumed to be associated with drug use in the abusers' natural environment. These studies have been referred to as naturalistic stimulus studies. The stimuli used in these experiments

consist of some combination of audiotapes, videotapes, and manual tasks with either drug- or nondrug-related content. A number of designs utilizing this basic procedure are possible.

TWO STIMULUS-ONE GROUP DESIGN

The most common design employing naturalistic stimuli exposes a single homogeneous group of drug-abusing subjects to both drug and nondrug stimuli. The studies discussed here examined either opiate abusers or cocaine abusers. In our two stimulus-one group design, stimulus sessions contained a videotape and a manual task. During drug-stimulus sessions, the videotape depicts a typical scene of drug use. For example, a heroin injector would view a tape in which a drug user is shown buying, preparing, and actually injecting a substance resembling heroin. The task in such a session would require the user to perform his usual drug preparation ritual but stop prior to the actual injection.

Control stimuli are typically nonarousing and nondrug-related. In our experiments, subjects watch a nature documentary and play the pong video game. Typically, both drug-related and nondrug-related stimuli are presented in a single experimental session.

A variety of physiological and self-report measures are taken both before and after presentation of the stimuli. The purpose of such studies is to examine whether drug-related stimuli produce greater changes in responding than do nondrug-related stimuli compared with baseline levels.

In designing these studies, we have created a standard set of stimuli for each type of drug use. Because these stimuli do not match perfectly with the experience of any individual drug abuser, the level of responding observed may actually underestimate responses to drug-related events in the addicts' real environments.

At the start of a session the subject is attached to a polygraph that is used to continuously monitor his heart rate, galvanic skin response (GSR), and skin temperature. He is then seated in the recording chamber for the remainder of the session. The initial portion of the session is a 10- to 15-min baseline period during which no events occur. Toward the end of the baseline perii, self-report measures are taken on the subject's state of high, craving, and withdrawal relative to his drug of abuse. Next, the subject views a 10-min videotape and

then performs a manual task. Following the completion of the task, self-report measures are again obtained.

This sequence of baseline, videotape, and task is then repeated during the remainder of the session. Half of the session consists of drug-related stimuli and the other half to nondrug-related stimuli.

Changes from baseline scores for each physiological measure used in these studies are analyzed. These scores are calculated by subtracting mean measures of responding during the last 5 min of the baseline period from those in the last 5 min of the video stimulus and the last 5 min of the task. In this way, change scores are calculated for each stimulus modality for both the drug-related and nondrug-related stimulus types.

Self-report scores in these studies consist of subjects' ratings of high, craving, and withdrawal on a scale of 1 to 10, where a score of 1 represents no effect and a score of 10 represents a maximum possible effect. The change in these ratings between the baseline and poststimulus periods are then analyzed.

Two diiferent kinds of analysis are performed on the change score data. First, we examine whether there is a difference between change scores during drug stimuli and change scores during nondrug stimuli. Second, we analyze whether individual change scores differ reliably from zero. This second analysis shows whether a signfficant change from baseline has occurred.

Responding to naturalistic stimuli has been assessed in our center using both heroin and cocaine abusers (e.g., Childress et al. 1988; O'Brien et al. 1990). The overall purpose of these studies was to evaluate various combinations of substance abuse treatments. The data shown here represent the pretreatment responses of the patients.

The three subject groups in this design were all inpatients at the Philadelphia VA Hospital. The groups were composed of 89 methadone-maintained opiate users, 15 drug-free opiate users, and 51 drug-free cocaine users. Table 1 depicts the results of these studies. The asterisks indicate cases where change from baseline scores significantly differed between the drug and nondrug stimulus presentations.

TABLE 1. *Significant differences in responding to drug-related and nondrug-related stimuli in three populations*

Measures	Population		
	Methadone Maintained (n = 89)	Drug-Free Opiate (n = 15)	Drug-Free Cocaine (n = 51)
Physiological			
Temperature	*	*	*
Galvanic skin response	*	*	*
Heart rate			*
Subjective			
Craving	*	*	*
Withdrawal	*		*
High			*

*$p < .05$

As shown in table 1, temperature and GSR are the most reliable physiological measures; there was a significant difference on these two measures between the drug- and nondrug-related stimuli for all three groups. Both skin temperature and GSR significantly decreased relative to baseline in response to the drug-related stimuli.

Craving is the most consistent subjective measure distinguishing the drug-related and nondrug-related stimuli in each of the groups. Relative to baseline, craving is significantly increased following the presentation of drug-related stimuli. In the methadone-maintained and cocaine groups, withdrawal is also significantly increased by the drug-related stimuli, as is the self-reporf of high in the cocaine subjects. It may seem inconsistent that both high and withdrawal scores increase in the cocaine patients. However, inspection of the data reveal that individuals who show the greatest self-report changes typically respond to only one of the questions.

Unfortunately, there is a general problem with interpreting the results of these studies. Because the stimuli that are brought into the laboratory already elicit responding, it is impossible to determine whether these responses represent conditioned or unconditioned effects. For example, a typical video stimulus shown to opiate users depicts an individual injecting himself with the drug. We

know from interviews with subjects in these studies that the scene showing the actual injection is considered arousing or unpleasant per se. Consequently, it is possible that the differential responding to drug-related and nondrug-related stimuli represents an unconditioned response and is unrelated to learned associations between the drug stimuli and actual drug use.

TWO GROUP-TWO STIMULUS DESIGN

A more elaborate naturalistic stimulus design would control for the unconditioned arousing nature of the drug stimuli. For example, drug-related and nondrug-related stimuli could be shown not just to drug users but also to individuals lacking a drug history. Any difference in responding to the two types of stimuli in the drug-naive group would then reflect unconditioned effects because this group lacks a conditioning history with the drug-related stimuli.

The optimal result in such a study would be to see greater responding to the drug-related stimuli than to the nondrug-related stimuli only in the drug-using group. However, as tong as the drug-using group shows a larger difference in responding to the different stimuli than does the drug-naive group, evidence of conditioning exists.

Although such a design is clearly an improvement on the first procedure, it unfortunately contains a more subtle confound. The problem with this design is that the two subject groups may generally differ in their responsiveness to the unconditioned effects of arousing stimuli. Although we are unaware of any studies that have examined the general arousability of opiate or cocaine abusers, there are data showing that alcoholics demonstrate more unconditioned responsiveness to nonalcohol-related salient stimuli than do social drinkers (e.g., Chandler et al. 1975). It may also be the case that drug users are more aroused by the disturbing drug videotape than are the drug-naive subjects, simply because drug users are more easily aroused in general. This possibility is especially likely given that these two subject groups are entirely self-selected.

THREE GROUP-THREE STIMULUS DESIGN

A better design for examining the role of conditioning in responding to drug-related stimuli involves exposing two different groups of drug users to cues for different kinds of drugs. The idea is to see if each group responds more to cues related to their drug of abuse than to cues related to a drug that they have

not previously used. More specifically, such a study might employ two drug-abuse groups, such as opiate abusers and cocaine abusers, and a third group of drug-naive individuals. All subjects would be exposed to three types of stimuli: stimuli related to use of one drug, e.g., opiates; stimuli related to use of a second drug, e.g., cocaine; and nondrug-related stimuli.

Additionally, it is important that the two drug-using groups administer their drug by different routes. This protocol is necessary to minimize generalization of conditioned responding across the two types of drug stimuli resulting from common stimulus elements.

The outcome of interest in this study involves showing that drug users are maximally responsive to drug cues relevant to their own drug-use histories. That is, drug 1 users should respond more to drug 1 cues than to either drug 2 or nondrug cues. Conversely, drug 2 users should show more responding to drug 2 cues than to drug 1 stimuli or nondrug cues. Drug-naive individuals should show equal responding to the three sets of stimuli.

This crossover pattern of results would deal with the kinds of problems associated with the other naturalistic stimulus designs, that is, that the groups being compared differ in their arousability or that the stimuli being compared differ in their unconditioned effects. One would be unable to argue that results are the consequence of different levels of arousability in the two drug groups because each group reacts to the appropriate drug stimuli. Similarly, one could not argue that one set of drug cues was intrinsically more arousing, because each type of stimulus would evoke responding in the appropriate group. Therefore, seeing this crossover data pattern in the two drug groups would provide a strong argument that responding to the drug stimuli is a specific function of a past conditioning history.

An implementation of this design, consistent with previous studies performed in our laboratory, would include cocaine-only users, opiate-only users, and drug-naive subjects. Cocaine-only users are individuals who are currently using cocaine and have no history of opiate use. Such subjects might well have an extensive history of alcohol or marijuana use, however. Similarly, opiate-only users are individuals using opiates who have no history of using cocaine.

Unfortunately, our patient population at this time includes few individuals who qualify for the opiate-only group. Consequently, we examined only two groups of subjects-cocaine users with no history of opiate use and subjects with no

history of using either cocaine or opiates. This reduced design prevents us from looking at the full crossover data pattern discussed above. However, it does allow us to determine whether differential responding to drug stimuli occurs in the cocaine group as a function of that population's history. In addition, the inclusion of a drug-naive group provides a better assessment of the unconditioned effects of our stimuli than has so far been available.

Method

Subjects. Male patients with a history of smoking cocaine and no history of opiate use were recruited from the inpatient drug dependency ward of the Philadelphia VAMC. Fifteen cocaine subjects have tested so far in this ongoing study. Nine drug-naive control subjects, employees of the hospital of similar ages as the inpatients, have also been tested.

Design. The subjects in this study were exposed to three kinds of stimuli. Cocaine-related stimuli were presented in one session, opiate-related stimuli in a second session, and nondrug-related stimuli in a third session. The order of the three sessions was counterbalanced across subjects.

The variables were physiological measurements of GSR, heart rate, and skin temperature; subjective self-reports of drug-specific high, craving, and withdrawal were also collected. Change from baseline scores were calculated for each of the measures in a manner similar to the one discussed earlier. Statistical tests were performed both to determine whether a stimulus presentation caused a significant change from baseline responding and to determine whether responsiveness differed between the stimuli. Although physiological data was collected during the entire session, only the physiological data collected during the video stimulus will be presented.

Procedure. After subjects had recording electrodes attached and were seated in the recording chamber, they were asked for their self-reports of high, craving, and withdrawal relative to both cocaine and opiate use.

Physiological recording was initiated at this point. After a 15-min baseline period during which no events occurred, subjects listened to an audiotape, watched a videotape, and performed a manual task. Within any one session, all three stimuli were either cocaine related, opiate related, or nondrug related. The videotape stimulus for the opiate session shows an individual buying, preparing, and injecting heroin. During the cocaine session, the videotape depicts two

users free-basing and smoking cocaine. The neutral videotape is a scene from a nature documentary. Following the completion of the manual task, subjects were again asked about their level of high, craving, and withdrawal related to the two drugs.

RESULTS AND DISCUSSION

Cocaine Subjects

Figure 2 depicts the physiological responding in the cocaine-only users-the mean change from baseline and standard error produced by the videotape stimulus for each physiological dependent measure in each session. The mean change in baseline level of cocaine craving is also depicted. The particular session associated with each data bar is indicated on the abscissa. In general, it should be noted that cocaine stimuli are more arousing in these subjects than are the other two sets of cues.

Specifically, cocaine users showed a significant heart rate increase to the cocaine stimuli when compared to prestimulus baseline responding ($t(13) = 3.09$, $p < .01$). By contrast, the opiate-related videotape did not produce a change in heart rate ($p > .05$), and the neutral videotape actually caused a significant decrease in heart rate ($t(13) = -2.98$, $p < .05$). Comparisons of responding between pairs of stimuli revealed a significantly greater increase in heart rate in response to the cocaine-related videotape than to either the opiate-related videotape ($t(13) = 4.48$, p .01) or the neutral videotape ($t(13) = 4.09$, $p < .01$).

For the GSR measure, the cocaine-related videotape caused a significant decrease from baseline ($t(14) = -2.98$, $p < .01$). The opiate- and nondrug-related videotapes had no significant effect ($p > .05$). Comparisons between stimuli showed that the cocaine-related videotape had a greater impact on GSR than the opiate-related videotape ($t(14) = -2.11$, $p = .05$) but failed to lower GSR significantly more than the neutral videotape ($p > .05$).

For skin temperature, both the cocaine- and opiate-related stimuli caused a significant decrease from baseline ($t(14) = -4.27$, $p < .01$ for the cocaine-related videotape; ($t(14) = -3.22$, $p < .01$ for the opiate-related videotape). However, the decrease in response to cocaine stimuli was significantly greater than the decrease to the opiate cues ($t(14) = -3.11$, $p < .01$). Responding to the cocaine-related and neutral videotapes differed as well ($t(14) = -3.84$, $p < .01$).

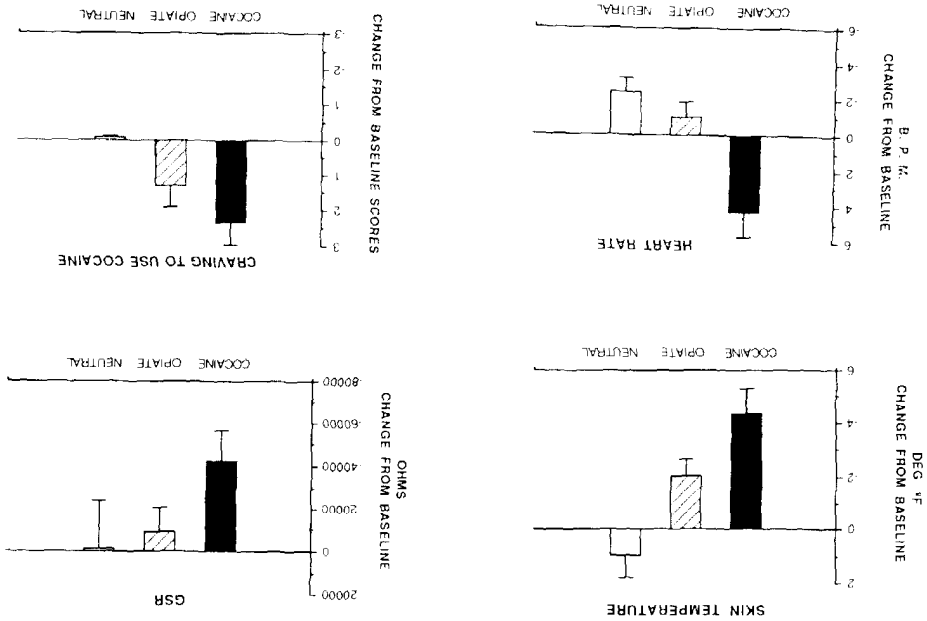

FIGURE 2. *Change from baseline scores during each of the three stimulus sessions (cocaine, opiate, neutral) for the cocaine-only group.*

Once again, this pattern of data is consistent with a conditioning interpretation of the response. Furthermore, the responsiveness to the opiate cues may reflect unconditioned arousal to injection scenes, as was mentioned earlier.

All analyses of the subjective data were performed using nonparametric tests. Data are presented only for craving because there were no significant changes in results for high or withdrawal. Figure 2 shows changes in the subjective state of cocaine craving produced by the stimuli. Craving to use cocaine increased significantly only in the cocaine session (Wilcoxon $T(10)=0$, $p < .01$). By comparison, no significant craving for opiates was reported by the cocaine subjects in any session *($p > .05$).*

Drug-Naive Subjects

In contrast to the pattern of responding observed in the cocaine subjects, the pattern among drug-naive subjects was characterized by a lack of differential responsiveness to the three stimuli across all the dependent measures (see figure 3). This pattern is consistent with a conditioning interpretation of the responding shown by cocaine subjects to the cocaine stimuli.

For example, while neither the cocaine nor the neutral stimuli caused a significant change in heart rate ($p > .05$), the opiate cues actually caused a significant decrease on this measure ($t(9) = -2.90$, $p < .05$). It should be noted that the direction of the effect of the opiate cues on heart rate is opposite that of the cocaine cues on heart rate in the cocaine group. The drug-naive subjects showed no significant changes in GSR to any of the cues (*p .05).*

With respect to skin temperature, it is of some interest to note that the drug-naive subjects showed a near significant ($p = .057$) decrease in skin temperature in response to the opiate cues but exhibited no change in response to the cocaine-related or neutral videotapes ($p > .05$). This decrease in temperature brought about by the opiate cues is similar to that seen in the cocaine subjects and supports the idea that the change in temperature may partially represent an unconditioned effect.

Not surprisingly, drug-naive subjects reported neither cocaine nor opiate craving in any of the three sessions ($p > .05$).

Although we were unable to test an opiate-only group in this design, the data discussed above from previous naturalistic stimulus studies are relevant to

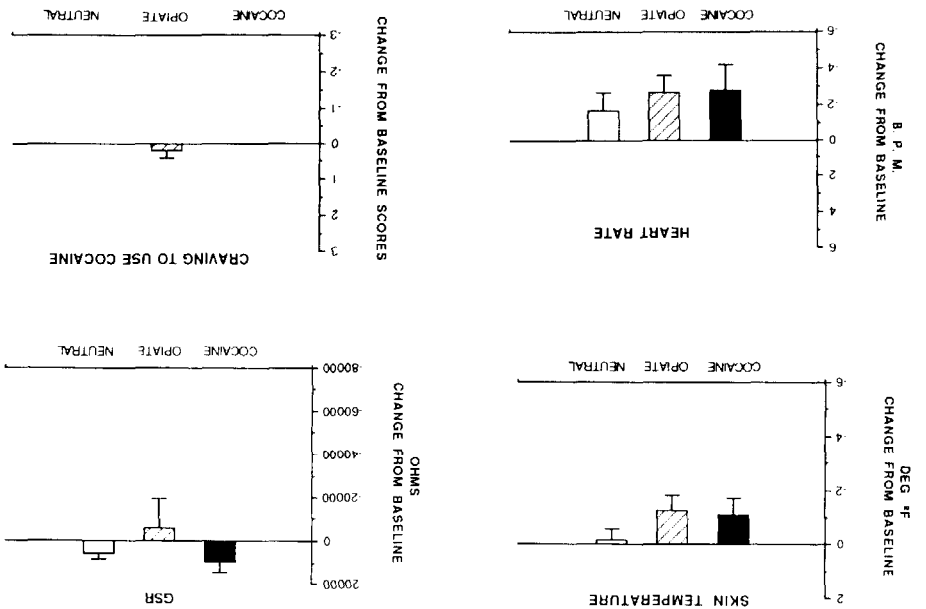

FIGURE 3. *Change from baseline scores during each of the three stimulus sessions (cocaine, opiate, neutral) for the drug-naive group.*

interpreting the current results. The present studies employed the same opiate cues that we have used previously. Consequently, the results of those earlier studies demonstrate that opiate-only subjects are responsive to the identical opiate cues that had a minimal impact on the cocaine and drug-naive subjects in the present study (see table 1).

For example, opiate-only subjects showed significant decreases in skin temperature and GSR in response to opiate stimuli but not to neutral stimuli. Similarly, these subjects reported significant increases in opiate craving in response to opiate stimuli but not to neutral cues (Childress et al. 1988). These results indicate that the opiate cues used in the current study are indeed evocative among individuals with the appropriate drug-use history.

This pattern of data reinforces the idea that the differential responsiveness to the drug stimuli observed in the cocaine-only group was not simply the result of unconditioned differences in evocativeness between the cocaine- and opiate-related stimuli.

CONCLUSION

The data represented here are the best evidence to date that responding to naturalistic stimuli results from a history of conditioning. Additional data comparing responsivity to nondrug-related arousing stimuli between groups of drug users and drug-naive individuals might also prove useful. Such data would provide an additional assessment of potential differences in arousability that may exist between these groups. In general, the current results encourage efforts to develop treatment strategies based on conditioning principles.

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State Dependency as a Mechanism of Central Nervous System Drug Action

Francis C. Colpaert

INTRODUCTION

A response acquired in a given state may not occur when the subject is in a different state. The phenomenon is referred to as state dependency (StD) (e.g., Overton 1983) and means that engrams that are stored in memory in a given state are often more accessible for recall in the same state than they are in a different state (e.g., Weingartner 1978).

The StD paradigm typically involves subjects that are trained to emit some classically conditioned or operant response while in a given (e.g., drug-induced) state; the StD of the acquired response is subsequently examined by determining to what extent the response occurs in different states (e.g., following the injection of a different drug or of the vehicle). Experiments using this paradigm have demonstrated the existence of StD with a wide variety of states that were induced either pharmacologically or otherwise (Overton 1982*a*).

A major problem arising with the StD paradigm is that it is difficult to obtain reproducible, robust evidence of the phenomenon in either human (Weingartner 1978) or animal subjects (Overton 1966). The recognition of this methodological problem, in the early seventies, was followed by decreased interest in the typical StD paradigm and increased use of the drug discrimination paradigm (Stolerman and Shine 1985). In the latter paradigm, subjects typically are trained to emit one response after drug administration, and to emit-under otherwise identical conditions-an alternative response after vehicle treatment (e.g., Colpaen et al. 1976). However, while some authors (Overton 1983) assume that the two paradigms define a single phenomenon, others have argued that the processes underlying drug discrimination and StD may differ (Colpaert et al. 1976).

We have recently described an StD procedure in which drug-to-saline or saline-to-drug state changes yielded robust and quantifiable response decrements following relatively low doses of chbrdiazepoxide (CDP) in rats (Colpaert 1986). The studies summarized here document the dose dependency of the CDP state and examine its possible relationship to tolerance, dependence, and the anxiolytic action of benzodiazepines. The data provide initial evidence that StD may in fact constitute the very mechanism of the central nervous system (CNS) actions of the benzodiazepines.

EXPERIMENTAL PROCEDURES

The state-dependency procedure and details of the experimental conditions have been described elsewhere (Colpaert 1990). Briefly, rats were trained in a food-rewarded, lever-pressing task until they could complete a fixed ratio (FR 10) requirement within the first 120 sec of the session, and were tested for the retention of this response requirement after having reached this criterion. The pharmacological treatment instituted at the time of tests was either the same as (same-state condition) or different from (different-state condition) the treatment used during acquisition.

In one further series of experiments, a conflict procedure was used in which rats were placed in a new environment containing a probe and allowed to explore (Meert and Colpaert 1986). Exploration of the probe is reduced when the probe is electrified, and anxiolytics disinhibit the behavior.

BENZODIAZEPINE STATE DEPENDENCY

Drug Effects on Acquisition

In the course of different experiments, a total of 188 rats were trained with saline injections that were given via various routes and at various time intervals. Six of the rats died prior to reaching criterion, and 15 failed to attain criterion in fewer than 20 sessions. The remaining 167 animals reached criterion after a median number of 10 sessions (95 percent CL (confidence unit), 8-14).

Analysis of the acquisition data that were obtained with the other treatments with which animals were trained failed to reveal any reliable effect on acquisition of any of these treatments, with the exception of cocaine. That is, after receiving 10 mg/kg of cocaine, as many as 22 rats exceeded the 20 acquisition sessions cut-off before 7 were found that reached criterion in less

than 20 sessions; acquisition with 10 mg/kg of cocaine thus required more sessions ($p < .01$; Mann-Whitney U test) than with saline (Siegel 1956).

Same-State Operant Performance

Control data collected throughout these experiments provided extensive evidence that rats trained to complete the FR 10 schedule of bar press responses for food reward within 120 set will reliably do so in test sessions if there is no state change between acquisition and test. That is, all 52 rats trained and tested with saline (subcutaneous [SC] over 30 min) completed the schedule within 120 set during the test session; that was also the case with 29 of 30 rats that were both trained and tested with 40 mg/kg of CDP (SC over 30 min). The response thus failed to occur within 120 set in only 1 of a sample of 82 animals (i.e., less than 5 percent) in which no state change was implemented.

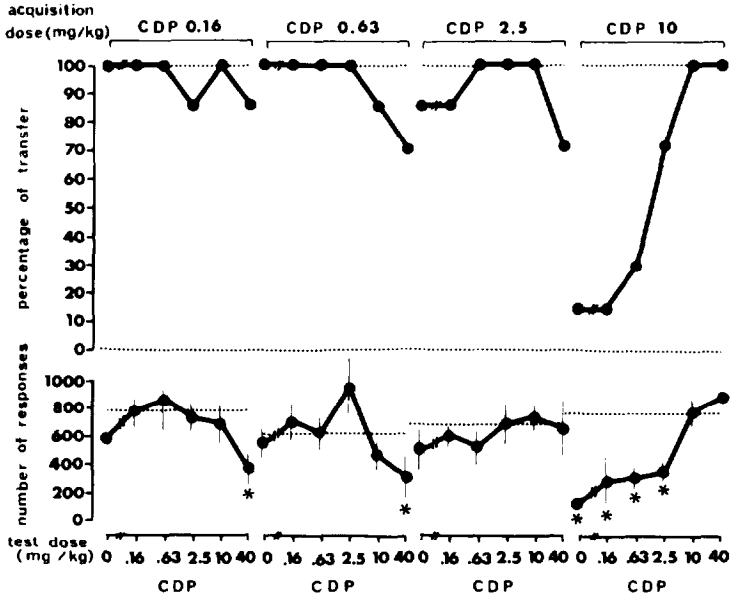
These data therefore indicate that the completion of the FR schedule within 120 set during the test constitutes a satisfactory criterion of transfer. Transfer will henceforth be defined as the completion of 10 lever press responses within 120 sec during a test session. The data described below will be expressed in terms of the percentage of animals in which transfer (as defined above) occurred.

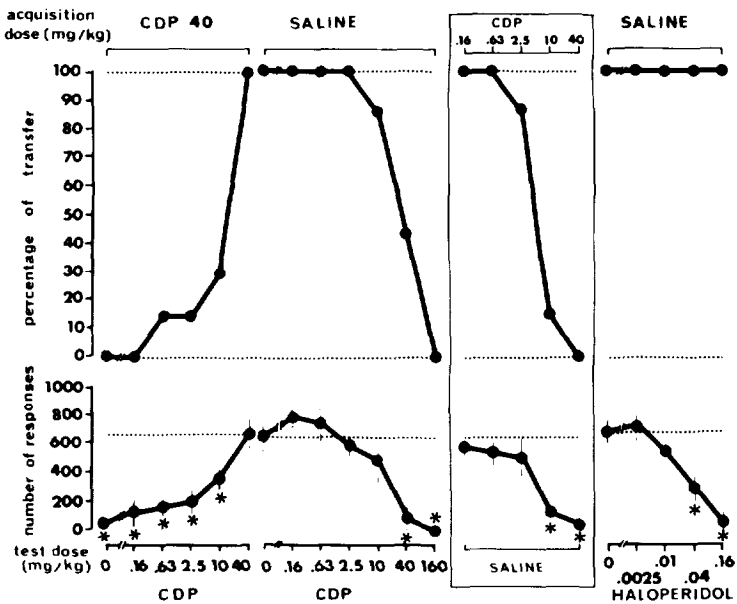
Chlordiazepoxide: Dose-Response Studies

Figure 1 shows that only a few failures to transfer occurred in rats trained with 0.16 to 2.5 mg/kg doses of CDP and tested with saline or 0.16 to 40 mg/kg CDP. Note that the 40 mg/kg test dose of CDP depressed overall response rate in rats trained with 0.16 or 0.63 mg/kg, but not in animals trained with 2.5 mg/kg or higher doses of CDP.

In rats trained with 10 mg/kg CDP, complete transfer occurred with test doses of 40 and 10 mg/kg; transfer then decreased, however, with test doses lower than 10 mg/kg. Transfer in 10 mg/kg trained rats was thus dose dependent, as indicated by Litchfield and Wilcoxon ED_{50} results of 0.96 mg/kg (95 percent CL, 0.34-2.7; Tallarida and Murray 1987). Test doses of CDP lower than 10 mg/kg, as well as saline, also depressed response rate in a manner that related inversely to the dose.

In rats trained with 40 mg/kg of CDP, transfer was similarly reduced in a dose-dependent manner at test doses smaller than 40 mg/kg; the ED_{50} of CDP for transfer in rats trained with 40 mg/kg CDP was 9.8 mg/kg (95 percent CL:


acquisition dose (mg/kg)
CDP 0.16
CDP 0.63
CDP 2.5
CDP 10
percentage of transfer
number of responses
test dose (mg/kg)
0 .16 .63 2.5 10 40
CDP


acquisition dose (mg/kg)
CDP 40
SALINE
CDP
.16 .63 2.5 10 40
SALINE
percentage of transfer
number of responses
test dose (mg/kg)
0 .16 .63 2.5 10 40
0 .16 .63 2.5 10 40 160
SALINE
0 .01 .16
.0025 .04
HALOPERIDOL
CDP

2.9-32). Test doses smaller than 40 mg/kg, again, depressed rate in a dose-dependent manner, and rate depression was virtually complete with saline.

In rats trained with saline, failure to transfer from saline to drug occurred in a dose-dependent manner at test doses of 10 to 160 mg/kg [ED_{50}, 29 mg/kg (95 percent CL: 11-75)].

Figure 1 (insert) shows how transfer in tests with saline varied as a function of the CDP dose used during acquisition. The ED_{50} acquisition dose of CDP at which drug-to-saline transfer failed to occur was thus found to be 5.0 mg/kg (95 percent CL: 2.9-8.6). This ED_{50} dose was also significantly lower *($p < .05$)* than the dose (i.e., 29 mg/kg) at which saline-to-CDP transfer failed to occur (Tallarida and Murray 1987).

The following are notable features of the total number of responses that were emitted in the course of the 15-min test sessions (figure 1). First, in tests in

FIGURE 1. *Results of transfer tests in rats that acquired a response in a given pharmacological condition and that were tested in the same or another pharmacological condition. Rats were trained until they completed a fixed ratio (FR 10) schedule of lever-press responses for food reward within 120 set after the beginning of a daily 15-min session. Training sessions occurred 30 min after subcutaneous (SC) injection of either saline (dose 0) or one of five different acquisition doses of chlordiazepoxide (CDP). Once trained, animals were given one test session 30 min after SC injection of saline or of one of the test doses of CDP. Upper panels: Percentage of transfer, i.e., the percentage of animals that completed the FR 10 schedule within 120 sec. during the test session. lower panels: meat ± 1 SEM of total number of responses made in the 15-min test session. Asterisks indicate significant differences from the group tested with the treatment that was also used during training (two-tailed $p < .05$; Mann- Whitney U test; Siegel 1956). The insert reiterates data that were obtained in tests with saline in animals that acquired the response with one of the different doses of CDP. Lower right panel: Data obtained in another series of experiments in which rats were trained with saline and tested with either saline or one of several different doses of haloperidol (SC over 30 min). Each data point is based on $n = 7$.*

which the test treatment was the same as that used in acquisition, total responding was similar (i.e., an average of about 700 responses), irrespective of whether the treatment was saline or any dose of CDP. Second, response rate generally correlated with transfer in experiments using CDP.

However, this pattern is not a general feature of the paradigm, because in saline-trained rats, 0.0025 to 0.16 mg/kg doses of haloperidol caused dose-dependent suppression of rate while leaving transfer intact (figure 1, bottom right). Third, saline lowered total responding in rats trained with 40 mg/kg CDP just as much as did 40 mg/kg CDP in rats trained with saline.

Chlordiazepoxide: Transfer After Extended Food Deprivation

In this experiment, rats were trained to criterion with CDP (40 mg/kg SC over 30 min) and were tested with either saline or CDP (40 mg/kg SC over 30 min) after 1, 2, 3, or 4 days of food deprivation. Seven animals were used with each test condition. The results are summarized in figure 2.

Rats tested with 40 mg/kg of CDP transferred after 1 day of food deprivation, and other rats also did so after 2 or 3 days of deprivation. Rats tested with saline failed to transfer regardless of the length of food deprivation preceding the test.

Six animals that were scheduled to be tested after 4 days of deprivation died before the tests were conducted, most likely as a result of malnutrition and dehydration. This outcome was entirely unexpected based on observations of wildlife (Hart 1980), indicating that rats can go without food for up to 10 days. At any rate, all experiments involving extended food deprivation were immediately discontinued at that point.

Thus, it appears that food deprivation, even when it is very extreme, did not overcome the failure of transfer of the food-rewarded response that occurred when the response was acquired in the 40 mg/kg CDP state and tested in the saline state.

Prolonged Testing In Switched State Conditions

In this experiment, two groups of seven rats each were trained to criterion with either saline or 40 mg/kg of CDP (SC over 30 min). The animals were then tested (1) for 1 session in the condition that was the same as that of training,

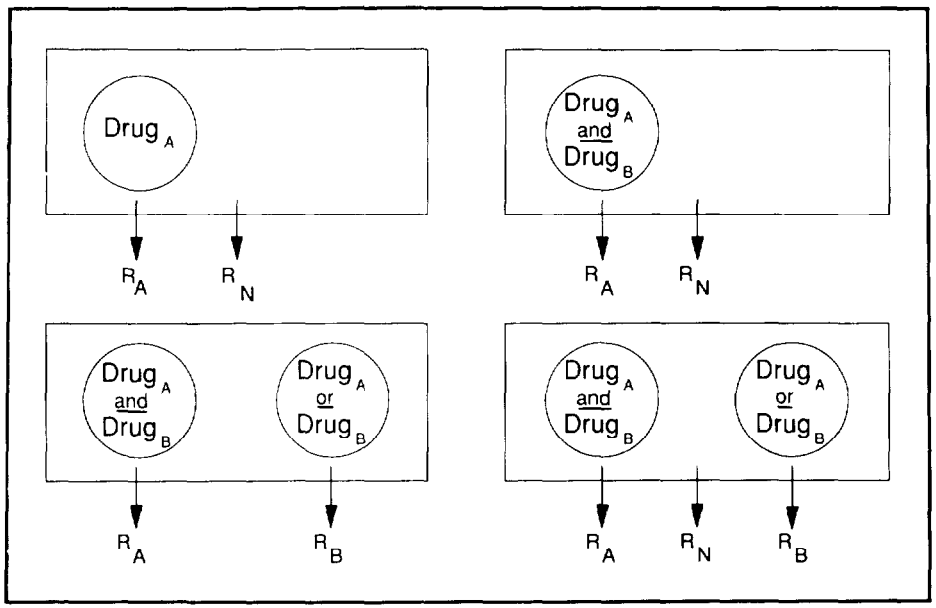


FIGURE 2. *Transfer in rats trained to criterion with 40 mg/kg of chlordiazepoxide (CDP) and tested with saline (open symbols) or 40 mg/kg CDP (closed symbols) after 1, 2, or 3 days of food deprivation. Percentage of transfer is the percentage of animals that completed the FR 10 schedule within 120 sec during the test session. Each data point is based on n = 7.*

(2) for 10 sessions in the different-state condition (i.e., 40 mg/kg of CDP or saline, respectively), (3) for 5 sessions again in the same state condition, and (4) for another 5 sessions in the different state condition.

The first switch from the same to the different state caused failure to transfer to occur in all seven CDP-trained rats and in four of seven saline-trained rats (figure 3). Continued testing or training in the different state resulted in complete transfer after about five sessions. The reinstitution of the same state had no apparent effect in CDP animals but caused a transient disruption in two

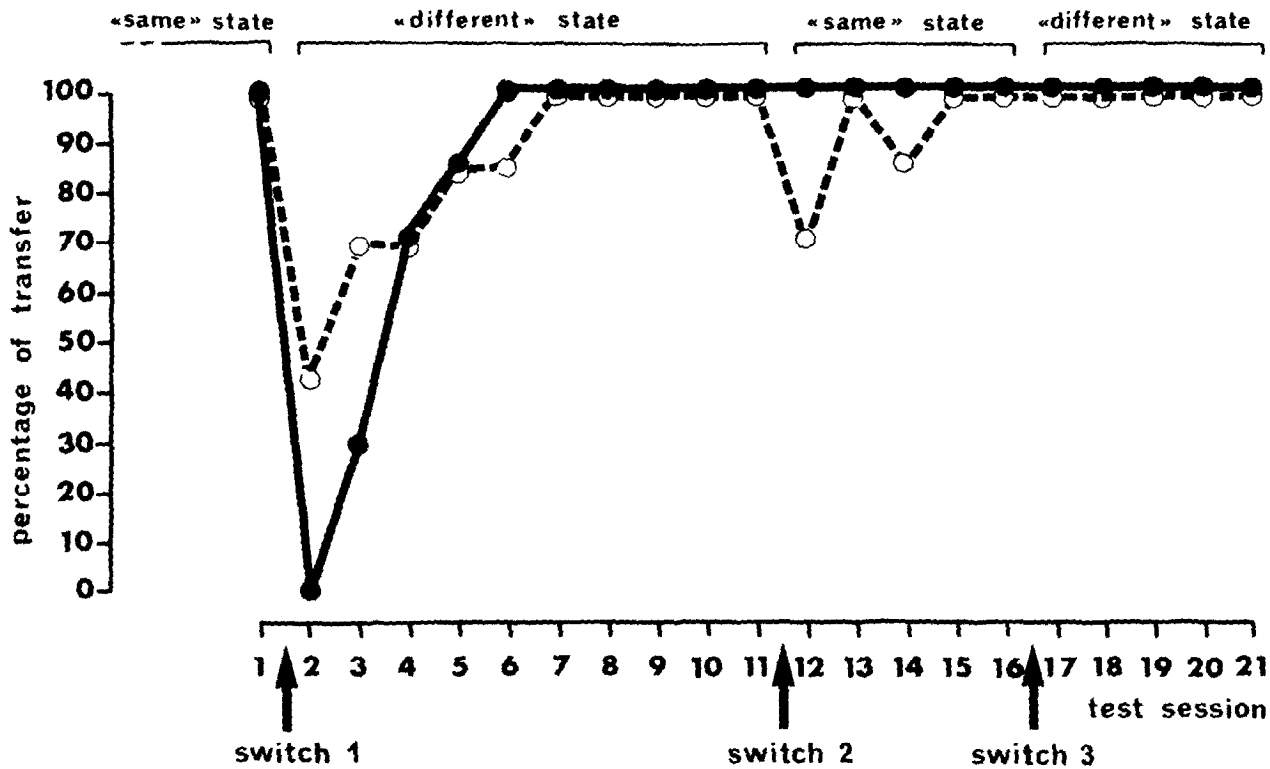


FIGURE 3. *Rats were trained to criterion with either saline (n = 7, open symbols} or 40 mg/kg CDP (n = 7, closed symbols) and then tested succesively in the "same" and the "different" state (i.e., saline or 40 mg/kg CDP, respectively). The number of subsequent sessions in which animals were tested in each phase and state is apparent from the abscissa. Ordinate: Percentage of transfer is the percentage of animals that completed the FR 10 schedule within 120 sec during the test session.*

saline-trained rats. Performance after switch 3 indicated that by then the response was, for all animals, retrievable in both states.

It is noteworthy that the data from this experiment further suggest that relearning after a saline-to-CDP state change requires fewer sessions (median, 2 sessions) than acquisition in experimentally naive animals (median, 10 sessions; see above).

Tolerance to CDP State Dependency

In this experiment, rats were trained to criterion with 40 mg/kg of CDP (SC over 30 min) and training was continued for 5, 10, 20, or 40 additional sessions before a test was carried out with either saline or 40 mg/kg of CDP.

Rats tested with 40 mg/kg of CDP continued to transfer. Rats tested with saline continued to fail to transfer, regardless of the number of additional training sessions that they had undergone with 40 mg/kg of CDP (figure 4).

In the preceding experiments, the performance of rats that were trained with saline and tested for transfer to 40 mg/kg of CDP on 10 consecutive sessions can, of course, also be viewed as conventional data on the development of tolerance, possibly through relearning, to depressant effects of CDP on the rate of operant behavior. The data (figure 4, insert) show CDP to markedly *($p < .01$*; Wilcoxon test) decrease rate in the first test (retraining) session. The depressant effect of CDP was no longer significant *($p > .05$)* in the second session, and disappeared entirely after three injections. Such rapid development of apparent tolerance stands in marked contrast with the main data in figure 4 showing that lack of CDP-to-saline transfer persisted after a total number of CDP injections that, including the approximately 10-session training phase, amounted to as many as 50.

CDP: State Dependence and Physical Dependence

Rats were trained to criterion with 40 mg/kg CDP (SC over 30 min) and then tested, in 10 consecutive sessions, with either saline (SC over 30 min; n = 7) or 40 mg/kg CDP (SC over 30 min; n = 7). Transfer and total responding were monitored, and rats were weighed 30 min before each daily session.

As in previous experiments (figures 1 and 4), the CDP-to-saline state change caused a failure to transfer, which now appeared to require five sessions to

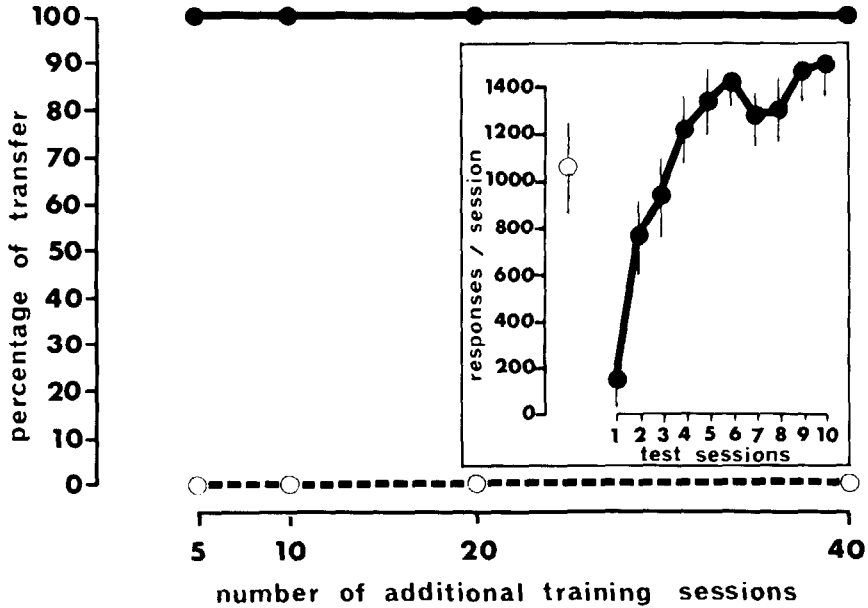


FIGURE 4. *Transfer in rats that were trained to criterion with 40 mg/kg of CDP and given an additional 5 to 40 sessions of training before being tested (SC over 30 min) with either saline (open symbols) or 40 mg/kg CDP (closed symbols). Transfer was defined as the percentage of animals that completed the FR 10 schedule within 120 sec during the test session. Each data point represents one test session in n = 7 rats. The insert shows data from the preceding experiment in which 7 rats were trained to criterion with saline and then tested (retrained) with 40 mg/kg CDP in 10 consecutive sessions. Data points represent the mean ± 1 SEM number of responses per 15-min session on the last saline session (open symbol) as well as on the 10 CDP injections (closed symbols).*

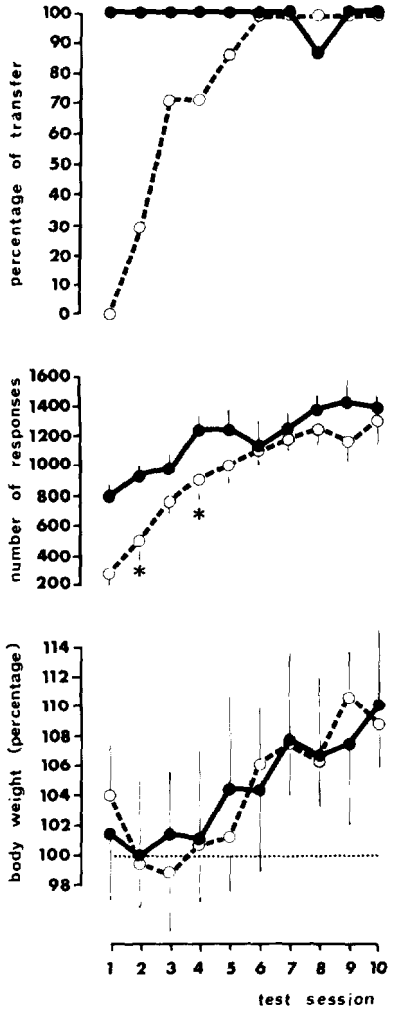


FIGURE 5. *Rats were trained to criterion with 40 mg/kg CDP and fesfed, in 10 consecutive sessions, with either 40 mg/kg CDP (n = 7, closed symbols) or saline (n = 7, open symbols). Transfer, total responding, and body weight were monitored daily. Body weight was expressed for each rat as a percentage of the body weight on the day criterion was reached; data points represent the mean ± 1 SEM. The asterisk indicates p < .05 for the difference between the two groups.*

recover (figure 5). Saline also depressed responding, and this depression, too, required about five sessions to disappear.

Relative to their weight on the day that criterion was reached, animals that continued to receive CDP steadily gained weight during the 10 days that test sessions took place. Animals receiving saline showed a slight decrease on Day 3 of testing. However, the weight of saline-treated rats failed to differ either from their own criterion weight (one-tailed $p > .05$; Wilcoxon test) or from that of animals that received CDP (one-tailed $p > .05$; Mann-Whitney U test). Note also that the drop in the weight of animals receiving saline on Day 3 failed to coincide with their maximal drop of transfer, which occurred on Day 1 (figure 5).

Benzodiazepine State Dependency: Receptor Specificity

In these experiments, several groups of rats were trained to criterion with two injections and tested with two injections of either the same or different injections; the two injections were SC and intraperitoneal (IP) and both were given 30 min before the session, as specified in table 1.

Table 1 shows that diazepam caused a dose-dependent failure to transfer from saline; its IP ED_{50} was 9.7 mg/kg (95 percent CL: 2.9-33).

Rats trained with 10 mg/kg of IP diazepam showed complete transfer with 40 mg/kg of SC CDP, but not with saline. Rats trained with 40 mg/kg of SC CDP failed to transfer either with saline or with a 0.63 mg/kg dose of IP diazepam. However, transfer did occur and increased dose-dependently as the diazepam test dose was increased; diazepam's ED_{50} (IP) in substituting for 40 mg/kg of CDP was 4.0 mg/kg (95 percent CL: 1.2-13).

Following Ro 15-1788, a benzodiazepine-receptor blocker that itself was ineffective in saline-trained animals, transfer failed to occur in CDP-trained rats. The compound antagonized the state induced by 40 mg/kg of CDP (table 1).

Finally, the following compounds (SC over 30 min) failed to induce transfer in all rats (n = 7 per test compound) that were trained with 40 mg/kg of CDP: yohimbine (10 mg/kg), haloperidol(0.16 mg/kg), cocaine (10 mg/kg), morphine (10 mg/kg), apomorphine (0.16 mg/kg), ± propranolol (10 mg/kg), clonidine (0.16 mg/kg), and methoxamine (2.5 mg/kg).

TABLE 1. *Transfer test results in rats (n = 7) trained with SC and IP injections over 30 min*[a]

Acquisition Treatment (mg/kg)		Test Treatment (mg/kg)		Percentage of Transfer[a]
SC	IP	SC	IP	
Saline	Saline	Saline	Saline	100
Saline	Saline	Saline	Diazepam (0.53)	100
Saline	Saline	Saline	Diazepam (2.5)	84
Saline	Saline	Saline	Diazepam (10)	43
Saline	Saline	Saline	Diazepam (40)	14
Saline	Diazepam (10)	Saline	Saline	0
Saline	Diazepam (10)	CDP (40)	Saline	100
CDP (40)	Saline	Saline	Saline	0
CDP (40)	Saline	Saline	Diazepam (0.63)	0
CDP (40)	Saline	Saline	Diazepam (2.5)	43
CDP (40)	Saline	Saline	Diazepam (10)	57
CDP (40)	Saline	Saline	Diazepam (40)	100
CDP (40)	Saline	Saline	Ro 15-1788 (10)	0
Saline	Saline	Saline	Ro 15-1788 (10)	100
CDP (40)	Saline	CDP (40)	Saline	100
CDP (40)	Saline	CDP (40)	Ro 15-1788 (10)	0

NOTE: COP = chlordiozepoxide

[a]The percentage of animals that completed the FR 10 schedule within 120 sec during the test session.

State Dependency With Nonbenzodiazepine Compounds

Rats that were trained to criterion with yohimbine and were tested with saline showed a failure to transfer that was proportional to the yohimbine dose used in acquisition (ED_{50} = 1.5 mg/kg; (95 percent CL: 0.68-3.1). (See table 2.) Failure to transfer also occurred in rats that were trained with saline and tested with

0.63 to 10 mg/kg doses of yohimbine (ED_{50} = 4.9 mg/kg (95 percent CL: 2.9-8.3).

In contrast, no failure to transfer occurred in either drug-saline or saline-drug transfer tests involving 2.5 and 10 mg/kg doses of cocaine (table 2). In rats trained with saline, tests with 0.0025 to 0.16 mg/kg doses of haloperidol also did not reveal any evidence of StD. These doses did, nonetheless, cause a dose-dependent depression of the total number of responses made during the 15 min sessions (figure 1, bottom right).

BENZODIAZEPINES: ANXIOLYTIC ACTION AND STATE DEPENDENCY

Out of 38 control rats given SC saline, 3 failed to initiate the session. The mean±SEM number of responses made during the session was 16±2.5 in unpunished SC saline controls (n = 15) and 4.1±0.66 in the 20 animals that served as punished SC saline controls. The number of rats that failed to initiate the session before 9 were found per dose that did, was 1, 0, 0, and 2 with CDP doses of 0.63, 2.5, 10, and 40 mg/kg, respectively. CDP (SC) increased punished responding in a dose-dependent manner, the effect being statistically reliable at 10 and 40 mg/kg (figure 6). The CDP dose at which the distance, along the Y axis, between punished and unpunished controls was covered halfway, was estimated by linear interpolation to be 30 mg/kg.

Out of 37 IP saline control rats, 4 failed to initiate the session. The 13 unpunished IP saline controls emitted a mean of 17±2.2 responses; the 20 punished IP saline controls averaged 3.1±1.6. The number of rats that failed to initiate the session before 9 were found per dose that did, was 1, 2, and 15 with diazepam doses of 0.63, 2.5, and 10 mg/kg, respectively. Diazepam (IP) increased punished responding dose dependently; its effects were significant at 2.5 and 10 mg/kg. The dose at which diazepam covered the distance between punished and unpunished controls halfway was 7.8 mg/kg.

The doses at which CDP and diazepam exerted anticonflict effects are important for at least two reasons. First, the 30 mg/kg dose at which CDP had a 50 percent anticonflict effect was considerably higher than the dose necessary to produce drug-to-saline transfer failure (i.e., 5.0 mg/kg; figure 1). The 50 percent anticonflict dose of diazepam (i.e., 7.8 mg/kg) was also higher than the dose (i.e., 4.0 mg/kg; table 1) at which diazepam substituted for CDP in CDP-trained animals. These data indicate that both benzodiazepines produced state dependency in drug-to-saline transfer tests at doses that were lower than

TABLE 2. *Transfer test results in rats trained to criterion with various pharmacological treatments*

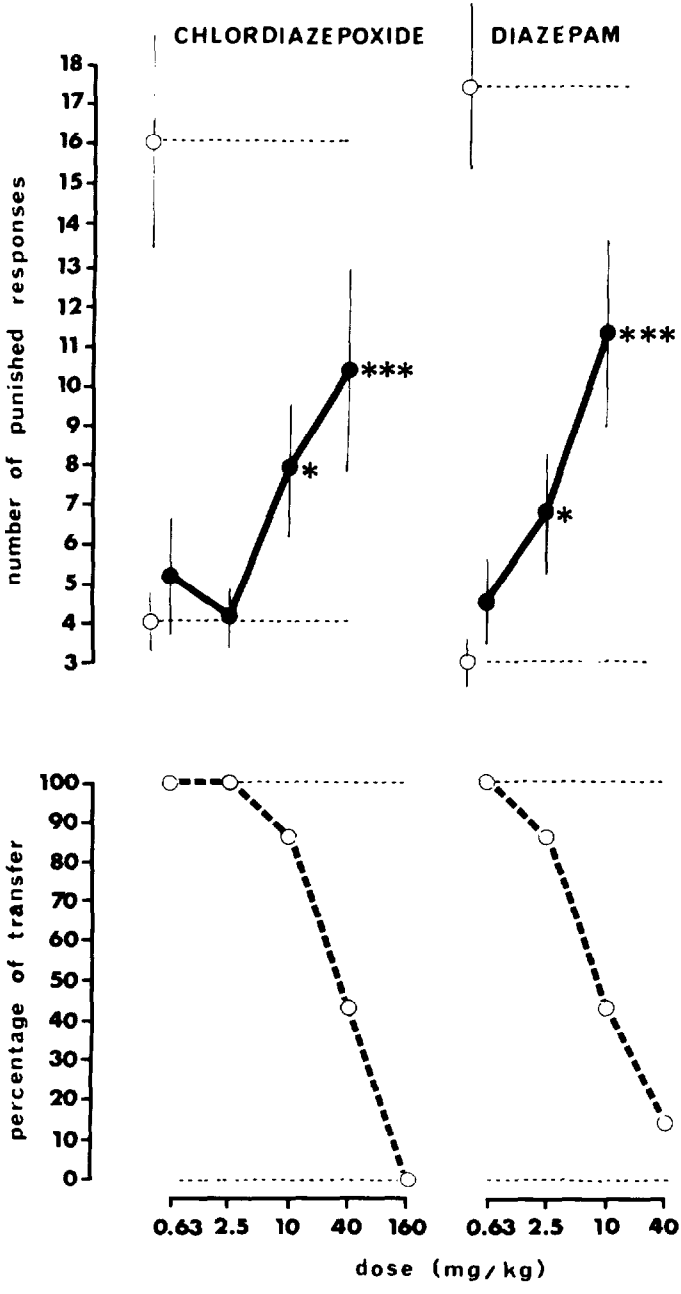
Acquisition Treatment				Test Treatment				
Compound	Dose (mg/kg)	Route	Time (min)	Compound	Dose (mg/kg)	Route	Time	Transfer[a]
Saline	-	IP	15	Saline	-	IP	15	100
Saline	-	IP	15	Yohimbine	0.63	IP	15	100
Saline	-	IP	15	Yohimbine	2.5	IP	15	66
Saline	-	IP	15	Yohimbine	10.0	IP	15	14
Yohimbine	0.04	IP	15	Saline	-	IP	15	100
Yohimbine	0.16	IP	15	Saline	-	IP	15	66
Yohimbine	0.63	IP	15	Saline	-	IP	15	86
Yohimbine	2.5	IP	15	Saline	-	IP	15	29
Yohimbine	10.0	IP	15	Saline	-	IP	15	0
Saline	-	SC	30	Saline	-	SC	30	100
Saline	-	SC	30	Cocaine	2.5	SC	30	100
Saline	-	SC	30	Cocaine	10.0	SC	30	100
Cocaine	2.5	SC	30	Saline	-	SC	30	100
Cocaine	10.0	SC	30	Saline	-	SC	30	100

[a]The percentage of animals that completed the FR 10 schedule within 120 sec during the test session.

the doses that produced anticonflict effects. Second, comparing the saline-to-drug failure-to-transfer data (figure 1; table 1) with anticonflict activity reveals the two curves to be mirror images with both CDP and diazepam (figure 6). In fact, the anticonflict doses of CDP and diazepam (i.e., 30 mg/kg and 7.8 mg/kg, respectively) were virtually identical to ED_{50} in causing a failure to transfer in saline-to-drug tests (i.e., 29 and 9.8 mg/kg, respectively; figure 1 and table 1).

GENERAL DISCUSSION

The present data indicate that the completion of an FR 10 schedule of food-rewarded lever presses within 120 sec constitutes a conditioned operant response requirement with which drug state changes can be shown to yield robust, reliable response decrements.


CHLORDIAZEPOXIDE
DIAZEPAM
number of punished responses
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
***
*
***
*
percentage of transfer
100
90
80
70
60
50
40
30
20
10
0
0.63 2.5 10 40 160
0.63 2.5 10 40
dose (mg/kg)

Three types of state changes involving CDP were analyzed in the present studies.

1. Drug-to-saline transfer tests in rats trained with one of several doses of CDP and tested with saline indicated StD occurred at an ED_{50} (acquisition) dose as low as 5.0 (95 percent CL: 2.9-8.6) mg/kg. Note that this dose is low indeed relative to the doses at which CDP exerts behaviorally depressant effects (figure 1, lower panel). These findings argue against the earlier view that StD effects can be obtained only at behaviorally toxic doses of drugs (Overton 1983).

2. Dose-to-dose transfer tests indicated lower doses of CDP substituted in a dose-dependent manner for a higher CDP dose used in acquisition (figure 1).

3. Saline-to-drug transfer tests revealed CDP disrupted the transfer of the saline-acquired response only at a dose (i.e., 29 [95 percent CL: 18-75] mg/kg) that was six-fold higher than that at which drug-to-saline transfer was disrupted. The interpretation of saline-to-drug transfer data has so far been hampered by the confounding effects that drugs may have on the rate of behavior (Overton 1983). These data indicate that it is arbitrary to ascribe any transfer failure in saline-to-drug tests simply to behaviorally depressant drug effects and the methodology presented, which clearly differentiates memory deficits due to state change from drug effects on the rate of behavior. Specifically, saline caused as large a rate-depressant effect in rats trained with 40 mg/kg CDP as did 40 and 160 mg/kg doses of CDP in saline-trained animals. Also, up to 0.16 mg/kg test doses of

FIGURE 6. *Anticonflict and state-dependency effects of chlordiazepoxide (CDP) and diazepam in rats. Upper left panel: Effects of CDP on punished responding. Data points represent the mean± 1 SEM of n = 9 with each dose (•); n = 15 for unpunished (o, upper) and 20 for punished (o, lower) saline controls. All injections were SC. With diazepam, data points are also based on n = 9 per dose; there were 13 unpunished and 20 punished saline controls; injections were made IP. Asterisks indicate one-tailedp to be <.05 (*) or < .001 (***) for the difference with punished saline controls (Mann-Whitney U test; Siegel 1956). Lowerpanels: Plot of saline-to-drug transfer data obtained with SC CDP (data from figure 1) and IP diazepam (data from table 1).*

haloperidol profoundly depressed response rates in saline-trained rats without causing any transfer failure (figure 1).

Another series of experiments substantiated the pharmacological specificity of the benzodiazepine state; the benzodiazepine receptor blocker Ro 15-1788 (Hunheler et al. 1981) antagonized CDP in producing the state, whereas the typical receptor agonist diazepam substituted for CDP (table 1). In contrast, various nonbenzodiazepine drugs that act on the CNS did not substitute for CDP in producing the benzodiazepine state. Data obtained with yohimbine (table 2) indicate that the StD procedure used here can also demonstrate StD with nonbenzodiazepine compounds. Cocaine, however, failed to produce StD in conditions that were otherwise similar to those in which cocaine is highly discriminable (Colpaett and Janssen 1982). The latter finding deserves further analysis in view of a longstanding theoretical controversy about whether StD and discriminative drug effects reflect the same phenomenon (Colpaert et al. 1976; Overton 1982*b*).

The data shown in figure 2 are dramatic evidence of the extent to which the memory for the response was locked into the CDP state; prolonged food deprivation, even to the point of starvation, failed to make the food-rewarded response transfer from the CDP to the undrugged state. Further evidence (figure 3) showed that it is nonetheless possible to render the response retrievable in both states by having it conditioned separately in the CDP and the undrugged state. Without such conditioning, however, the response, if acquired in the CDP state, remained unretrievable in the undrugged state, even when subjects were overtrained by up to 40 sessions (figure 4). Clearly, these data fail to satisfy the operational definition of tolerance, which requires that the repeated administration of the drug leads to a demonstrable loss of its initial effect (Carlton 1983). That state dependency persisted after as many as 50 injections in all of 40 mg/kg CDP is all the more remarkable as only 3 injections were sufficient (figure 4, insert) to cause an apparently complete tolerance to CDP's known depressant effects on behavior (Cook and Sepinwall 1975). A similar loss of saline's behaviorally depressant effects developed at a similar rate in rats trained with 40 mg/kg of CDP (figure 5). Note that these data also add to previous evidence demonstrating that the learning (or relearning) of the engram can account for apparent tolerance to drug effects on behavior (Colpaert and Shearman 1988). Using body weight as a measure of physical dependence (Martin et al. 1963) no evidence was obtained in the present experiments that CDP-StD was associated with physical dependence (figure 5).

Our data suggest that there may be a relationship between CDP-StD and drug dependence. Drug dependence can be defined as a situation in which the physiological or psychological integrity of the subject is conditional upon a drug (Balster 1985; Tatum et al. 1929). That CDP-trained animals require CDP to be able to emit vital, food-reinforced behavior (figure 2) can thus be taken to suggest that StD can operate as a mechanism of drug dependence. The marked resistance to tolerance to the CDP state (figure 4) makes it possible for this potential mechanism of benzodiazepine dependence to be extremely powerful and persistent.

Further experiments explored the possible relationship between StD and the anxiolytic action of benzodiazepines. Anxiolytic activity was assessed in rats using a modification (Meert and Colpaert 1986) of the conflict paradigm (Geller and Seifter 1960) that has predictive validity for the anxiolytic potency of benzodiazepines in humans (Cook and Davidson 1973; Sepinwall and Cook 1978). The data indicated (figure 6) that the prototypical benzodiazepines, CDP, and diazepam produce anticonflict effects at doses (i.e., 30 and 7.8 mg/kg, respectively) that, if anything, were higher than those at which drug-to-saline transfer failure occurs (i.e., 5.0 and 4.0 mg/kg, respectively) and that were essentially identical to those at which saline-to-drug transfer failure occurs (i.e., 29 and 9.7 mg/kg, respectively).

Two important considerations would seem to arise from these findings. One is that any use of benzodiazepines as anxiolytics may be associated with StD; some storage of information in memory, or perhaps much of the storage that takes place while subjects are on anxiotytic doses of benzodiazepines, may fail to transfer to the normal state. Thus, StD provides a possible explanation for what is conventionally referred to as widespread retrograde amnesia produced by the benzodiazepines (Lister 1985; Weingartner 1985). Second, the finding that benzodiazepines produce anticonflict activity at precisely the doses at which saline-to-drug transfer fails makes it possible to consider StD as the very mechanism of the benzodiazepines' anxiolytic activity. Unlike such primary drives as hunger and thirst, anxiety has been characterized by behavioral theorists (Miller 1955; Mowrer 1939) as a "chiefly secondary," or an acquired, drive; anxiety is progressively learned, and stored in memory, in the course of ontogeny. The implication is that for anxiety to be operative at a mature stage, the subject must retrieve what it has learned about the anxiety drive in its undrugged past. Our data show that an undrugged-to-drugged state change produced with benzodiazepines can make such retrieval fail. An interesting prediction from this StD mechanism of benzodiazepine anxiolysis is that the

dose at which anxiolysis occurs is the same dose at which saline-to-drug transfer failure occurs; the anxiolytic dose, therefore, should not vary with specific behavioral contingencies. We have shown previously (Meert and Colpaert 1986) that CDP's anxiolytic dose is indeed constant and does not change with varying levels of shock intensity in a rat conflict procedure. It is interesting to ask whether similar mechanisms may apply to engrams that are acquired phylogenetically.

In summary, the research discussed here indicates that the retrieval from memory of a hunger-driven operant response can be made drug-state dependent; robust retrieval failures occurred in drug-to-saline and in saline-to-drug tests for transfer involving benzodiazepines. Although the learned behavioral response was reinforced by food, food deprivation to the point of starvation was unable to overcome the retrieval failure that occurred with a drugged-to-undrugged state change. Up to 50 injections of CDP also failed to prevent the drugged-to-undrugged retrieval failure, indicating that the CDP state is remarkably resistant to tolerance. A new theory of benzodiazepine drug action results: the state dependency of memory retrieval may constitute a parsimonious, integrative explanation for, and mechanism of, the anxiolytic and untoward (amnesic, drug dependence) actions of these drugs.

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State-Dependent Learning With Social Drugs

Geoff Lowe

INTRODUCTION

Drug-induced state-dependent learning (SDL) is now a well-established phenomenon (Overton 1978). The term is used to describe the finding that behavior learned in one drug state is better remembered when retention is tested in the same drug state. This refers to an ability to access information under the same or a different set of retrieval cues and, indeed, might be more accurately described as state-dependent retrieval. Even drinking immediately *after* sober learning may affect consolidation and result in recall deficits if there is drug-state dissociation between storage and retrieval phases (Lowe 1982).

In studies with human subjects, alcohol (Lowe 1981), marijuana (Darley et al. 1974) barbiturate and amphetamine (Bustamente et al. 1970), methylphenidate (Swanson and Kinsboume 1976) and nicotine (Peters and McGee 1982) have all been shown to produce SDL effects. In some cases, the effect was asymmetrical. That is, recall tended to be poorest when the acquisition stage was under a drug (D) state but the retrieval stage was drug free (ND). With drug-free acquisition and drug-state retrieval (ND-D), recall is also poor but less so than under D-ND conditions. However, in studies reporting asymmetrical effects, it is worth noting that there were slight drug-induced deficits in Day 1 learning, compared with drug-free learning. When equivalence of original learning between D and ND states is obtained, symmetrical SDL effects have been observed, at least in the case of alcohol (Lowe 1981).

The combined use of alcohol and cigarettes, and subsequently caffeine, is a common occurrence (Carmody et al. 1985), and concern has been shown for the disproportionate increase in susceptibility to disease in populations where the incidence of joint consumption is high (e.g., Keller 1979, 1977; Walton 1972). However, few researchers have examined the psychopharmacological interactions of alcohol, nicotine, and caffeine, particularly in relation to SDL.

The interaction between alcohol and stimulant drugs is generally regarded as complex, with antagonistic (e.g., Knott and Venables 1979, 1977) synergistic (e.g., Lee and Lowe 1980; Leigh 1982), and negligible or mixed (e.g., Tong et al. 1974) effects being reported. Since both alcohol (A) and nicotine (N) have separately been shown to produce SDL in man, it is reasonable to expect that the A+N combination should produce similar state-dependent effects. Although caffeine (C) has not yet been shown to produce SDL in man, cognitively disruptive effects of alcohol-caffeine combinations have been observed (Lee and Lowe 1980) and, thus, SDL effects would also be expected from the A+C combination. However, in terms of the possible antagonistic/synergistic interaction, of particular interest is the recall performance of those subjects given a single drug on Day 2 after previously undergoing the acquisition phase under the influence of an alcohol-nicotine/caffeine combination. Recall decrements (or otherwise) on Day 2, when either alcohol or nicotine/caffeine is absent, should shed more light on the nature of these drug combination interactions, at least in relation to the phenomenon of SDL.

EXPERIMENT 1: ALCOHOL AND NICOTINE

Method

Twenty-four undergraduate students, ages 18 to 28, volunteered as subjects. There were 12 females and 12 males, all of whom were smokers who regularly combined use of alcohol and cigarettes. Their smoking histories ranged from 8 months to 7.5 years (mean, 2.84 yr). Their mean consumption was 9.2 cigarettes per day and 24.5 units of alcohol per week (a “unit” is defined as one measure of spirits or one half-pint of beer or equivalent).

The alcoholic beverage consisted of vodka (37.5 percent alcohol per volume) mixed with an equal volume of Schweppes Russchian aromatic tonic water. The subject was given 4.4 ml of this mixture for every kg of body weight, so the alcohol dose was moderate (0.66 g/kg), and designed to produce a blood-alcohol concentration (BAC) of around 80 mg/100 ml (the United Kingdom’s legal limit for driving). In the placebo drink, the vodka was replaced by an equivalent amount of water, and the rim of the drinking glass was smeared with vodka to give an identical initial olfactory cue. Nicotine administration consisted of subjects smoking two Benson & Hedges Pure Cold middle tar cigarettes within a 12-min period. These cigarettes have a rated average nicotine delivery of 1.4 mg. In the placebo condition, two Honeyrose

herbal cigarettes (nicotine-free) were given. Although puff frequency was monitored, no direct measure of inhalation was possible.

Design and Procedure

On Day 1 (learning), all subjects received the same drug treatment (A+N) immediately prior to the acquisition stage. On Day 2 (retrieval) male and female subjects were randomly allocated to one of four conditions to receive either (1) both drugs (A+N); (2) nicotine plus placebo (N+O); (3) alcohol plus placebo (A+O); or (4) no drugs (O+O). There were three males and three females in each subgroup.

Subjects were asked to refrain from smoking and from consuming caffeinated beverages for at least 3 hr before each daily session. They were also required to consume no alcohol for at least 18 hr before the experiment. Upon arrival in the laboratory, each subject was weighed and required to complete a questionnaire on smoking and drinking history. The subjects were then tested with an Alcolmeter AE-D1 (Lion Laboratories) in order to check that they were alcohol-free and to familiarize them with this instrument.

Pairs of subjects consumed their alcoholic beverages and smoked two cigarettes within a 20-min period. Approximately 40 min after starting drinking, subjects were retested with the Alcolmeter before undertaking the learning task, which consisted of a simplified geographical map visually displayed with a 19-item set of auditory instructions about a particular route (Lowe 1981). An arbitrary criterion of at least 14 correct items was adopted as a learning measure. If after four learning trials, the subject had not attained this level, then the highest number correct in any of the four trials was taken as the learning performance score. This learning session lasted approximately 5 min, and was followed by another Alcolmeter reading. Day 1 ended with a final Alcolmeter reading, approximately 50 min after commencing the drinking session.

The procedure for Day 2 (recall) was the same as that for Day 1 (learning) except for the different drug conditions of the subgroups and the fact that there was only one recall trial. The number of correctly recalled items from the Day 1 presentation of the route map was recorded as the recall performance score.

Results and Discussion

Table 1 shows the mean number of items correct from the route map for learning (Day 1) and recall (Day 2) sessions, and for each condition. Mean BACs during route map performance are also shown. A one-way analysis of variance on the learning scores of Day 1 revealed no significant difference among the four subgroups (F[3,20] = 1.053, $p > .05$). Table 1 shows the mean decrements between learning and recall for each condition. Apart from the slightly increased recall performance on Day 2 in the A+N condition, all other conditions resulted in significant recall decrements due to alcohol (F[1,20] = 23.09, $p < .0001$) and to nicotine (F[1,20] = 6.08, $p < .05$). Although the nicotine effect was more marked in the alcohol condition, the A+N interaction was not statistically significant (F[1,20] = 3.36, $p > .05$).

The lack of recall decrement when subjects ingested both alcohol and nicotine on Day 2 supports the notion that SDL was induced by an alcohol-nicotine combination. Both the double placebo (O+O) and nicotine only (O+N) conditions resulted in the largest dissociation decrements from the (A+N) learning state. There was just as much dissociation when nicotine was ingested on Day 2 as when no drugs were taken. This suggests that the major SDL effect was due to alcohol. However, it seems likely that nicotine had some influence because when nicotine was absent during the alcohol session on Day 2 (A+O), the dissociation decrement was about halfway between that of the combination (A+N) and nicotine only (O+N) conditions. In other words, the alcohol-alone state was insufficient to prevent a recall decrement on Day 2; the

TABLE 1. *Alcohol and nicotine*[a]

Condition	Items correct		Mean Recall	Mean BAC	
Day 2)	Day 1 (Learning)	Day 2 (Recall)	Decrements	Day 1	Day 2
A + N	12.25	12.75	-0.5[b]	69.5	80.1
A + O	12.75	8.00	4.75[c]	87.4	73.0
O + N	15.00	6.67	8.33[c]	74.2	-
O + O	12.00	4.00	8.00[c]	81.4	-

[a]BAC = blood alcohol content; A = alcohol; N = nicotine; O = placebo.
[b]Not significant.
[c]$p<.01$.

absence of nicotine led to a partial dissociation from the Day 1 (A+N) learning state.

If, as Leigh (1982) suggests, the combination of alcohol and nicotine can result in a synergistic interaction, it seems likely that the nicotine-alone state is discriminatively different from the combination, and much more so than the alcohol-alone state. But the difficulty of assessing the relative degree of SDL produced by a mixture of two drugs when each is tested at only one dose must be acknowledged, especially in the case of nicotine when no biological markers are taken.

EXPERIMENT 2: ALCOHOL AND CAFFEINE

Method

Sixteen undergraduate students, ages 18 to 28 yr, volunteered for this experiment. There were eight males and eight females, and all were regular alcohol and coffee drinkers.

The experimental design and general procedure were identical to those of Experiment 1 except that the nicotine conditions were replaced by caffeine conditions. Subjects drank two cups of Gold Blend (Nescafe) coffee, each containing 1.5 5-ml teaspoons, approximating a total dose of 300-375 mg caffeine. This was consumed within a 10-min period subsequent to the alchol/placebo drinking period. In the placebo condition, Gold Blend decaffeinated coffee was administered at the same concentration.

On Day 1 all subjects received the same drug treatment (A+C) immediately prior to the acquisition stage. On Day 2 (retrieval) subjects were randomly allocated to one of four conditions to receive either (1) both drugs (A+C); (2) placebo plus caffeine (O+C); (3) alcohol plus placebo (A+O); or (4) no drugs (O+O). See table 2.

Results and Discussion

A one-way analysis of variance on the learning scores of Day 1 revealed no significant differences among the four subgroups (F[3,12] 0.196, $p > .05$). Dependent t-tests (comparing Day 1 with Day 2) were computed for the performance scores for each condition. In the caffeine conditions (A+C; O+C)

TABLE 2. *Alcohol and caffeine*[a]

Condition (Day 2)	Items correct		Mean Recall Decrements	Mean BAC	
	Day 1 (Learning)	Day 2 (Recall)		Day 1	Day 2
A + C	14.25	14.00	0.25[b]	78.0	84
A + O	14.25	10.75	3.55[c]	79.5	81
O + C	14.75	13.75	1.00[b]	79.0	-
O + O	13.75	8.25	5.50[c]	80.5	-

[a]BAC = blood alcohol content; A = alcohol; C = caffeine; O = placebo.
[b]Not significant.
[c]$p < .01$.

there were no significant differences. But whenever caffeine was absent on Day 2 (A+O; O+O), significant recall decrements were observed.

The lack of recall decrement when subjects ingested both alcohol and caffeine on Day 2 supports the notion that SDL was induced by an alcohol and caffeine combination. Both the double-placebo (O+O) and alcohol-only (A+O) conditions resulted in the largest dissociation decrements from the (A+C) learning state. There was almost as little dissociation when only caffeine was ingested on Day 2 as when both drugs were ingested. This finding suggests that the major SDL effect was due to caffeine, although the relatively high dose used could be the major determinant. The alcohol-alone state was insufficient to prevent a recall decrement on Day 2; the absence of caffeine led to a partial but significant dissociation from the Day 1 (A+O) learning state.

If, as Waldeck (1974) suggests, the combination of alcohol and caffeine can result in a synergistic interaction, it appears that the caffeine-alone state is not discriminatively different from the combination, whereas the alcohol-alone state does seem to be. This finding contrasts somewhat with the results of Experiment 1 using alcohol-nicotine combinations, which showed alcohol to be the dominant drug.

GENERAL DISCUSSION

These studies offer some initial insights and confirm the complex nature of social drug combinations in an area of cognitive behavior (SDL) in which these particular drug combinations have not previously been applied. Demonstrations of state-dependent effects of social drugs are of practical interest only if they involve dosages and combination practices typically used outside the laboratory. Our results indicate that SDL can occur not only in carefully designed animal laboratory experiments but also under more naturalistic conditions of moderate social drinking, smoking, and caffeine use. The clinical implications of the phenomenon of state-dependent learning are potentially large. There is anecdotal evidence from drug users suggesting that state dependence may play a role in their use of drugs. Users often report a continuity of desirable thoughts, perceptions, and feelings from one occasion of drug use to another and relative amnesia for the pleasant feelings and thoughts experienced in between. Overton (1978) has outlined and reviewed several models linking state dependence with drug abuse.

One theory proposes that an altered repertoire of drug-state responses develops with repeated drug use. If the drug-specific behaviors are more reinforcing than normal “undrugged” behaviors, then the user may ingest drugs to gain access to the drug-response repertoire rather than because of any intrinsically reinforcing drug effects. For instance, other people may treat a person differently (and possibly more favorably) when he is intoxicated than when he is sober. Alternatively, drug effects may alter the user’s sensitivity to social reinforcement so that reinforcing contingencies are effectively changed even in the absence of any real change in the external environment (Lowe 1984).

Another theory treats the discriminative effects of psychoactive drugs as a conditioned stimulus that evokes a reinforcing effect. Yet another theory proposes that SDL is a direct causal factor in drug dependence and alcoholism. The notion is that the dissociative barrier prevents recall in the sober or nondrug state for many of the negative consequences of drug use. Only the initial (usually positive) effects of ingestion are well recalled. Evidence supporting this proposal has been obtained by Tamerin et al. (1971), who showed that alcoholics selectively fail to remember much of the dysphoric content of their drinking episodes.

A final point relates to cognitive anticipation involved in drug effects. It is a well-established fact that people in Western cultures have distinct expectations about the effects of social drugs on behavior and feelings (e.g., Brown et al. 1980; Southwick et al. 1981). A prominent hypothesis assumes that many acute effects of alcohol, for instance, are mediated by such expectations alone (Marlatt and Rohsenow 1980), and a recent meta-analysis indicates substantial empirical support for this contention (Hull and Bond 1986).

The extent of such cognitive influences should not be underestimated, as was amply illustrated in Marlatt and Rohsenow's review of balanced-placebo-design studies (1980). In the majority of studies, expectations alone produced certain behaviors, but pharmacological effects alone did not. Hence, there is convincing evidence that some alcohol-related behaviors are actually the consequence of cognitive factors or of other factors that have no basis in pharmacology. Whether such cognitive factors influence the outcomes of state-dependent learning studies reported in the present paper remains to be seen. We are currently running a series of experiments designed to explore such possibilities.

In conclusion, a significant proportion of everyday forgetting could be due to SDL effects, and drug combinations offer an increased range of dissociation possibilities. It would thus be helpful if people became more aware of the conditions that can limit the potential range of retrieval cues available when recall of information is required.

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Discriminative Stimulus Effects of Drug Mixtures in Rats

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INTRODUCTION

It is widely recognized that the discriminative stimuli that many drugs produce may have a compound nature. In dealing with exteroceptive stimuli it is often easy to generate simple, unitary stimuli, such as light of a defined wavelength. Drugs are different. Almost all compounds have multiple effects, both at the level of receptors and in terms of subjective effects, and recognition of this fact has led to the widespread use of the term “discriminative stimulus complex” in such work. The processing of drug-produced stimuli may therefore be more like the processing of compound than of simple exteroceptive stimuli. Few studies have addressed the implications of this situation, although there are several cases in which the complex nature of stimuli have been unraveled to various extents. For example, cyclazocine produces discriminative stimuli with multiple components that seem to parallel the effects of this drug at both the mu and the kappa subtypes of opioid receptors (reviewed by Holtzman 1985). Other results suggest that clonidine has both cocaine-like and noncocaine-like activity (Wood et al. 1985) and imply that chlorphentermine acts on both dopamine and serotonin mechanisms (Young 1988). There are also cases of generalization to mixtures of drugs in circumstances when the component drugs failed to generalize fully; Holloway et al. (1985) and Gauvin et al. (1989) have reported full generalization from amphetamine or cocaine to certain mixtures of caffeine with phenylethylamines. The present experiments represent an attempt to use mixtures of drugs to generate compound interoceptive stimuli, the components of which may be manipulated independently by varying the doses of drugs in the mixtures.

Figure 1 shows some examples of situations in which mixtures of drugs may serve as discriminative stimuli. The different paradigms are shown according to the conceptualization of Järbe and Swedberg (1982) in comparison with a

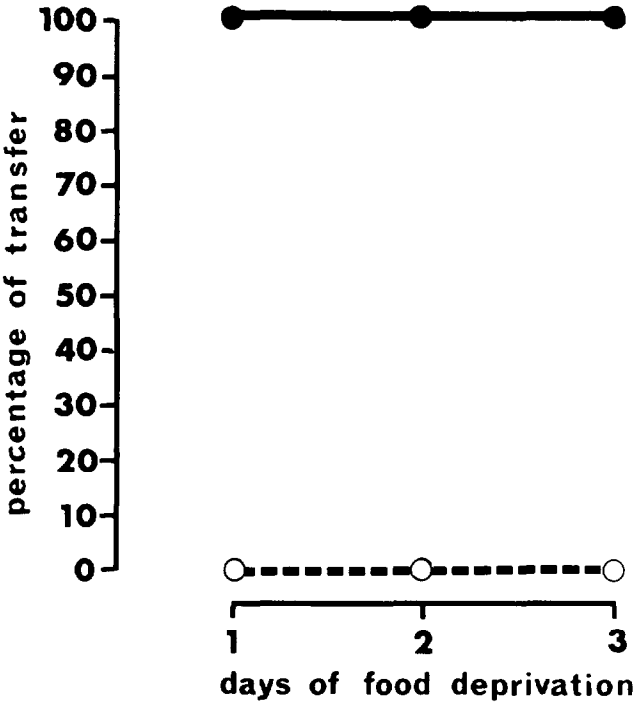

FIGURE 1. *Schematic outline of four different drug discriminations produced by extending the conceptualization of Järbe and Swedberg (1982). Upper left section shows a drug versus nondrug discrimination. RA = response associated with a particular dose of the training drug. RN = response associated with absence of drug Upper right section shows basic mixture discrimination in which RA is associated with particular doses of drug A and drug B. lower left section extends this conceptualization to discriminate explicitly between mixture and component drugs by making RB = response associated with drug A or drug B. Lower right shows one of several further extensions, in this case to a three-choice paradigm with responses associated with and, or, and nondrug states.*

simple form of discrimination based on a single drug. In the latter case (figure 1, upper left section), one response is associated with the presence of the drug and a second response is associated with its absence. Figure 1 (upper right section) shows an equivalent concept of a discrimination based on the effects of a mixture of two dissimilar drugs. Here, one response is associated with the simultaneous presence of both drugs (i.e., drug A and drug B) and a second

response is associated with the absence of both drugs; this procedure is subsequently called the “AND” discrimination. Figure 1 shows only two of the many other possible concepts involving mixtures of drugs. The lower left section of figure 1 shows the particularly interesting case of an “AND-OR” discrimination where one response is associated with a mixture of drugs A *and* B and the second response is associated with the effects of either drug A *or* drug B. Figure 1 (lower right section) shows an elaborated form of the AND-OR discrimination in which a third response is associated with the absence of both drugs. (Two different responses, one for each component drug, could be used in a further elaborated model.)

These conceptual models are important because they focus attention on functional differences between the different paradigms; drug discriminations developed in the different paradigms may well exhibit different characteristics as a result of these varying functional relationships. This paper is limited mainly to studies of the AND paradigm for mixtures (figure 1, upper right), plus results for some preliminary studies with an AND-OR discrimination (figure 1, lower left); it also expands the concepts and data put forward by Stolerman et al. (1987*a,b*).

A number of earlier investigations also shed light on the discrimination of drug mixtures, but usually in ways rather different from the studies reviewed here. The earliest study that has come to light is that of Overton (1966), who developed an AND discrimination based on a mixture of atropine and pentobarbitone using a shock-escape procedure in a T-maze. Subsequently, Järbe and Johansson (1976) studied the AND discrimination with a mixture of the anticholinesterase physostigmine and the muscarinic blocking mixture Ditran (which was itself a mixture). However, studies with drugs that partly cancel each other’s effects are difficult to interpret unless the characteristics of noninteracting drugs are understood. The present studies therefore attempt first to define the main features of a discrimination based on a mixture of nicotine *and* midazolam. Discriminations based on these drugs given singly had previously been studied in some depth under similar conditions and there was no reason to expect interactions through pharmacological mechanisms. The studies conceptually closest to the present experiments seem to be those of Hanlin and Appel(1985), but these have appeared only in abstract form.

Witkin et al. (1980) carried out some work with a mixture of amphetamine *and* pentobarbitone. One group of pigeons was trained to discriminate a mixture of amphetamine and pentobarbitone from amphetamine alone (an AND

discrimination within a drug versus drug design). Other pigeons were trained to discriminate a mixture of amphetamine and pentobarbitone from pentobarbitone alone. In previous behavioral experiments, amphetamines and barbiturates were found to interact in additive, synergistic, or antagonistic ways, depending on behavioral baselines and other factors (Branch 1974; Rushton and Steinberg 1983). In drug discrimination experiments, such mixtures might exhibit unusual characteristics that could contribute to understanding why they are particularly subject to abuse; studies directed to this end have been carried out. Preliminary work with mixtures of pentazocine *and* tripelennamine (T's and blues), caffeine and phenylpropanolamine (formerly an over-the-counter substitute for amphetamine), and morphine *and* nicotine has also been performed to test the generality of findings to date.

Snoddy and Tessel (1983) studied mixtures of amphetamine and nisoxetine, but there was cross-generalization between these drugs in simple drug discrimination experiments, and studies on such mixtures can make a limited contribution to understanding compound interoceptive stimuli. Glennon and Young (1984) studied the discrimination of a drug mixture without really intending to do so. In the wurse of investigations in which racemic methylene dioxyamphetamine (MDA) was used in training, it became apparent that the (-)- and (+)-isomers in the mixture generated qualitatively distinct stimuli. It was suggested that (-)-MDA acted mainly through serotonergic receptors whereas (+)-MDA acted on dopaminergic mechanisms. Many other drug discriminations based on racemic mixtures may also involve multiple mechanisms, and a full understanding of such discriminations will therefore depend on knowledge of how compound stimuli produced by drugs are processed.

The main questions to be answered about the processing of such compound interoceptive stimuli include the following: Can such complex procedures yield orderly, readily interpretable results? Are stimuli produced by mixtures of drugs perceived and processed in terms of the component drugs or as new homogeneous entities? What are the effects of altering the doses and relative amounts of drugs used for training? Does the use of mixtures for training influence the specificity of the discriminations obtained? What are the effects of antagonist drugs that selectively affect responses to the individual drugs used to establish the discrimination? What is the importance of psychological processes such as overshadowing and blocking, which have been found to play key roles in discrimination of compound exteroceptive stimuli? Answers to some of these questions are emerging from the work summarized here.

METHODS

Animals

Male, Lister hooded rats were housed individually in rooms maintained at about 20°C with a regular light-dark cycle (light from 8 a.m. to 8 p.m.). Initially, the rats weighed 210-280 g, but throughout the experiments they were fed restricted amounts of food to maintain their weights at about 80 percent of normal. Water was available in the living cages at all times.

Apparatus

Standard experimental chambers (Campden Instruments, London, UK) were contained in sound-insulated, ventilated enclosures. The chambers were fitted with two retractable response bars separated by a recess in which 45 mg pellets of food could be presented. White noise was present at all times to mask external sounds. The experiments were controlled by programs written in ONLIBASIC, running on CUBE microcomputers (Control Universal, Cambridge, UK).

Training Procedure

The procedure was based on that described previously for establishing discriminations based on single drugs (Garcha et al. 1985; Pratt et al. 1983). Rats were trained to press bars for food reinforcers before any injections were given. Then, after injections of two drugs, animals were reinforced for pressing one of the two bars; presses on the other bar were reinforced in sessions after saline injections. The dose of drugs, the route by which they were administered, and the interval between injections and training depended on the drugs used, as detailed below. Training sessions were of 15 min duration. Drug and saline training sessions took place in random order, with the restriction that no more than three sessions with the same treatment occurred in succession. The final schedule of food reinforcement was tandem variable-interval 1 min fixed-ratio 10; under this schedule, food was presented following the tenth consecutive response on the correct bar after a randomly determined interval (mean = 1 min). The doses of drugs were based on preliminary experiments and previous work.

Generalization Test Procedure

Discriminative effects of drugs and drug mixtures were determined with groups of 8-12 rats in 5-min extinction tests. Such tests took place twice weekly, with training sessions continuing on the intervening days. Tests with different doses of the training drugs or with other drugs normally took place in random order. The index used to assess discriminative effects was the number of responses on the bar appropriate for the mixture of drugs used for training, expressed as a percentage of the total number of responses on both bars. A minimum of 10 responses was required for this index to be calculated.

Variations In Training Doses

In some experiments, the relative amounts of drugs in mixtures used to maintain discriminations were varied. After first establishing discrimination with a particular mixture and carrying out tests for stimulus control as detailed below, the dose of one of the drugs used to maintain the discrimination was altered. The rats were trained for at least 12 further drug and saline sessions (in random order), and then stimulus control was examined again by means of extinction tests. Several different dose ratios were used in succession to maintain discriminations in this way, as detailed in the Results section.

Drugs

Nicotine bitartrate (BDH, Poole, Dorset, UK), midazolam maleate (Hoffman-La Roche, Basle, Switzerland), (+)-amphetamine sulfate (Smith Kline and French, Welwyn Garden City, UK), sodium pentobarbitone, pentazocine, tripelennamine hydrochloride, phenylpropanolamine hydrochloride (all from Sigma, Poole, Dorset, UK), morphine hydrochloride (May and Baker, Dagenham, UK), and quipazine maleate (Miles, Slough, UK) were dissolved in isotonic saline. Caffeine (Sigma) and phencyclidine hydrochloride (NIDA) were dissolved in distilled water. All injections were given in volumes of 1 ml/kg, and all doses were calculated as those of the base. Pentazocine and tripelennamine were administered 30 min before sessions. All other drugs were administered 15 min before sessions except as noted below.

RESULTS AND DISCUSSION

Stimulus Control With Compound Drug Stimuli and Their Elements

A variety of different mixtures engendered strong stimulus control in a manner generally similar to single drugs. Such results have been obtained in rats trained with mixtures of nicotine *and* midazolam, amphetamine *and* pentobarbitone, morphine *and* nicotine, pentazocine andtripelennamine, and caffeine *and* phenylpropanolamine. Rats acquired strong discriminative responses cued to the drug states with an accuracy of at least 85 percent. The results with the mixtures were compared with results of component drugs tested separately at the doses used to maintain discriminations. Such tests with single drugs serve as tests of stimulus control by elements of a compound interoceptive stimulus.

Examining first results for rats trained with nicotine (0.4 mg/kg SC) and midazolam (0.2 mg/kg SC), figure 2 shows that these drugs separately produced about 60 percent drug-appropriate responding, as compared with 88 percent after the mixture and 8 percent after saline (Stolerman et al. 1987). These results set a pattern that was repeated with only minor variations in the studies with mixtures of other drugs. Figure 3 shows that in tests with amphetamine (0.4 mg/kg SC) or pentobarbitone (10 mg/kg SC), there was about 75 percent drug-appropriate responding in rats trained to discriminate a mixture of these drugs from saline. Generally similar results were obtained with pentazocine (8 mg/kg IP) *and* tripelennamine (8 mg/kg SC), with morphine (3 mg/kg SC) *and* nicotine (0.4 mg/kg SC), and with caffeine (20 mg/kg IP) and phenylpropanolamine (20 mg/kg IP) in rats trained with mixtures of each pair of drugs at the doses stated. In these experiments only, morphine was administered 30 min before sessions.

These experiments suggest that in rats trained with mixtures of dissimilar psychoactive drugs, the separate drugs at the doses used for training engender considerable amounts of mixture-appropriate responding. This principle may be very generalizable because such findings have been obtained with five different mixtures.

Dose-Response Studies With Drugs Used for Training

Full dose-response data have been obtained only for mixtures of nicotine *and* midazolam and amphetamine *and* pentobarbitone. Figure 4 shows the results of

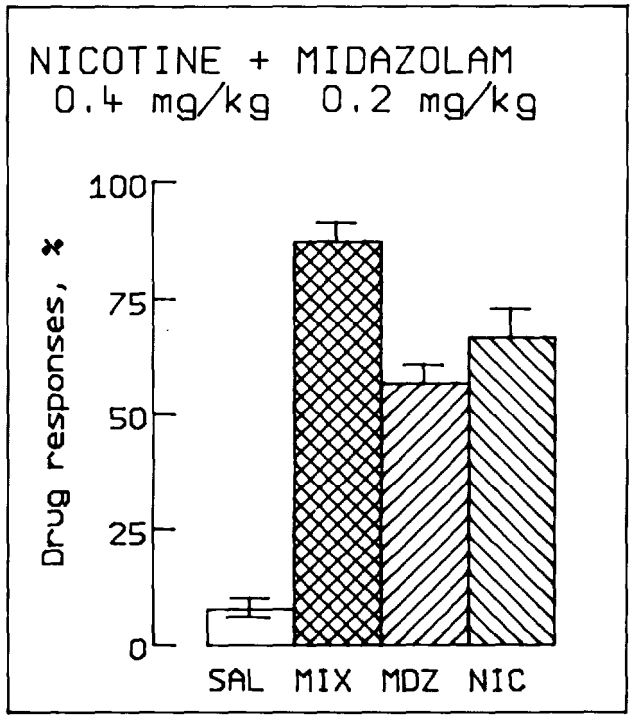

FIGURE 2. *Tests of responses to the training drugs in rats trained to discriminate a mixture of nicotine and midazolam from saline (AND discrimination). Results are shown for a group of 8-9 rats tested with saline (SAL), nicotine* and *midazolam (MIX), midazolam alone (MDZ). or nicotine alone (NIC) at doses shown. All data were obtained in 5-min extinction tests and are expressed as mean percentages of drug-appropriate responding ± SEM. (Data from Stolerman et al. 1987).*

these studies in rats trained to discriminate nicotine (0.4 mg/kg SC) and midazolam (0.2 mg/kg SC). Administering different amounts of the mixture, while keeping constant the ratio of the doses of the component drugs, yielded a progressive increase in the percentage of drug-appropriate responding (Stolerman et al. 1987). The dose-response curve resembled typical curves for subjects trained on single drugs. Nicotine administered alone, up to the amount in the mixture used for training (i.e., in doses of 0.04-0.4 mg/kg), increased the percentage of drug-appropriate responding in a dose-related manner. Figure 4

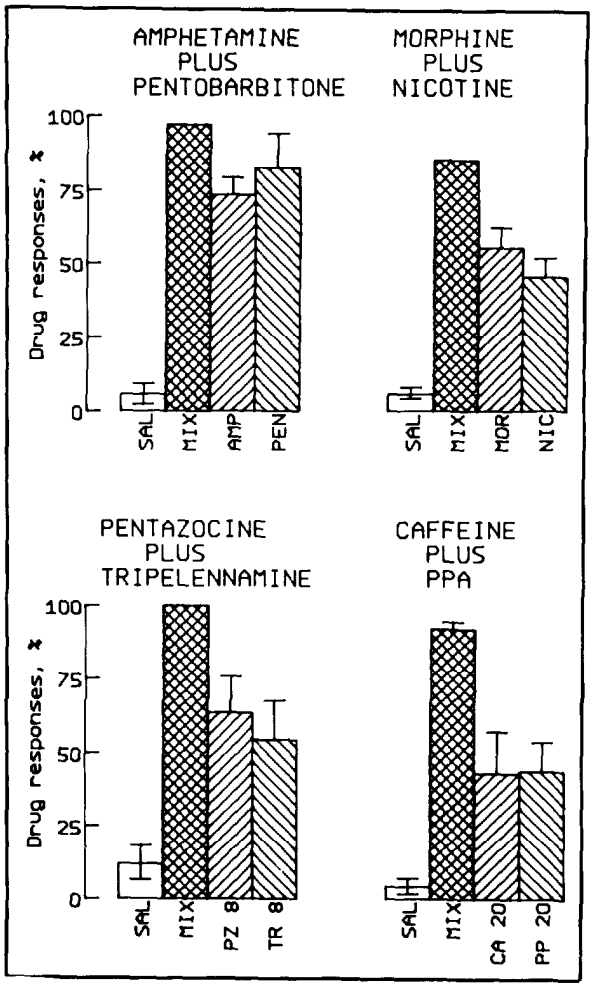

FIGURE 3. *Tests of responses to the training drugs in four groups of rats trained to discriminate different mixtures of drugs from saline (n = 8-10, AND discrimination). In each case, results are shown for saline (SAL), mixtures of drugs (MIX), and component drugs, as follows. Upper left: amphetamine (0.4 mg/kg, AMP) and pentobarbitone (10 mg/kg, PEN). Upper right: morphine (3 mg/kg, MOR)* and *nicotine (0.4 mg/kg, NIC). Lower left: pentazocine (8 mg/kg, PZ)* and *tripelennamine (8 mg/kg, TR). Lower right: caffeine (20 mg/kg, CA)* and *phenylpmpanolamine (20 mg/kg, PP).*

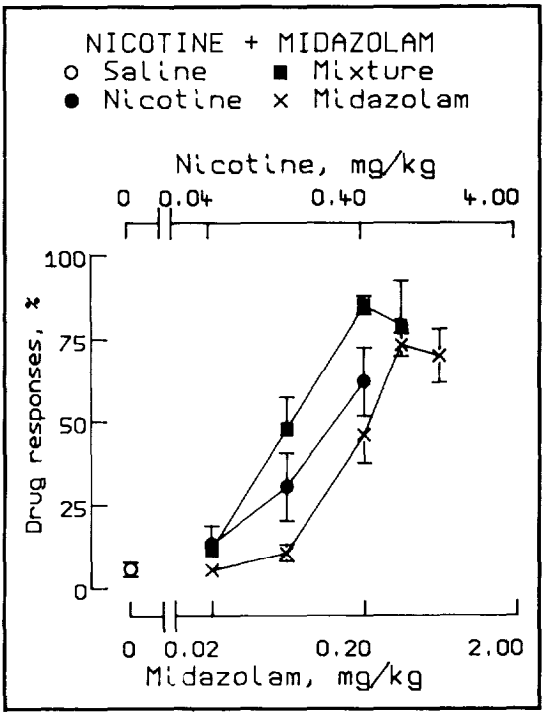

FIGURE 4. *Dose-response curves from rats trained to discriminate a mixture of nicotine (0.4 mg/kg) and midazolam (0.2 mg/kg) from saline (n = 9, AND discrimination). Results are shown for discriminative effects of mixtures with the dose ratio for component drugs held constant(■), for nicotine alone (X), and for midazolam alone (●). Responding after saline is also shown (O). After large doses of drugs some animals did not respond sufficient/y for discriminative effects to be assessed and data are not shown where n < 7. All data are shown as means ± SEM, with overlapping SEM omitted for clarity. (Data from Stolerman et al. 1987.)*

shows that the scores were slightly below those for the mixture. Midazolam (0.02-0.2 mg/kg) also increased drug-appropriate responding in a dose-related manner, and again the scores were below those for the mixture. However, increasing the dose of midazolam to 0.36 mg/kg (i.e., above the dose in the training mixture) further increased drug-appropriate responding, which then

approached the highest levels obtained after administering mixtures. Data for nicotine at doses above that used for training could not be obtained because such doses reduced response rates too severely. Both nicotine and midazolam reduced response rates in these experiments; their effects in mixtures were not additive, but were between those seen with either drug separately. Stolerman et al. (1987) have described the changes in response rates in detail.

Similar experiments have been carried out in rats trained to discriminate amphetamine (0.5 mg/kg SC) and pentobarbitone (12 mg/kg SC) from saline. Figure 5 shows that the mixture increased drug-appropriate responding in a dose-related manner. Amphetamine (0.05-0.73 mg/kg SC) and pentobarbitone (1.2-18 mg/kg SC) given separately also increased drug-appropriate responding, although in each case the effect was less than for amounts of the mixture containing the same dose of each drug. When either of these drugs was given at a dose above that used for training, drug-appropriate responding approached the highest levels seen after administering the mixture. However, the number of rats for which these data could be obtained was reduced because the large doses of the drugs severely reduced overall response rates.

These experiments confirm that rats trained to discriminate mixtures of drugs can respond to the discriminative effects of the component drugs when they are given separately. Furthermore, the responses are dose related and, in some instances, there is almost complete generalization from mixtures to components.

Studies With Antagonists

A further potentially powerful approach to analyzing discriminative effects of mixtures of drugs utilizes specific pharmacological antagonists. Stolerman et al. (1987) examined the effects of a nicotine antagonist and a benzodiazepine antagonist in rats trained with a mixture of nicotine and midazolam. These animals were trained to discriminate a mixture of nicotine (0.4 mg/kg SC) and midazolam (0.2 mg/kg SC) from saline. Tests with the benzodiazepine antagonist flumazenil (Ro 15-1788) and the nicotine antagonist mecamylamine showed that there was no generalization to the antagonists, either when they were administered separately or together as a mixture (figure 6). Administering either flumazenil or mecamylamine reduced the discriminative effects of the mixture of nicotine and midazolam, but the percentage of drug-appropriate responding did not fall below about 60-70 percent. However, administering the antagonists together completely blocked the discriminative effect of the mixture. The doses of antagonists used had previously been shown to be more than

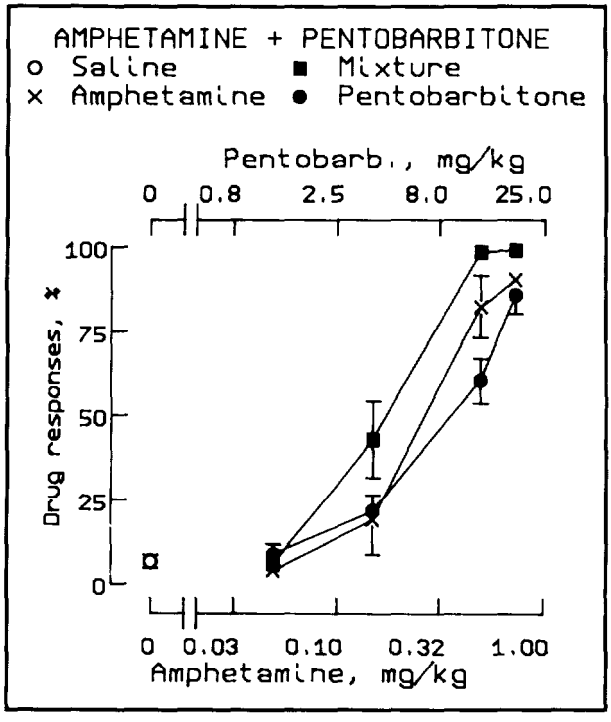

FIGURE 5. *Dose-response curves from rats trained to discriminate a mixture of amphetamine (0.5 mg/kg and pentobarbitone (12 mg/kg) from saline (n = 10, AND discrimination). Results are shown for discriminative effects of mixtures with the dose ratio for component drugs held constant (■), for amphetamine alone (X), and forpentobarbitone alone (●). Responding after saline is also shown (0). After large doses of drugs some animals did not respond sufficiently for discriminative effects to be assessed and data are not shown where n < 4. All data are shown as means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted for clarity.*

sufficient to fully block the discriminative effects of their respective agonists in rats trained in conventional, single-drug discrimination procedures (Stolerman et al. 1983, 1966).

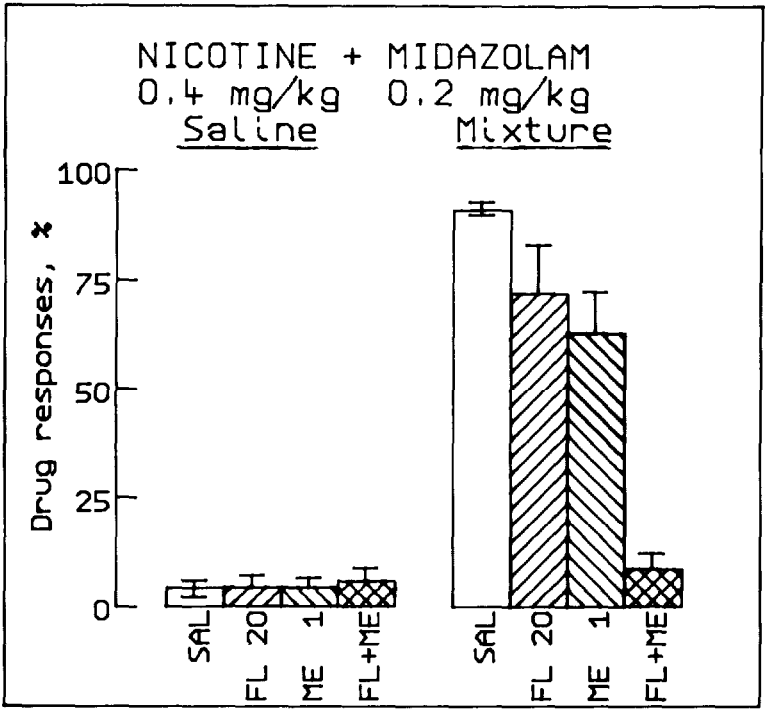

FIGURE 6. *Tests with antagonists in rats trained to discriminate a mixture of nicotine (0.4 mg/kg) and midazolam (0.2 mg/kg) from saline (AND discrimination). Left section shows results of tests for generalization with saline (SAL), flumazenil (20 mg/kg, FL), mecamylamine (1 mg/kg, ME), and both antagonists at these doses (FL + ME). Right section shows tests for antagonism of training mixture by the same doses of flumazenil and mecamylamine. All data were obtained in 5-min extinction tests and are expressed as mean percentages of drug-appropriate responding ± SEM. (Data from Stolerman et al. 1987.)*

It is notable that neither the benzodiazepine antagonist nor the nicotine antagonist alone produced more than a marginal and unconvincing block of the discriminative effects of the mixture, despite the fact that each antagonist alone blocked an important element of the stimulus complex. Such marginal degrees of antagonism are commonly interpreted as negative outcomes in pretreatment

studies. The present findings suggest that when a drug produces a compound interoceptive stimulus, weak degrees of antagonism may reflect real effects that should not always be ignored. Appel et al. (1978) reported that the discriminative effects of pentazocine were fully blocked by a mixture of a dopamine antagonist and a narcotic antagonist, and that either drug alone produced only partial blockade.

Much more extensive work will be needed to develop fully the insights that may be attainable through the use of antagonists in such studies. The data available are very restricted; even with the benzodiazepine and nicotine antagonists employed in the study detailed above, there are no dose-response data available for mixture experiments. Such work will need to include cross-antagonism tests to determine whether antagonists are fully selective in their effects on the appropriate agonists. The effects of antagonists in cases where the training drugs interact may be particularly complex, and they may also depend on the methods used to establish the discrimination in the first place.

Role of Training Dose Ratio

Results are presented for three studies with generally similar designs but involving different mixtures of drugs. The first study examined discrimination of mixtures of nicotine *and* midazolam and the sequence of training doses studied is shown in table 1; the doses of both drugs were varied in the course of the study. In this experiment, midazolam was injected 5 min before sessions. Discrimination between saline and the mixtures was well maintained throughout and was not greatly dependent on the dose ratio used to maintain the discrimination (Garcha and Stolerman 1989).

TABLE 1. *Sequence of training doses used to maintain discrimination of a nicotine-midazolam mixture in rats (n = 8).*[a]

Test Set	Nicotine (mg/kg)	Midazolam (mg/kg)	Ratio
1	0.20	0.10	2.0
2	0.32	0.10	3.2
3	0.32	0.06	5.3
4	0.32	0.04	8.0
5	0.32	0.10	3.2

[a]A set of extinction tests was carried out after a minimum of 12 sessions of discrimination training with each mixture and saline. All drugs were given by subcutaneous injection.

At first, the discrimination was maintained by a mixture in which the ratio of the doses (nicotine:midazolam) was 2:1. Extinction tests with the drugs given separately showed that the response to the midazolam was much greater than that to the nicotine (figure 7). Thus, at these doses, midazolam seemed to produce a more salient stimulus than nicotine. When the dose ratio was increased to 3.2:1, there was a strong discriminative response to each drug. Further increases in the dose ratio enhanced the response to nicotine and decreased the response to midazolam. With a dose ratio of 8:1, the discrimination of the mixture could be attributed mainly to the nicotine and the role of the midazolam was minimal. Thus, a fourfold change in dose ratio, from 2:1 to 8:1, was sufficient to reverse the relative contributions of the two drugs to stimulus control by the mixture. The final set of extinction tests was carried out after reinstating a training dose ratio of 3.2:1. As in the initial tests with this dose ratio, the responses to the nicotine and midazolam were approximately equal in magnitude, suggesting that the nonrandom sequence of training doses was not a major confounding factor (Garcha and Stolerman 1989).

The next experiment examined discriminations maintained by mixtures of amphetamine *and* pentobarbitone. In this study, the dose of one drug, pentobarbitone, was varied while the dose of the other drug, amphetamine, was held constant at 0.4 mg/kg (SC). At first, discrimination was maintained by a mixture in which the ratio of the doses (pentobarbitone:amphetamine) was 12.5:1; extinction tests with the drugs given separately showed that the response to the amphetamine was much greater than that to the pentobarbitone (figure 8). Thus, at these doses, amphetamine seemed to produce a more salient stimulus than pentobarbitone. When the dose ratio was increased to 25:1, there was a strong discriminative response to each drug. Further increases in the dose ratio enhanced the response to pentobarbitone and decreased the response to amphetamine. With a dose ratio of 50:1, the discrimination of the mixture could be attributed mainly to the pentobarbitone and the role of the amphetamine was minimal. Thus, a fourfold change in dose ratio, from 12.5:1 to 50:1, was sufficient to reverse the relative contributions of the two drugs to stimulus control by the mixture.

The final experiment of this type examined discriminations maintained by mixtures of morphine *and* nicotine. In this study, the dose of one drug, nicotine, was varied while the dose of the other drug, morphine, was held constant at 3 mg/kg (SC). At first, the discrimination was maintained by a mixture in which the dose of nicotine was 0.1 mg/kg (SC); extinction tests with the drugs given separately showed that the response to the morphine was much greater than

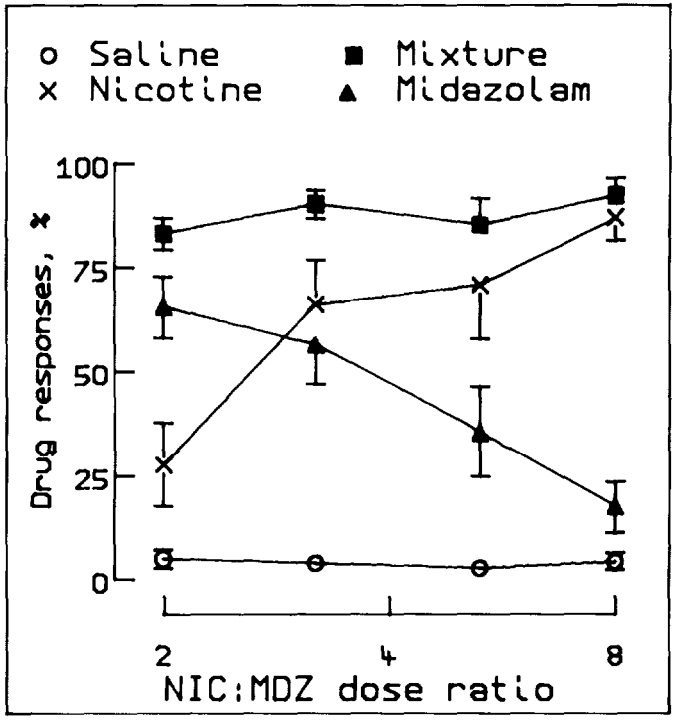

FIGURE 7. *Discriminative stimulus effects of nicotine and midazolam in rats trained to discriminate mixtures of these drugs from saline (n = 8). Responses to nicotine, midazolam, and mixtures are shown for four sets of tests carried out while stimulus control was maintained by mixtures containing different doses of drugs (AND discrimination). Responding after saline is also shown. Abscissa, ratio of dose of nicotine to that of midazolam, with data shown for ratios of 2:1, 3.2:1, 5.3:1, and 8:1 (doses of both drugs were varied, as detailed in table I). Ordinate, responses on drug-appropriate bar expressed as percentage of total responses on both bars. All data were obtained in 5-min extinction tests. Results are means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted for clarity. (Data from Garcha and Stolerman, 1989.)*

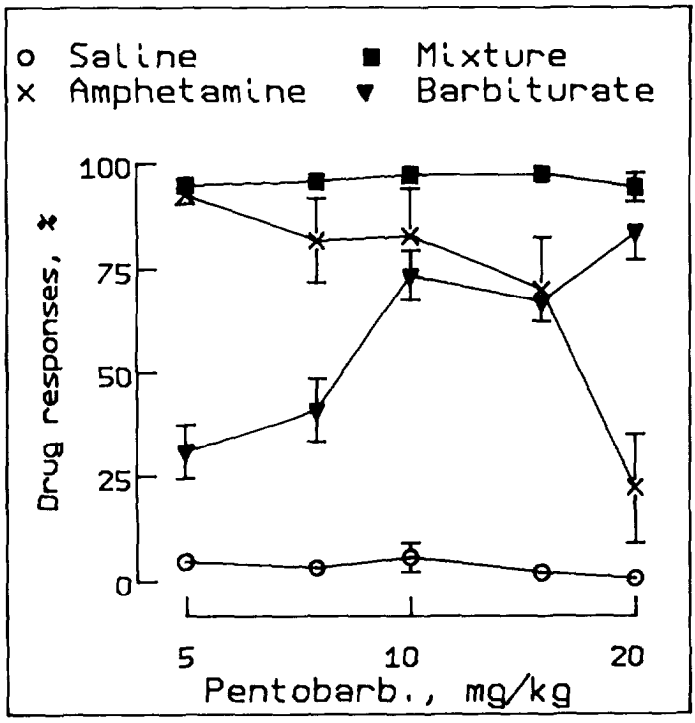

FIGURE 8. *Discriminative stimulus effects of amphetamine and pentobatbitone in rats trained to discriminate mixtures of these drugs from saline (n = 8). Responses to these drugs are shown for five sets of tests carried out while stimulus control was maintained by mixtures containing different doses of pentobarbitone (AND discrimination). Dose of amphetamine was 0.4 mg/kg throughout. Responding after saline is also shown. Abscissa: dose of pentobarbitone. Ordinate: responses on drug-appropriate bar expressed as percentage of total responses on both bars. All data were obtained in 5-min extinction tests. Results are means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted for clarity.*

that to the nicotine (figure 9). Thus, at these doses, morphine seemed to produce a more salient stimulus than nicotine. When the dose of nicotine was increased to 0.4 mg/kg, there was a strong discriminative response to each drug. A further increase in the dose of nicotine enhanced the response to

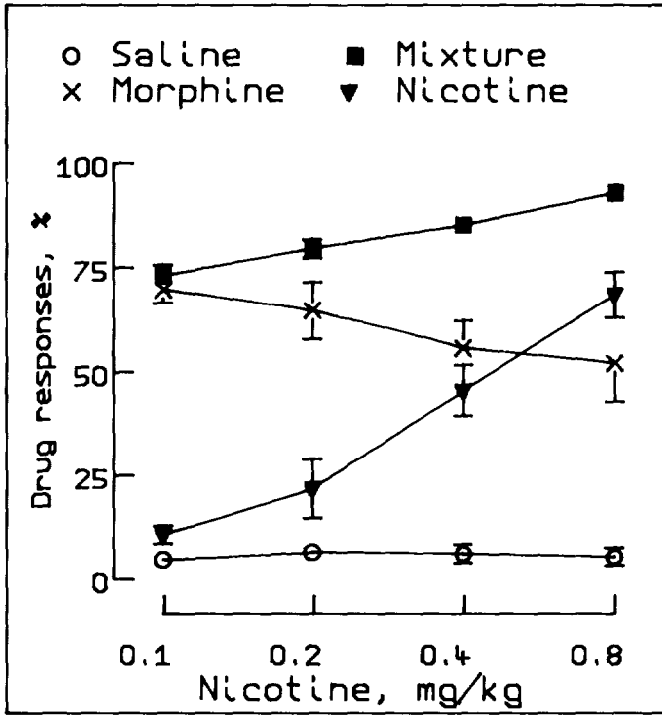

FIGURE 9. *Discriminative stimulus effects of morphine and nicotine in rats trained to discriminate mixtures of these drugs from saline (n = 8). Responses to these drugs are shown for four sets of tests carried out while stimulus control was maintained by mixtures containing different doses of nicotine (AND discrimination). Dose of morphine was 3 mg/kg throughout. Responding after saline is also shown. Abscissa: dose of pentobarbitone. Ordinate: responses on drug-appropriate bar expressed as percentage of total responses on both bats. All data were obtained in 5-min extinction tests. Results are means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted for clarity.*

nicotine and decreased that to morphine, but there was still a marked response to the morphine alone. Thus, in these experiments, even an eightfold increase in dose ratio was not sufficient to completely reverse the relative contributions of the two drugs to stimulus control by the mixture.

These experiments demonstrate the critical importance of the ratio between the training doses of drugs in mixtures as a determinant of the characteristics of the cue obtained. Relatively small fourfold shifts in dose ratio were sufficient to entirely reverse the dominant component in cues produced with mixtures of nicotine *and* midazolam and amphetamine *and* pentobarbitone. With mixtures of nicotine and morphine, even an eightfold change in the dose ratio had a less pronounced effect, although similar trends were apparent. The reason for this difference is unclear. The studies reported should be regarded as only a preliminary exploration of the role of training doses in discrimination of mixtures. At least three major manipulations remain unexamined. These include (1) dose-response determinations by means of extinction tests at different training dose ratios and (2) the consequences of training with relatively large or small amounts of mixtures with the dose ratio held constant. It can also be expected that (3) profiles of generalization to other compounds will be critically linked to training dose ratios and amounts.

Specificity of Cues Based on Mixtures

Koek and Slangen (1982) and Overton (1982) have shown that under certain conditions the specificity of discriminations based on single drugs may be influenced by training the alternative response not simply with vehicle but with multiple drugs (in different sessions). Although the functional parallel is inexact, this raises the possibility that training a discrimination with two or more drugs in a mixture may influence specificity. Studies to test this issue have been carried out in rats trained to discriminate mixtures of nicotine *and* midazolam and of amphetamine *and* pentobarbitone from saline.

Figure 10 shows the results of dose-response (generalization) tests with amphetamine, morphine, and quipazine in rats trained to discriminate nicotine (0.32 mg/kg SC) and midazolam (0.1 mg/kg SC) from saline. In this experiment, midazolam was injected 5 min before sessions. The test drugs did not increase the percentage of drug-appropriate responding above the level associated with injections of saline. The responses to saline and to the mixture of nicotine and midazolam confirmed the persistence of stimulus control by the training mixture throughout the period of tests for specificity. Amphetamine, morphine, and quipazine all reduced the total numbers of responses in a dose-related manner (Garcha and Stolennan 1989).

Similar experiments have been carried out in rats trained to discriminate amphetamine (0.5 mg/kg SC) *and* pentobarbitone (12 mg/kg SC) from saline.

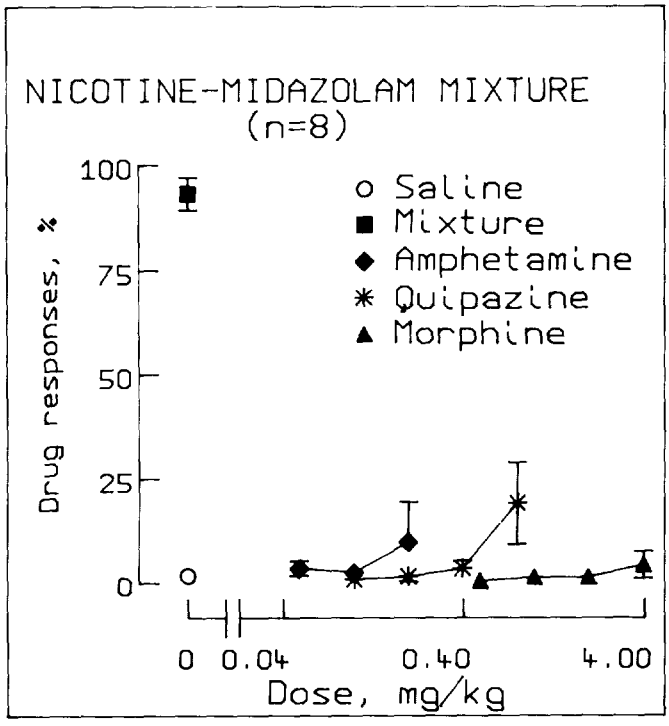

FIGURE 10. *Tests of responses to novel drugs in rats trained to discriminate a mixture of nicotine (0.32 mg/kg) and midazolam (0.1 mg/kg) from saline (n = 8, AND discrimination). Results are shown for different doses of amphetamine, quipazine, and morphine. Abscissa: dose of drug. Ordinates: responses on drug-appropriate bar expressed as a percentage of total responses. Control data above 0 on the abscissa show responses to saline and the mixture used for training as means for three sets of such tests carried out during work with the novel drugs. (Data from Garcha and Stolerman, 1989.)*

The results were compared with those obtained in two additional groups of rats trained to discriminate the same doses of amphetamine or pentobarbitone from saline in a conventional, single-drug discrimination procedure. Tests for generalization to morphine, quipazine, and phencyclidine were carried out. Generalization to a test drug was never greater in rats trained on the mixture than in rats trained on the component drugs. All drugs were tested at doses that markedly reduced overall rates of responding.

The results of the tests to date suggest that there is no general loss of specificity that is necessarily associated with the use of mixtures for training discriminations. The possibility of certain unusual profiles of generalization with particular mixtures of drugs that interact cannot be precluded. It would also be reasonable to expect generalization to many drugs that normally share stimulus properties with either training drug, possibly resulting in generalization to a wider range of compounds than would typically be the case for single-drug discriminations.

Another Paradigm for Mixtures: The AND-OR Discrimination

The findings outlined above may be related to the use of one particular paradigm for training the preceding discriminations based on drug mixtures. This paradigm is laid out in figure 1 (upper right) according to the scheme of Järbe and Swedberg (1982). Several other paradigms are also possible and the lower left section of figure 1 illustrates one on which work has begun. In this procedure, subjects are trained to discriminate the simultaneous presence of two drugs (AND) from the presence of either drug alone (OR), called the AND-OR discrimination. According to this nomenclature, the preceding work with mixtures may be called the AND discrimination. It may be noted that OR discriminations have been established previously and in these cases OR referred to one of the following situations: either of two drugs was associated with one response and vehicle with another response (Colpaert and Janssen 1982); one of several drugs was associated with one response and just one drug with another response (Overton 1982); or three doses of one drug were associated with one response and vehicle with the other response (Stolerman et al. 1984). Therefore, it seemed that there would be no fundamental difficulty in establishing the OR element of the discrimination, but there appeared to be no previous report of an AND-OR discrimination.

The first question about the AND-OR discrimination was simply whether it could be established. This question has been answered positively because rats have been trained to discriminate amphetamine (0.4 mg/kg SC) and pentobarbitone (10 mg/kg SC) from the same doses of amphetamine or pentobarbitone. This AND-OR discrimination was acquired more slowly than AND discriminations studied previously with the same drugs, but mean accuracy for all 10 rats reached 83 percent after 48 training sessions. Dose-response curves for amphetamine, pentobarbitone, and the mixture were then determined in the eight most accurate rats and figure 11 shows the main findings. Different amounts of the mixture (with the ratio between the doses of the component

drug held constant) produced increasing amounts of mixture-appropriate responding, up to a maximum of 90 percent. None of the tested doses of either amphetamine or pentobarbitone produced any appreciable amount of mixture-appropriate responding. Thus, the capacity of the rats to perform the AND-OR discrimination was confirmed and the distinction between mixture and component drugs was maintained even when the doses of component drugs were increased slightly above those used for training. Figure 11 shows another very intriguing characteristic of this AND-OR discrimination: Saline was generalized with the single drugs since it failed to produce any appreciable amount of mixture-appropriate responding.

From the preceding results, it would appear that rats trained in an AND-OR discrimination procedure can treat the effects of drug mixtures as distinctly different from those of any dose of the component drugs. It is debatable whether the mixture should be considered as producing a novel, distinct stimulus under such conditions, but operationally the rats behaved as if it did. Whether most drug mixtures can function in such a way remains to be determined; amphetamine-barbiturate mixtures can produce unusual patterns of behavioral effects under other conditions (Branch 1974; Rushton and Steinberg 1968) and this form of drug interaction may be contributing to the acquisition and performance of the AND-OR discrimination.

After completion of the dose-response determinations described above, the training procedure for all 10 rats was changed to the AND procedure used in all the earlier studies. The doses of amphetamine and pentobarbitone associated with one response were held constant as before, but the second response was associated with saline. After 22 training sessions under these conditions, the dose-response determinations were repeated. Discrimination between mixture and saline remained strong and dose related, as before (96 percent accuracy). Amphetamine alone failed to increase mixture-appropriate responding appreciably (maximum of 24 percent). Pentobarbitone slightly increased mixture-appropriate responding, but to no more than 46 percent. Thus, a previous history of training under AND-OR conditions greatly influenced the characteristics of a discrimination based on a drug mixture, as compared with its characteristics in rats trained and maintained on an AND discrimination throughout their experimental history (as described earlier). Barrett and Olmstead (1989) have described how testing history influences the characteristics of a discrimination based on spiroxatrine, a drug that acts as a dopamine antagonist and a serotonin agonist. Studies are now needed to

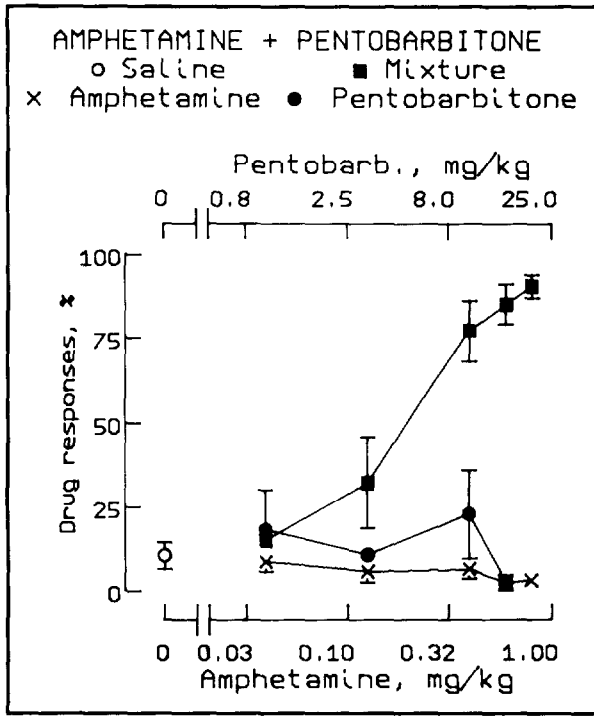

FIGURE 11. *Dose-response curves from rats trained to discriminate a mixture of amphetamine (0.4 mg/kg) and pentobarbitone (10 mg/kg) from either drug separately (n = 8, AND-OR discrimination). Results are shown for discriminative effects of mixtures with the dose ratio for component drugs held constant (■), amphetamine alone (X), and pentobarbitone alone (●). Responding after saline is also shown (0). After large doses of drugs some animals did not respond sufficiently for discriminative effects to be assessed and data are not shown where n < 4. All data shown as means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted for clarity. These results should be compared with those for similar experiments in rats trained in an AND discrimination with the same drugs.*

investigate the role of behavioral history in the discrimination of drug mixtures in a more systematic manner.

DISCUSSION

Orderly Form of the Data

The most surprising feature of the results is their orderly nature despite the complexity of the experiments and the use of several mixtures of drugs thought to have complex, interactive effects. Thus, readily interpretable findings have been obtained in tests of generalization to component stimuli, in dose-response studies, in pilot studies with antagonists, in tests of generalization to novel drugs, and when training dose ratios have been altered. This orderliness bodes well for the further use of drugs when analyzing the processing of compound interoceptive stimuli.

The AND Discrimination

In all cases of the AND discrimination studied to date, involving nine drugs in five different mixtures, rats trained with the mixtures have responded strongly to the component drugs. Rats therefore seem to identify and process the mixture stimulus on the basis of its elements rather than as a new and homogeneous entity. This result was obtained first with drugs that are not abused as a mixture, which on theoretical and empirical grounds are thought to produce different, nonoverlapping stimuli, and which do not interact pharmacologically (i.e., nicotine and midazolam; Stolerman et al. 1987). These findings have now been extended to include drugs that are abused as mixtures and are thought to interact in complex ways that await full definition (amphetamine and pentobarbitone, pentazocine and tripelennamine, caffeine and phenylpropanolamine). All these consistent results have been obtained with the AND discrimination (drug A *and* B versus vehicle); this functional relationship may be crucially important.

The AND-OR Discrimination

Results for amphetamine and pentobarbitone in rats trained in an AND-OR discrimination suggest a radically different picture. In this case, total discrimination between a mixture and its elements was obtained, even in dose-response studies. There was no generalization of responding between mixture and component drugs regardless of the dose of the latter. Such a result is, perhaps, not surprising in view of the training conditions imposed. Nevertheless, this complex discrimination was learned with little difficulty, and it supports the view that under particular conditions of use mixtures may have a

unique profile of behavioral effects. It will be important to determine the generality of this finding with regard to other classes of drugs, to examine yet other paradigms involving mixtures, and to test whether training that yields different discriminative effects also influences other effects of the drugs (e.g., on response rates) and the receptors on which they act.

History

Different behavioral and pharmacological histories can markedly influence reactions to drugs (Barrett et al. 1989). In a more limited context, this principle also applies to discriminative stimulus effects (Barrett and Olmstead 1989). The present experiments demonstrate the role of behavioral pharmacological history in a novel, orderly, and predictable manner. Rats transferred from AND-OR discrimination to AND discrimination responded to amphetamine and pentobarbitone in a manner radically different from rats maintained on an AND discrimination throughout their experimental history. The characteristics of the AND-OR discrimination persisted to a great extent after the programmed contingencies were changed. The limits of this phenomenon need to be fully explored. Within the context of drug abuse, it could imply that the manner in which the effects of mixtures are perceived will depend on the precise circumstances surrounding their previous use. It is noteworthy that possibly the first demonstration of a history effect in behavioral pharmacology also used an amphetamine-barbiturate mixture (Steinberg et al. 1961).

Overshadowing and Blocking

Interactions between drugs are normally interpreted in pharmacological terms, by means of pharmacodynamic and pharmacokinetic processes. There are complementary mechanisms based on behavioral constructs such as stimulus masking, overshadowing, and blocking (Mackintosh 1974). These constitute a rich new field with enormous potential for adding to our knowledge of interactions between both clinically used and abused mixtures of drugs. Stimulus masking has not appeared to be a pervasive phenomenon in drug discrimination experiments, but Gauvin and Young (1989) have demonstrated its occurrence with amphetamine and morphine in certain conditions. Järbe et al (1983*a*, 1983*b*, 1989) have clearly demonstrated mutual overshadowing and blocking of drug-produced discriminative stimuli by conditioning with more salient exteroceptive stimuli. No studies, however, seem to have explicitly looked for overshadowing and blocking between pairs of drug-produced stimuli

(i.e., with drug mixtures). The present experiments contain indications that such phenomena can occur and are worthy of systematic study.

Normally, rats can reliably discriminate nicotine in doses of 0.1-0.2 mg/kg from saline (Stolerman et al. 1984). However, very little stimulus control accrued to these doses of nicotine when conditioning occurred in the presence of midazolam (figure 7) or morphine (figure 9). Similarly, figure 8 shows that little stimlus control accrued to amphetamine (0.4 mg/kg) in the presence of pentobarbitone (20 mg/kg), and figure 9 shows that nicotine (0.8 mg/kg) weakened stimulus control by morphine (3 mg/kg). It is suggested that overshadowing may be the common mechanism underlying these diverse interactions. Overshadowing (Mackintosh 1974) is shown as weakening of conditioning to a normally adequate stimulus by conditioning with a compound stimulus in which there is another, more salient element. It is thought unlikely that pharmacological interactions could account for the observations because the drugs used in the mixtures are not agonist-antagonist pairs and are thought to act through diierent receptor mechanisms. Extensive tests for pharmacological interactions between nicotine and midazolam have been carried out in drug discrimination experiments under conditions similar to the present work, and they have yielded negative results (Stolerman et al. 1987). There are, however, some indications that amphetamine may weaken a pentobarbitone stimulus and that pentobarbitone may weaken an amphetamine stimulus (Kline and Young 1986; Mariathasan et al. 1990). These are rather fragile interactions with limited generality and they may reflect either a pharmacological mechanism or stimulus masking. Fully controlled overshadowing and blocking experiments like those carried out with exteroceptive discriminative stimuli are needed to clarify the issues raised by the present findings.

Overshadowing may also be a key factor in the acquisition of discriminations produced by single drugs with multiple effects; it may contribute to the general tendency for such discriminations to be based on the major effects of the drugs, rather than on an extremely complex mixture of main effects and side effects.

Clinically Used Mixtures of Drugs

The complexities and uncertainties of psychiatric diagnosis, coupled with the limited clinical efficacy of many psychoactive drugs, lead many clinicians to prescribe mixtures of drugs. Often, little psychopharmacological study has been done on such mixtures because there were no compelling reasons to expect

any exceptional interactions between their components. The coexistence of depressive symptoms with those of schizophrenia and anxiety is common, leading to the combined use of neuroleptics, antidepressants, and anxiolytics in different permutations. Studies of the discriminative stimulus properties of mixtures of such drugs may contribute to understanding the effects of such widely used but poorly understood mixtures.

CONCLUSIONS

There are numerous areas of application for discriminations based on mixtures of drugs; the phenomenon is not a laboratory curiosity without practical relevance. It provides an approach to identifying general principles that underlie the perception and processing of compound interoceptive stimuli. It may help us understand why certain mixtures of drugs are particularly prone to abuse as mixtures, which is one aspect of the pervasive phenomenon of polydrug abuse that has often been neglected in laboratory studies. Drug discrimination based on mixtures of drugs may shed new light on certain clinically used mixtures of drugs, and it will provide the methodology needed to test the roles of overshadowing and blocking in ways that may extend and complement classical pharmacological accounts of drug interactions. It also provides a novel approach to testing how behavioral and pharmacological histories of subjects may combine to produce marked individual variations in response to mixtures of drugs. Finally, much of what has been said about mixtures of drugs may also apply to single drugs with multiple effects.

ACKNOWLEDGMENT

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Application of Drug Discrimination With Drugs of Abuse To Develop New Therapeutic Agents

Theo Frans Meert

INTRODUCTION

The drug discrimination test procedure is a behavioral technique that can preclinically characterize the subjective effects of known and new substances. The test is based on a classical conditioning paradigm in which a drug is used as a conditioning stimulus. The internal effects (or cueing properties) evoked by a drug are used as discriminative stimuli for different ways of responding. Typically, in order to obtain food pellets, food-deprived rats are required to press one lever after treatment with the training drug and another lever after treatment with saline (or the vehicle).

The drug discrimination procedure has several advantages compared to classical behavioral tests, in that it is completely standardized with fixed parameters and well-defined conditions. The procedure is not limited to interactions at one particular neurotransmitter system. Depending on the training drug employed, responding may represent interactions at various systems including serotonin, dopamine, acetylcholine, noradrenaline, and opiate. Also, compounds acting at different receptor subtypes of one neurotransmitter system can be studied and differentiated. Furthermore, complex stimuli without any obvious behavioral reaction and often not limited to a single neurotransmitter system also fall within the scope of this procedure.

Often, drugs of abuse are used as training drugs. For several of these compounds, a correlation has been demonstrated between the similarity of the discriminative stimulus properties of these drugs in rats and their specific subjective effects in humans (Colpaert and Slangen 1982). For instance, it was demonstrated that stimulus generalization with lysergic acid diethylamide (LSD)

in rats represents a necessary, albeit insufficient, condition to predict hallucinogenic activity in man (Appel et al. 1982; Glennon et al. 1984). Drug discrimination studies with drugs of abuse in animals, and also in humans, may have some predictive value for the abuse potential of new chemical substances. Typically, generalization experiments are performed to test whether a new compound is recognized by rats trained on standard drugs of abuse. The same drug discrimination training also allows the characterization of new therapeutic agents. In antagonism studies, compounds are tested for their ability to attenuate the stimulus effects of the drugs of abuse. This process can identify antidotes for the drugs of abuse and also clinically relevant new compounds.

In order to demonstrate the usefulness of the drug discrimination test procedure, examples using new neuroleptic and antiepileptic agents will be presented. Ritanserin, a selective serotin-2 (5-HT_2) antagonist (Awouters et al. 1988) with abuse-reducing properties in rats (Meert et al. 1990*a*, 1990*b*) will also be considered.

In all studies reported, rats used a two-lever, food-reinforced drug discrimination test procedure. The equipment, training, and test procedures have been described in detail in previous papers (Meert et al. 1989, 1990*c*).

USE OF DRUG DISCRIMINATION TO DETECT NEW NEUROLEPTIC AGENTS

All clinically active neuroleptics, such as haloperidol, have the ability to antagonize central dopamine (DA) receptor activation (Niemegeers and Janssen 1979). Clinically, these drugs are effective against the positive but not the negative symptoms of schizophrenia. They all have the risk of inducing extrapyramidal side effects (EPS) (Berger 1978). Selective 5-HT_2 antagonists such as ritanserin, given in combination with neuroleptics, can overcome the negative symptoms of schizophrenia and reduce EPS (Gelders 1989; Reyntjens et al. 1986). Blockade of $5HT_2$ receptors seems to increase therapeutic activity and reduce the side effects of D_2 receptor blockade. Risperidone is a potent serotonin 5-HT_2 and D_2 antagonist. Its serotonin antagonism is more pronounced than its antidopaminergic activity (Janssen et al. 1988). Clinically, risperidone has been shown to be active against both the positive and negative symptoms of schizophrenia, with a very low incidence of EPS (Casteleao et al. 1989; Mew et al. 1989; Mesotten et al. 1989). Recently, R 79 598, a benzisoxazolyl derivative (Janssen et al. 1990), was characterized

as a potent neuroleptic with equipotent 5-HT_2 and D_2 antagonist properties. In rats, R 79 598 was at least as potent as haloperidol and several times more potent than risperidone in inhibiting dopaminergic overactivity. In dogs, R 79 598 was even more potent than haloperidol. In order to further characterize the pharmacological profile of R 79 598 and to preclinically confirm its possible neuroleptic activity, the drug was compared with risperidone and haloperidol in the drug discrimination test procedure. The selected training drugs included LSD, 1-(2,5-dimethoxy-4-methyphenyl)-2-aminopropane (DOM), cocaine, and *d*-amphetamine.

The serotonin agonist DOM was selected because it is a 5-HT_2 agonist that can produce hallucinations in humans (Glennon et al. 1983, 1984). LSD produces cardiovascular changes and feelings of tension, anxiety, and depressed mood (Cohen 1967; Freedman 1968, 1969) as well as hallucinations, especially at high doses (Isbell 1959). Potent LSD antagonists such as risperidone (Meert et al. 1989) have been clinically shown to possess antipsychotic activity. Both *d*-amphetamine and cocaine produce stimulatory effects with psychotic-like symptoms in man (Beamish and Kiloh 1960; McLellan et al. 1979). There are, however, basic differences in the stimulus properties of cocaine and &amphetamine. Whereas D_2 antagonists such as haloperidol can antagonize the discriminative stimulus effects of *d*-amphetamine, these compounds only partially antagonize the cocaine cue (Meert et al. 1990*c*). The selected training conditions in this study included a discrimination of either 0.63 mg/kg DOM (intraperitoneal [IP]; time [T]-15 min), 0.16 mg/kg LSD (IP; T-15 min), 10.0 mg/kg cocaine (IP; T-15 min), or 1.25 mg/kg *d*-amphetamine (subcutaneous [SC]; T-30 min) versus saline.

Within each training group, complete dose-response functions for stimulus generalization and antagonism were established for haloperidol, risperidone, and R 79 598. The stimulus generalization experiments were performed after 1 hr whereas for the antagonism studies, time intervals between 1 and 8 hr were used. Also, the ED_{50} values for stimulus generalization of the training drugs with the training condition were established.

In terms of stimulus generalization (table 1), all training drugs produced a complete stimulus generalization 1 hr after subcutaneous treatment. The ED_{50} value of DOM in 0.63 mg/kg DOM-trained rats was 0.17 mg/kg. For 0.16 mg/kg LSD, 10.0 mg/kg cocaine, and 1.25 mg/kg *d*-amphetamine, the corresponding ED_{50} values were 0.26, 1.55 and 0.51 mg/kg, respectively. Except for LSD, the ED_{50} values for stimulus generalization were 2.45 (*d*-amphetamine) to 6.45

TABLE 1. *ED_{50} values (plus 95 percent confidence limits) of the training drugs, haloperidol (HALO), risperidone (RISP), and R 79 598, for stimulus generalization with the training conditions*[a]

Training Condition[b]	Training Drug[c] (dose m mg/kg)	HALO[d]	RISP[d]	R 79 598[d]
DOM (063;IP;T-15)	0.17 (0.11-0.25)	0.63	0.63	0.16
LSD (O.16;IP;T-15)	0.26 (0.16-0.41)	0.63	0.63	0.16
Cocaine (10.0;IP;T-15)	1.55 (1.03-2.31)	0.63	0.63	0.16
d-Amphetamine (1.25;SC;T-30)	0.51 (0.34-0.76)	0.63	0.63	0.16

[a]AII generalization experiments, including those of the training bugs with the mining conditions, were performed 1 hr after subcutaneous treatment
[b]DOM = 1-(2,5dimethoxy-4-methylphenyl)-2-aminopropane; LSD= lysergic acid diethylamide; IP = intraperitoneal; SC = subcutaneous.
[c]Doses in mg/kg.
[d]All values are "greater than."

(cocaine) times lower than the training dose. For 0.16 mg/kg LSD, the ED_{50} for stimulus generalization was 1.63 times higher. Two elements can account for the higher ED_{50} of LSD. First, LSD may have a relatively short duration of action. As a consequence, the dose of LSD must be increased to obtain a generalization when the pretreatment time is increased from 15 to 60 min. Second, the alteration in route of administration (IP for the training and SC for the stimulus generalization conditions) may cause a difference in ED_{50} values.

Neither R 79 598, risperidone, nor haloperidol resulted in stimulus generalization to any of the training drugs (table 1). Thus, up to doses that severely reduced response rate, no selection of the drug lever occurred.

The antagonism studies are summarized in table 2. In terms of DOM antagonism, the ED_{50} of R 79 598 to antagonize the stimulus properties of 0.63 mg/kg DOM after 1 hr was 0.0027 mg/kg. For risperidone, the corresponding ED_{50} was 0.024 mg/kg. Haloperidol, at doses up to 0.63 mg/kg, which clearly reduced response rate, did not antagonize DOM. A similar result was obtained

TABLE 2. *Antagonism studies of R 79 598, risperidone (REP), and haloperidol (HALO) on DOM, LSD, cocaine, and d*-amphetamine[a]

Training Drug Treatment[b]	Time (hr)	R 79 598[c]	RISP[C]	HALO[C]
DOM (0.63 mg/kg;IP/T-15)	1	0.0027 (0.0018-0.0040)	0.024 (0.016-0.037)	> 0.63
LSD (0.16 mg/kg;IP;T-15)	1	0.097 (0.072-0.13)	0.028 (0.015-0.051)	> 0.63
	2	0.021 (0.01-0.043)	0.028 (0.015-0.051)	> 0.63
	4	20% at 0.63	0.064 (0.037-0.12)	
	8		0.45 (0.23-0.85)	
Cocaine (10.0 mg/kg;IP;T-15)	1	> 0.31	> 2.50	40% at 0.63
	2	60% at 0.63	40% at 2.50	> 0.63
d-Amphetamine (1.25mg/kg;SC;T-30)	1	40% at 0.31	0.34 (0.21-0.54)	0.13 (0.086-0.19)
	2	0.043 (0.0264069)	(0.24-.81)	(9.091-0.14)

[a]ED_{50} values (plus 95% confidence limits) are presented over time for antagonism of the training condition or the maximal percentage of blockade measured.
[b]DOM = 1-(2.5-dimethoxy-4-methylphenyl)-2-aminopropane; LSD = lysergic acid diethylamide; IP = intraperitonsal; T = time in min; SC = subcutaneous.
[c]Doses in mg/kg, administered subcutaneously.

with haloperidol against LSD after both 1 and 2 hr. On the contrary, risperidone completely antagonized LSD with a lowest ED_{50} of 0.028 mg/kg after both 1 and 2 hr. Even after 8 hr, there was still a complete antagonism with an ED_{50} of 0.45 mg/kg. Risperidone is therefore characterized as a potent and long-acting LSD antagonist (Meert et al. 1989). The lowest ED_{50} of R 79 598 for LSD antagonism was 0.021 mg/kg after 2 hr. After 4 hr, however, only a partial antagonism of 20 percent was obtained at 0.63 mg/kg. Therefore, R 79 598 was as potent as risperidone in terms of the lowest ED_{50}, but the drug had a shorter duration of action. The data presented here on the antagonism of LSD confirm the idea that the discriminative stimulus properties of LSD are both serotonin-

(especially $5HT_2$) and catecholamine-(D2 and noradrenaline) mediated (Meert et al. 1989, 1990*c*).

None of the tested compounds completely antagonized the discriminative stimulus properties of 10.0 mg/kg cocaine. A partial antagonism of 40 percent was obtained with 0.63 mg/kg haloperidol (1 hr) and with 2.50 mg/kg risperidone (2 hr). At 0.63 mg/kg R 79 598, there was a partial antagonism of 60 percent (2 hr). These results confirm earlier studies indicating that the discriminative stimulus properties of 10.0 mg/kg cocaine cannot simply be antagonized by either D_2 or mixed D_2/5-HT_2 antagonists (Meert et al. 1989, 1990*c*).

In terms of an antagonism of the discriminative stimulus properties of 1.25 mg/kg *d*-amphetamine, a complete antagonism was obtained with all three test compounds. The ED_{50} values of haloperidol after 1 and 2 hr were 0.13 and 0.11 mg/kg, respectively. For risperidone the corresponding values were 0.34 and 0.44 mg/kg. R 79 598 only partially antagonized the cueing properties of 1.25 mg/kg *d*-amphetamine after 1 hr. At 0.31 mg/kg R 79 598, a dose with rate-reducing effects, only a 40 percent antagonism was obtained. After 2 hr, however, the response rate reductions were less pronounced, and a lowest ED_{50} of 0.043 mg/kg was measured. Thus, in terms of lowest ED_{50} for *d*-amphetamine antagonism, R 79 598 was 2.56 and 7.91 times more potent than haloperidol and risperidone, respectively. Globally, these results on the antagonism of d-amphetamine indicate that D_2 antagonists are able to antagonize the discriminative stimulus properties of 1.25 mg/kg *d*-amphetamine (Meert et al. 1989).

In terms of response rate, a second parameter measured in the drug discrimination studies, haloperidol by itself reduced response rate at doses $\geq$ 0.16 mg/kg. In the antagonism studies, doses $\geq$ 0.04 mg/kg were active. Similar results were obtained with R 79598. However, for R 79 598, the rate-reducing effects disappeared over time. After 2 hr, an antagonism without effects on response rate was measured. It is therefore possible that the rate reducing effects of R 79 598 did mask the antagonist properties, especially at short time intervals. For risperidone, reductions in response rate were observed in both the generalization and antagonism studies at doses $\geq$ 0.16 mg/kg. In addition, response rate reductions disappeared over time, and, at intervals $\geq$ 2 hr, an antagonism without any rate reduction could be obtained.

Globally, the results presented here indicate that R 79 598 is a potent DOM, LSD, and d-amphetamine antagonist. The data confirm earlier studies indicating that R 79 598 has 5-HT_2 and D_2 antagonist properties. The antagonist properties of R 79 598 were most pronounced after 2 hr when the effects of the drug on response rate were minimal. As compared to haloperidol and risperidone, lower ED_{50} values were obtained with R 79 598 for the antagonism of DOM and *d*-amphetamine. Because DOM and *d*-amphetamine are respectively, $5HT_2$- and D_2-mediated, R 79 598 was clearly more potent than both other drugs in terms of 5-HT_2 and D_2 antagonism. With regard to LSD, R 79 598 was equipotent to risperidone but showed a shorter duration of activity.

Effectiveness against both the positive and negative symptoms of schizophrenia with a low rate of extrapyramidal side effects can be predicted for this new compound for the following reasons: R 79 598 has D_2 and 5-HT_2 antagonist properties; the drug antagonizes the stimulus properties of various compounds with psychotomimetic symptoms in man; and R 79 598 to some extent resembles risperidone.

DRUG DISCRIMINATIVE ANALYSIS OF THE ANTIEPILEPTIC AGENT LORECLEZOLE

Loreclezole was introduced recently as an antiepileptic agent (Wauquier et al. 1990). It is a triazole derivative with a broad spectrum of activity in different animal species and is unrelated to prototype antiepileptic drugs. The compound is active against audiogenic seizures and chemically or electrically induced seizures. An antagonism of both tonic and clonic seizures was obtained. Loreclezole increased the threshold for both behavioral and electroencephalographic seizures. The drug was found to be nonteratogenic in rats (Wauquier et al. 1990).

In order to further characterize the pharmacological profile of foreclezole, the drug was compared with the antiepileptic agents clobazam and carbamazepine and the benzodiazepine chlordiazepoxide in rats trained to discriminate either 5.0 mg/kg chlordiazepoxide (IP, T-15 min) or 20.0 mg/kg metrazol (IP, T-15 min) from saline. All generalization and antagonism studies with the four compounds were performed 1 hr after oral treatment. For the generalization experiments of the training drug with the training condition, data on subcutaneous injections after 1 hr are presented.

The ED_{50} value of chlordiazepoxide (SC; 1 hr pretreatment) in 5.0 mg/kg trained rats was 3.08 mg/kg. For metrazol the corresponding ED_{50} value in the 20.0 mg/kg metrazol group was 9.36 mg/kg.

In terms of generalization to 5.0 mg/kg chlordiazepoxide, a dose-related substitution was observed with chlordiazepoxide, clobazam, and loreclezole (figure 1). The corresponding ED_{50} values for stimulus generalization 1 hr after oral treatment were 3.55, 24.73, and 18.76 mg/kg, respectively. No generalization was observed with carbamazepine at doses up to 40.0 mg/kg. A significant reduction in response rate was present only at 80.0 mg/kg loreclezole.

In terms of substitution for metrazol (figure 2), none of the tested compounds produced a stimulus generalization at doses $\geq$ 40.0 mg/kg after oral treatment. Reductions in response rate were present at 10.0 and 40.0 mg/kg chlordiazepoxide, 40.0 mg/kg carbamazepine and clobazam, and $\geq$ 40.0 mg/kg loreclezole in metrazol-trained rats. In chlordiazepoxide-trained animals, rate reductions were measured only at 80.0 mg/kg loreclezole. Thus, the training condition appears to codetermine the degree of response rate reduction observed with the various compounds.

Regarding the antagonism of the discriminative stimulus properties of 20.0 mg/kg metrazol (figure 3), a complete antagonism after oral treatment was obtained with chlordiazepoxide and loreclezole. The ED_{50} of metrazol antagonism was 2.69 mg/kg for chlordiazepoxide and 8.17 mg/kg for loreclezole. Clobazam revealed a partial antagonism. At 40.0 mg/kg, the highest dose tested, a partial antagonism (60 percent) was obtained. Carbamazepine at doses up to 40.0 mg/kg was inactive. No rate-reducing effects were observed for any of the four compounds at the doses tested.

The results for loreclezole in the drug discrimination test procedure indicated a complete generalization with 5.0 mg/kg chlordiazepoxide and an antagonism with 20.0 mg/kg metrazol. A comparable effect was obtained with the benzodiazepine chlordiazepoxide at lower doses. Clobazam had a somewhat similar profile. Carbamazepine, on the contrary, was completely different. These drug discrimination data, together with the results obtained in animal models of anxiety and in procedures measuring side effects of the benzodiazepines (Meert et al. 1990*d*), support the idea that loreclezole possesses a pharmacological profile different from catbamazepine and, to some extent, comparable to the benzodiazepines clobazam and chlordiazepoxide.

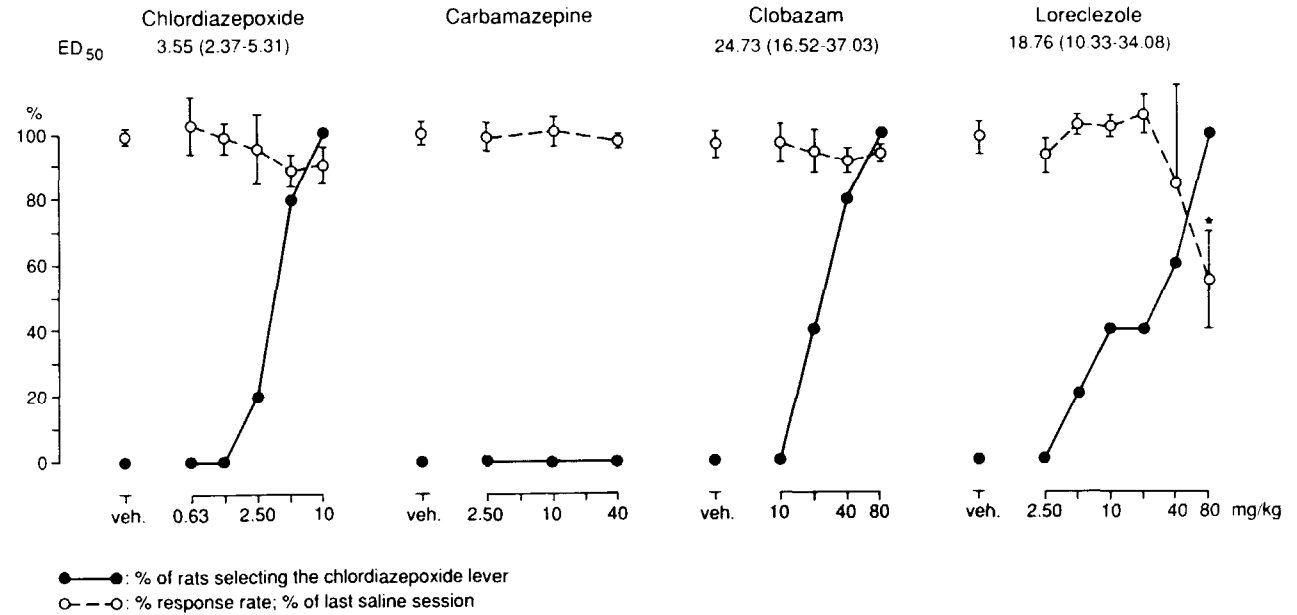

FIGURE 1. *Generalization experiments in 5.0 mg/kg chlordiazepoxide (IP, over 15 min) trained rats. Number of rats selecting the drug lever and response rate are expressed as a percentage of last saline session. Drugs were given orally 1 hr prior to testing. At each dose, results for five rats are given. Doses and ED_{50} values are given in mg/kg. Statistical differences were evaluated with the Wilcoxon test (two-tailed; * = $p < .05$).*

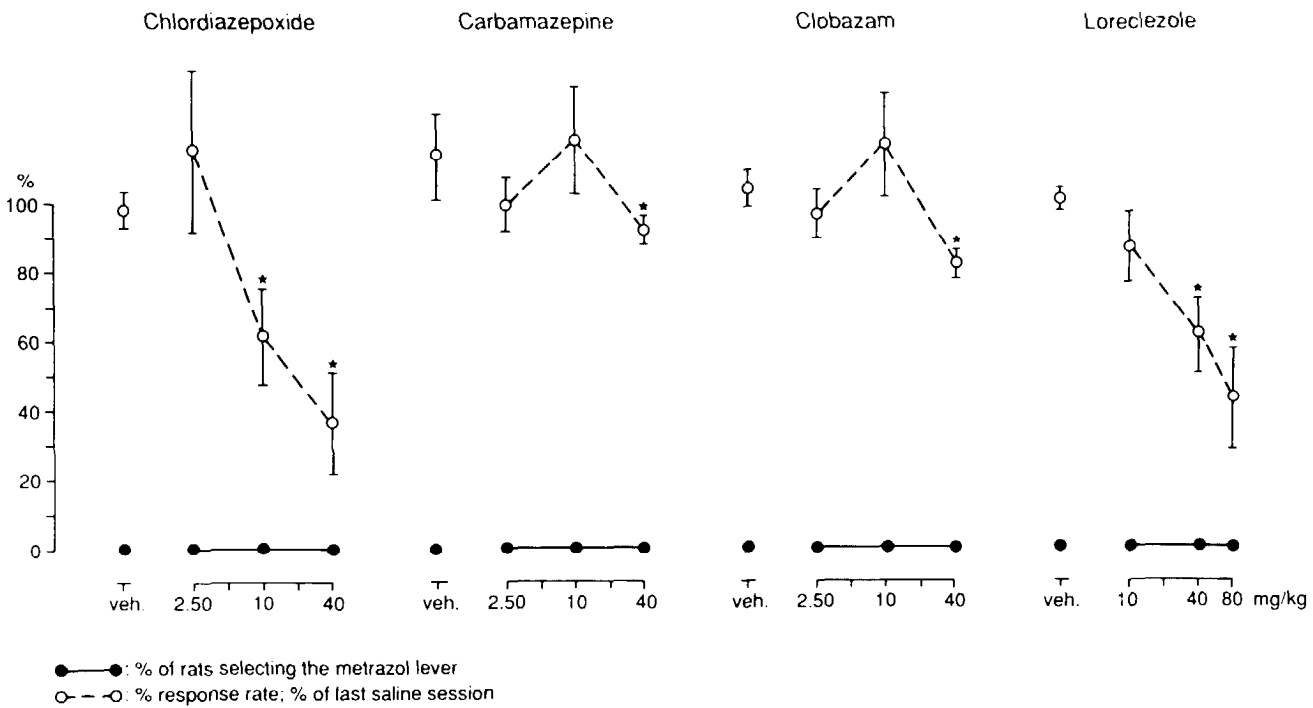

FIGURE 2. *Generalization experiments in 20.0 mg/kg metrazol (IP, over 15 min) trained rats. See also legend to figure 1.*

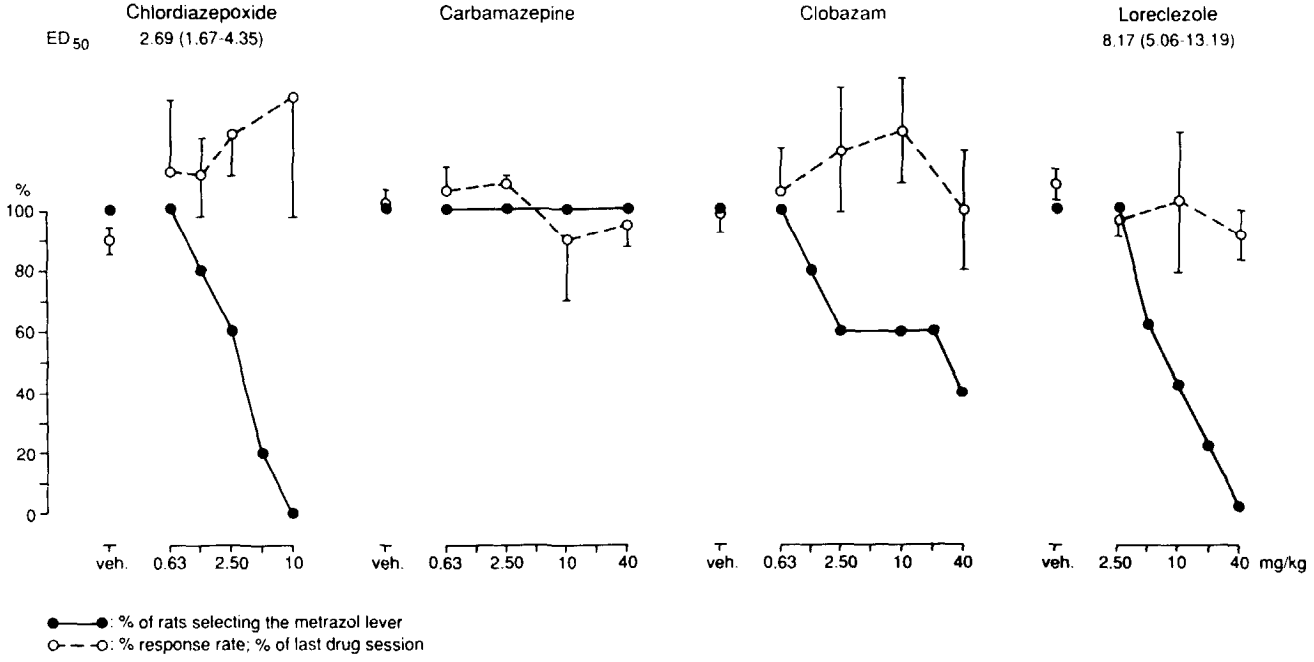

FIGURE 3. *Antagonism studies in 20.0 mg/kg metrazol (IP, over 15 min) trained rats. Number of rats selecting the drug lever and the response rate are expressed as a percentage of last drug session. Compounds were given orally at 1 hr before testing and at 45 min before intraperitoneal injection with metrazol. See also legend to figure 1.*

Globally, the studies with both the neuroleptics and the antiepileptfc drugs illustrate how drugs of abuse can be used to select and characterize therapeutic agents. Besides the selection of new compounds, the drug discrimination test procedure can also help to clarify the mechanism of action of therapeutic agents. Furthermore, in an early phase the procedure can reveal information about the abuse liability of a new substance. The following example illustrates this.

DRUG DISCRIMINATIVE ANALYSIS OF RITANSERIN: A PHARMACOLOGICAL APPROACH FOR ADDICTION

Ritanserin is a specific 5-HT_2 antagonist belonging to a series of piperidine derivatives (Awouters et al. 1988). In rats, low doses on the order of 0.1 mg/kg antagonize the behavioral effects induced by serotonergic stimulants such as tryptamine, mescaline, or 5-hydroxytryptophan (5-HTP). Similar doses increase deep slow wave sleep (Dugovic et al. 1989) and markedly disinhibit behavior induced by natural aversive stimuli (Meert and Janssen 1989). At somewhat higher doses, an activity is observed in models used to screen antidepressants (Meert and Janssen 1989). Normal behavior in rats is not affected up to high doses, which indicates that central 5-HT_2 antagonism by itself is neurobehaviorally silent (Awouters et al. 1988; Leysen et al. 1989). In humans, rftanserin also markedly increases slow wave sleep (ldzikowski et al. 1986) and improves mood and drive in dysthymic patients (Reyntjens et al. 1986). It has been suggested that restoration of physiological sleep promotes improvement of neurotic symptoms (Janssen 1987, 1988). Occasional observations in the past 5 years also indicated that ritanserin may be of value in subjects withdrawing from drugs of abuse, who may experience problems similar to dysthymic patients in coping with everyday activities. Therefore, experiments were performed to test the effectiveness of rftanserin against drug abuse. A typical example of these experiments is given in figure 4. The effects of rftanserin versus 3 percent alcohol, 0.1 mg/ml cocaine, and 0.02 mg/ml fentanyl are presented.

In these three experiments, rats were allowed access to a drug solution of either alcohol, cocaine, or fentanyl as the only available drinking fluid for 10 days. On Day 11, the rats were randomly assigned to treatment groups receiving subcutaneous injections of either vehicle or one of the various doses of rftanserin between 0.01 and 2.5 mg/kg. Both drug solution and water were made available in identical graduated bottles immediately following treatment. The injections were given on 5 consecutive days at 8 a.m. Individual

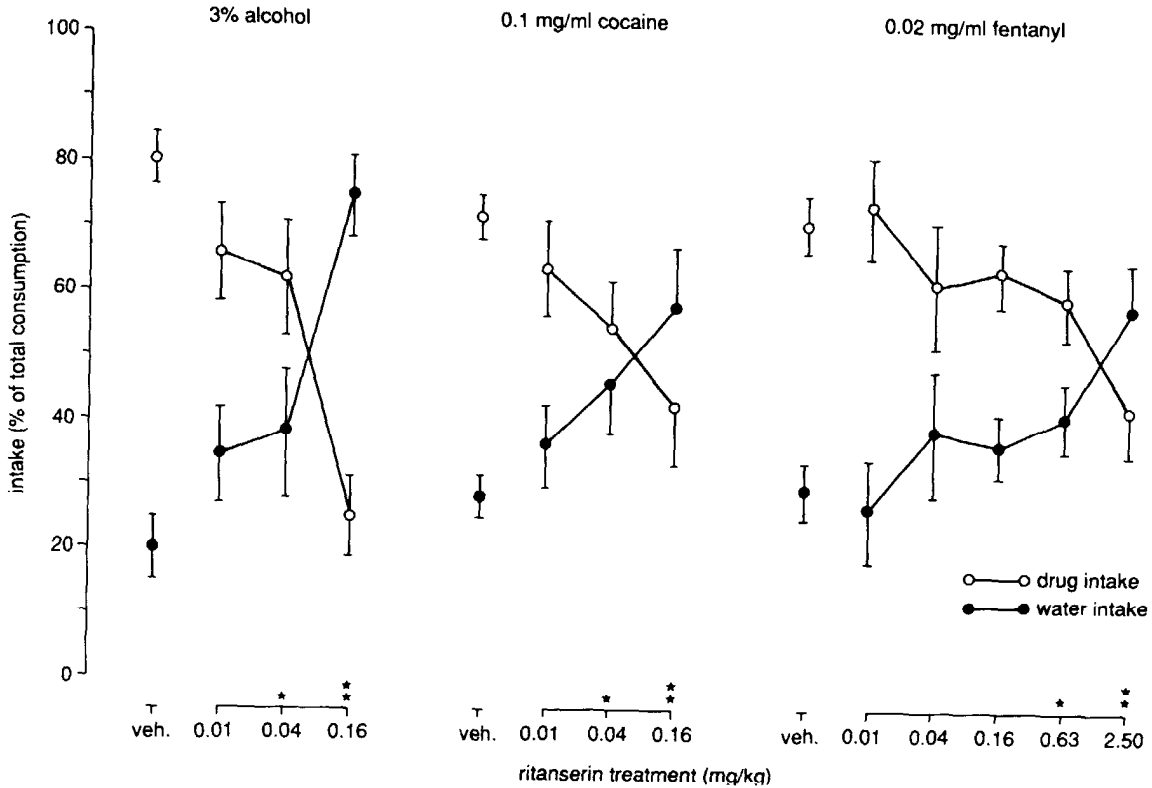

FIGURE 4. *Mean fluid intake (percent of total SEM) of drug solution or wafer during the period of treatment with vehicle or various doses of ritanserin. Statistical differences from corresponding vehicle controls: (Mann-Whitney u-test, two-tails) * = p < .05; ** = p < .07.*

consumption of drug and water was measured every 24 hr following each injection. The results of the treatment are shown in figure 4. Total fluid consumption, set at 100 percent for the control rats, did not change in the ritanserin dose range, which produced marked changes in the drinking pattern. Drug consumption was 71 to 80 percent of total fluid intake in the control rats of the three sessions. Alcohol intake decreased significantly at 0.04 mg/kg, and at 0.16 mg/kg a further pronounced fall was observed. The decrease in cocaine consumption was very similar within the same dose range of ritanserin. For fentanyl, reductions in drug intake were measured at doses ≥ 0.63 mg/kg. In all three experiments, water consumption was the mirror image of drug consumption. Because ritanserin was active against three pharmacologically different drugs of abuse, the effects of ritanserin might reflect rather a general mechanism of action rather than a direct interaction with one of the drugs of abuse.

To rule out the possibility that ritanserin substitutes for the drugs of abuse or that the drug directly potentiates the activity of the substances of abuse, riianserin was tested against different drugs of addiction in the drug discrimination procedure. Different groups of rats were trained to discriminate either 40.0 (SC, T-30 min) or 5.0 (IP, T-15 min) mg/kg chlordiazepoxide, 0.16 mg/kg LSD (IP, T-15 min), 0.04 mg/kg fentanyl (SC, T-30 min), 10.0 mg/kg cocaine (IP, T-15 min), 1.25 mg/kg *d*-amphetamine (SC, T-30 min), 0.63 mg/kg DOM (IP, T-15 min), or 1,000 mg/kg ethanol (IP, T-15 min) from saline. With ritanserin, at doses up to 40.0 mg/kg, no stimulus generalization was observed in any of these training groups. Furthermore, for those tested, no shift in the dose response functions of the training drugs was observed. In other studies using different doses of ritanserin (e.g., 1.25 mg/kg given IP at T-15 min or 10.0 mg/kg given SC at T-60 min), it was demonstrated that rats could not be trained to discriminate between the presence and absence of riianserin (Meert and Janssen 1989). These studies thus indicate that riianserin does not substitute for any of the current types of drugs of abuse and that the drug is devoid of internal stimulus properties. It may be that ritanserin is devoid of an abuse potential. Furthermore, in rats, ritanserin is not self-administered (Meert and Janssen 1989). Because ritanserin is a drug with a high safety, lacks an abuse potential, and reduces the abuse of various drugs of addiction (Meert et al. 1990*a*, 1990*b*), the compound may be a supportive pharmacotherapy for subjects who wish to abstain from drugs.

CONCLUSION

The three examples presented here indicate that the drug discrimination procedure with drugs of abuse as training stimuli is a valuable behavioral technique. The procedure could be used with almost any drug of abuse and the variety of experiments that could be conducted would all have typical outcomes. In generalization experiments, the drug discrimination procedure can be used to determine whether substances substitute for a particular drug of abuse. Results from these studies, together with information on discriminative stimulus properties, can provide some insight into the abuse liability of novel agents. In antagonism studies, direct antagonists of specific drugs of abuse can be tracked. These antagonists might be useful as antidotes and, especially those of drugs of abuse that can introduce symptoms resembling psychiatric states, might be useful in psychiatric disorders. Finally, mechanisms of action of drugs can be identified through these discrimination studies. The drug discrimination test procedure is thus a valuable and widely applicable behavioral technique, the outcomes of which can contribute to addiction research as well as to clinical medicine.

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Intracranial Stimulation as Reinforcer for Neuropeptide Discrimination

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INTRODUCTION

In establishing drug discrimination behavior, at least three variables must be controlled: the pharmacological class of the drug; the animal's behavior; and the unconditioned stimulus that reinforces the behavior.

These variables are not strictly independent. Drugs can produce motor effects that interfere with discrimination acquisition, and they can also possess strong reinforcing properties of their own. Therefore it would seem useful to establish a method by which discrimination and reinforcing properties of drugs could be assessed at the same time. Classical rewarding brain stimulation can be used as an unconditioned stimulus to evaluate the rewarding value of numerous drugs. Intracranial self-stimulation gives direct access to the reward centers in the brain and any modification of the activity in the reward centers by drugs becomes measurable by the rate of bar presses per brain stimulation. Because the discrimination of drugs results in access to a reward that reinforces the correct behavior, reinforcing brain stimulation could conceivably be used in a classical drug discrimination paradigm to study some qualities of reinforcement and discriminative properties of drugs at the same time.

The purposes of this study were to describe a design using brain stimulation as the reinforcer in a drug discrimination paradigm; to study the effects of varying the intensity of the brain reinforcer; and to test the use of a neuropeptide as discrimination stimulus to obtain the reward, i.e., reinforce brain stimulation.

DESIGN AND ADVANTAGES FOR DRUG DISCRIMINATION USING BRAIN STIMULATION REINFORCER

Male albino Wistar rats from a random breeding, individually housed under standard laboratory conditions (12 hr light/dark cycle, temperature 22-24°C humidity 55 percent), and maintained ad lib with laboratory food pellets, were used. The animals weighed 300 g at the time of surgery and were implanted with a monopolar nickel-chrome electrode (0.25 mm) insulated except for the cross section of the tip. The electrode was implanted stereotaxically according to the following coordinates: A-3.5 mm behind bregma, L-1.2 mm, and H-8.3 mm below the skull surface (lateral posterior hypothalamus). This brain area was chosen for the consistent rate of self-stimulation that can be obtained there. The indifferent electrode was placed 2 mm in front of the bregma. The animals were allowed 1 week to recover and were then trained to self-stimulate. They were allowed to self-stimulate in modified Skinner boxes using brain stimulations of 0.2 sec duration, 0.2 msec negative pulses being delivered at a frequency of 100 Hz. Threshold for self-stimulation behavior ranged from 60 to 200 μA with a mean of 130 μA. Rats were given daily training sessions until the bar-pressing rate became steady (1200 bar presses per 15-min session).

The drug discrimination procedure began with injection of drug or vehicle. Animals were placed in an illuminated and sound-attenuating chamber containing two levers. The appropriate lever was connected to the brain stimulator which automatically delivered one stimulation train after the required number of lever presses had been made; this number varied from 1 to 10 in the acquisition process of the fixed ratio (FR) requirement. The electrical parameters of the rewarding brain shock were always monitored by means of an oscilloscope, and the number of bar presses on both levers was recorded. Subjects were placed in the test chamber for a 15-min session 10 min after an intraperitoneal injection of ethyl alcohol (1 g/kg) or saline. Injections of alcohol (A) or vehicle (V) were given according to two weekly alternating sequences, i.e., Week 1: V-A-A-V-A and Week 2: V-V-A-V-A. The right lever was associated with alcohol and the other lever with saline.

Discrimination training began simultaneously with training on the FR 10 schedule. The following data were recorded: number of bar presses on the incorrect lever before the first reward was delivered, number of bar presses for the entire session, and number of rewards received during each session (De Witte 1982).

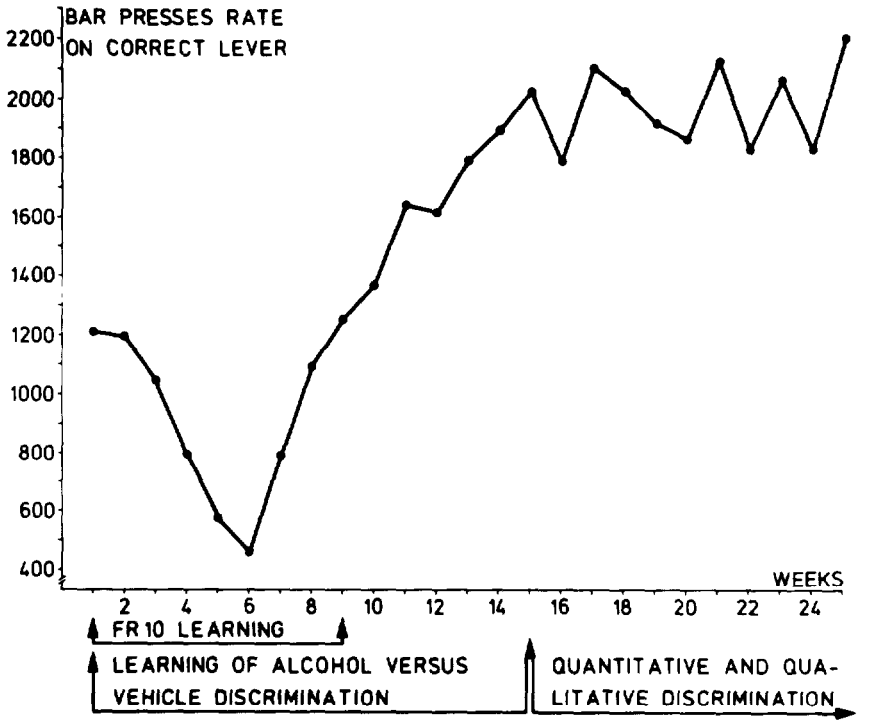

FIGURE 1.

A correct choice was defined as a mean percentage of correct responses beyond 82 percent (and no more than two presses on the incorrect lever before obtaining the first reward). The criterion of acquisition was set at 8 such correct choices out of 10 consecutive training sessions. Animals (six out of eight) acquired the FR 10 lever press response for brain stimulation after a mean period of 9 weeks. Figure 1 shows that response rates initially increased as the FR requirement increased. The mean percentage of correct responses increased from 56 percent at the end of the FR 10 learning period to attain the criterion of 82 percent in the 15th week, and from this week on, the percentage always remained above criterion.

This experiment shows that electrical brain stimulation can serve as a reward in a drug discrimination paradigm. While comparison to published results (Chipkin et al 1980, Kubena and Barry 1969, Schechter 1978, Winter 1977) on alcohol discrimination remains difficult, the use of brain stimulation as a reinforcer

TABLE 1. *Number of responses on the appropriate lever*[a]

Brain Electrical Intensity[b]	Low Dose (0.5 g/kg)		High Dose (2 g/kg)
Threshold	611 (±53)	<	900 (±102)[c]
+20µA	877 (±112)	=	932 (±83)[d]
+40µA	1604(±188)	>	1057 (±124)[c]

[a]15-min session.
[b]Mean number of bar presses (± standard deviation) for brain stimulation at the threshold and after increasing the intensity (+ 20 and + 40 µA).
[c]Significantly ($p < .01$) greater performance, using Student's f-test for dependent samples.
[d]Not significant.

would seem to delay the FR 10 learning (9 weeks). Nevertheless, once animals learned the task, discrimination remained stable for the entire experiment.

The main advantages of our method of using direct intracranial brain stimulation as the reward include the following. (1) Because rats are nondeprived, they have normal body weight. (2) The high number of responses (about 2000 bar presses per session) enables stabilization of the discriminative conditioning. (3) In addition to the analysis of the discriminative properties of drugs, the effects of the drug on the brain reward centers are estimated by the number of brain stimulations. After lever selection had occurred, rats self-stimulated, using FR 10 procedure, for the remainder of the 15-min session.

VARIATIONS OF THE BRAIN REINFORCER AND EFFECTS ON THE DISCRIMINATIVE PROPERTIES

Drug self-administration procedures have clearly shown that drugs can possess strong reinforcing effects in addition to strong discriminative properties. In a drug discrimination procedure this reinforcing property of the drug by itself, added to the reinforcer used to discriminate, could differentially affect the drug and saline sessions. Indeed, classically, an asymmetry was found to reach the discrimination criterion, and animals reached this criterion more rapidly under drug training sessions than under saline sessions (Colpaert et al. 1980).

The use of intracranial stimulation allows study of the effects of variations of magnitude of the reinforcement itself on the generalization gradients and the performance on the appropriate lever in a drug-drug discrimination (alcohol

2 g/kg vs. alcohol 0.5 g/kg). After estimating the generalization gradient, the intensity of the electrical reward was increased by 20 μA for each rat. The level of self-stimulation increased and was computed for 2 weeks of normal drug training (table 1). Then the generalization gradient was estimated using the same test doses. Following this, the intensity of the electrical brain reinforcer was raised another 20 μA (i.e., + 40 μA from the threshold intensity) and the generalization gradient was again estimated for the same doses (figure 2). The resulting generalization gradients diverged concurrently with the frequency of reinforcement. Cur results show that the obtained ED_{50} progressively slipped to the right; i.e., the greater the intensity of the brain reinforcer, the flatter the gradients became (De Witte and Gewiss 1986).

In a classical two-lever discrimination paradigm, the reinforcement used was food delivered to deprived animals and given asymmetrically and equally for responding on the two levers. Reinforcement rates were different between drug and saline training sessions in a way that animals received more reinforcements during drug sessions than during saline sessions (Colpaert and Janssen 1981; Koek and Slanger 1982).

By using electrical brain stimulation as the reinforcer and manipulating the intensity of the electrical stimulation, we have shown that the absolute number of reinforcements received varies under low- and high-dose training sessions (table 1). The number of reinforcers obtained using brain stimulation increased along with the increase in current intensity only for the low training dose; the performance remained constant at high ethanol dosage training sessions. By manipulating the intensity of the brain stimulation, we can control the rate of reinforcement, thus obtaining the same frequency of reinforcement delivered on both levers (i.e., the case of + 20 μA in table 1).

Cur experiment shows that, independent of the action of alcohol on brain reward systems, the use of brain reinforcing stimulation as reward in a conditioned paradigm produces a quantitative generalization gradient with the delivery of the same number of rewards after low dose or high dose of the pharmacological substance injected. This represents an important variable to control before studying qualitative generalization to other substances in the research of the pharmacological profile when using a two-lever drug discrimination paradigm.

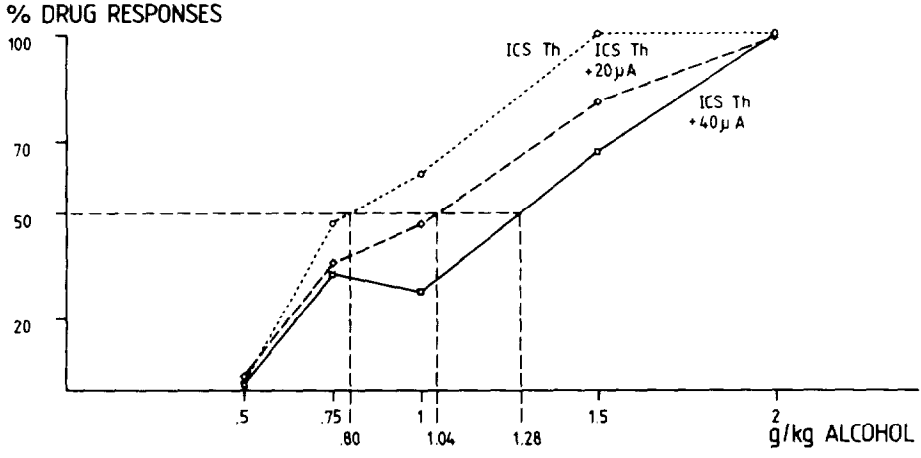

FIGURE 2. *Mean sample score on the drug lever (ordinate) after injection of different test doses of alcohol (abscissa). Different gradients are obtained successively depending on the intensity of the brain electrical stimulation. This figure also shows the mean ED_{50} for each obtained gradient.*

NEUROPEPTIDE AS DISCRIMINATION STIMULUS

In a drug discrimination procedure, drugs can induce internal unconditioned stimuli, which can serve as discriminative stimuli for responses that lead to reinforcement. We used a procedure in which the discrimination of the presence of the neuropeptide caerulein (CER), a cholecystokinin analog (CCK-8), was the condition to obtain the reward (a reinforcing brain stimulation, which reinforced the organism when performing the correct discrimination between CER and its vehicle). The use of direct electrical stimulation in a rewarding brain center producing short (0.2 sec) and highly powerful repetitive rewards without satiety offers the advantage of training animals of normal body weight without food deprivation. This last characteristic is essential since CER and CCK-8 are neuropeptides acting on feeding (i.e., inducing satiety). We can thus suppose that these peptides will be differently perceived under a deprived state than under a normal steady state. Animals reached the criterion of acquisition of the discrimination 12 to 18 weeks of training. At this point the experiment shows that while a neuropeptide such as CER can serve as a cue in a drug

discrimination paradigm, more sessions are required to reach the discrimination criterion with CER than with classical pharmacological substances.

It is known that drugs producing only peripheral action are harder to discriminate than centrally acting drugs (Lal 1977), but the mechanism by which CCK and CER exert their wide-ranging effects on the CNS remains controversial. It is also unclear whether CCK peptides cross the blood-brain barrier. In this regard, immunoreactivity of CCK-8 is not observed in rabbit CSF following IV injection. However, behavioral and biochemical studies show that peripherally administered peptides may indeed alter central dopamine (DA) mediated processes. These actions could occur through an effect mediated by the vagus without necessarily having to cross the blood-brain barrier. Thus, the neural information from peripheral to central sites implicates the ascending sensory fibers of the vagus nerve to the nucleus tractus solitarius (NTS) (Crawley and Schwaber 1984). From this subdivision of the NTS, projections ascend to the lateral hypothalamus. In turn, the lateral hypothalamus returns projections to the NTS and then to the efferent motor fibers of the vagus nerve to the gut (Schwaber et al. 1982).

A few years ago, cholecystokinin carboxyl terminal octapeptide immunoreactive neuronal cell bodies were discovered in the rat substantia nigra and ventral tegmental areas. They were present in such large numbers that coexistence of CCK with DA was suggested in these neurons (Hökfelt et al. 1980). Following this discovery of DA-CCK-8 coexistence, a large core of behavioral data suggested that CCK-8 had neuroleptic-like properties (De Witte it al. 1985; Van Ree et al. 1963; Zetler 1983). Moreover, clinical data suggested a potential involvement of this peptide in several psychiatric disorders, particularly schizophrenia. Nevertheless, the origins of the neuroleptic-like properties of CCK-8 remain unknown and the potential role of dopaminergic systems in the discriminative properties of the neuropeptide cholecystokinin has not yet been studied experimentally. In this context, the search for common cueing properties between CER, a CCK-8 analog, and unsulfated CCK; DA agonists, such as amphetamine (AMPH) and apomorphine (APO); and antagonists, such as chlorpromazine (CPZ) and haloperidol (HAL) could help define the possible pharmacological role of CCK (table 2).

CCK-8 in its sulfated form shares properties with the training drug CER, while CCK-4 and the unsulfated form of CCK-8 present no pharmacological properties similar to CER. These results thus confirm previous work using other

TABLE 2. *Results of statistical analyses of treatment effects with chi-square test*

Training Condition	Number Responding on Drug Lever/Number Tested	Mean Responses on Selected Lever (15-min session)
Training Drug		
Caerulein		
3 µg/kg	1/6*	1,088
Test Drugs		
Caerulein		
1 µg/kg	1/6*	922
2µg/kg	5/6	731
Cholecystokinins		
CCK-4		
10 µg/kg	0/6*	1,122
20 µg/kg	0/6*	1,373
200 µg/kg	0/6*	1,412
CCK-8		
5 µg/kg	0/6*	987
10 µg/kg	3/6*	1,011
20 µg/kg	6/6	932
CCK-8 (unsulfated)		
10 µg/kg	0/6*	1,267
20 µg/kg	0/6*	962
200 µg/kg	1/6*	817
Dopamine Agonists		
d-Amphetamine		
0.32 mg/kg	0/6*	1,673
0.64 mg/kg	0/6*	2,370
1.28 mg/kg	0/6*	1,655
Apomotphine		
0.05 mg/kg	0/6*	978
0.10 mg/kg	1/6*	251
0.20 mg/kg	0/6	300
0.60 mg/kg	0/6*	0
Dopamine Antagonists		
Chlorpromazine		
0.5 mg/kg	2/6*	728
1.0 mg/kg	2/6*	473
2.0 mg/kg	5/6	182
Haloperidol		
0.03 mg/kg	5/6	621
0.05 mg/kg	5/6	268
0.20 mg/kg	0	0

*Probability of difference from training drug (caerulein 3 µg/kg) being due to chance; $p < .05$ chi-Square test.

behavioral techniques showing differential effects between those forms of cholecystokinin (De Witte et al. 1987; Hsiao et al. 1984).

Both DA agonists AMPH and APO are generalized to the vehicle lever. Animals generalize HAL 30 µg/kg to CER, possibly the result of the neuroleptic-like properties of CER. The other DA antagonist, CPZ, is also completely generalized to CER but at a different dosage than HAL, i.e., 2 mg/kg CPZ versus 30 µg/kg HAL. Thus, a 67 times greater dose of CPZ is required to obtain an equi-active cue with HAL similar to the training CER. These equi-active doses of HAL and CPZ are in the range of those obtained using the APO-antagonism test in dogs and in clinical use. The large difference in dosage between HAL and CPZ in the generalization to the CER may be the result of the different pharmacological properties of these two DA antagonists.

Thus our experiment clearly shows that peripheral injection of HAL and CPZ induces pharmacological cues similar to those of CER, while injections of AMPH and APO do not share pharmacological cues *with* CER. Peripherally injected CCK-8 is thus generalized with HAL. Similarly, the neuroleptic effect of peripherally administered CER in the rat is similar to the effect of haloperidol. However, since CCK behaves like a direct DA agonist when injected directly into the caudal part of the accumbens (De Witte et al. 1987), the effect of intraperitoneally or intraventricularly injected CER might be an indirect consequence of CCK-8 excitation of some projections to the caudal accumbens known to antagonize the effect of DA in that nucleus. According to this assumption, CCK-8 differs in many actions from neuroleptics and this could explain the discrepancies observed in the studies of its clinical pseudoneuroleptic effect on human beings (De Witte et al. 1988).

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Drug Discrimination Used To Study Drug Withdrawal

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INTRODUCTION

Withdrawal from the long-term use of psychoactive drugs produces a variety of readily discernible phenomena in humans. In clinical terms, these phenomena can be classified as those that have an objectively verifiable basis (signs) and those that are subjective in nature (symptoms). Symptoms of drug withdrawal have been described as mixtures of anxiety, dysphoria, and drug craving, and at least some of these symptoms are shared across classes of drugs (Marks 1978; Wikler 1980). Even when drugs produce obvious signs of withdrawal, the symptoms continue long after signs have abated. Thus, symptoms of drug withdrawal may play a significant role in promoting the continuation of drug dependence: they provide motivation for continued drug use, and they occur prior to or in the absence of physical signs of withdrawal. In order to clarify the role of subjective symptoms of withdrawal in drug dependence, animal models have been developed that may address this problem (for reviews see Emmett-Oglesby et al. 1990; File 1990). Clinically, these models may be particularly important for testing new treatments for withdrawal and studying the biological basis of drug dependence.

This paper will focus on efforts to study withdrawal using drug discrimination methodology. Two approaches have been reported for these drug discrimination models of withdrawal. The first stems from the observation that withdrawal in humans frequently causes anxiety. Thus, if animals could be taught to discriminate an anxiogenic drug, it is conceptually plausible that such a discrimination might generalize to drug-withdrawal stimuli. The discrimination of pentylenetetrazole (PTZ) has been reported to have predictive validity for identifying both anxiogenic and anxiolytic drugs, and, in subjects trained to detect PTZ, withdrawal from benzodiazepines substitutes for this stimulus. These data have been extensively reviewed elsewhere (e.g. Emmett-Oglesby

et al. 1987, 1990) and in general support the idea that the discrimination of PTZ may be useful for detecting withdrawal from a variety of drugs of dependence, particularly those of the sedative-hypnotic class. For example, withdrawal from benzodiazepines or ethanol substitutes fully for PTZ, and withdrawal from cocaine, morphine, or nicotine substitutes partially for the stimulus properties of PTZ.

A second approach to drug discrimination models involves training animals to detect directly the stimulus effects arising during withdrawal, and the primary aim of this chapter is to review and evaluate the utility of this paradigm. These experiments maintain subjects on a baseline of chronic drug of dependence such as morphine and attempt to train the stimulus properties of an antagonist such as naloxone. In general, subjects are trained to respond on one of two levers and food is presented for correct responses. They are then maintained chronically on a drug of dependence, and withdrawal is presumably precipitated by using an antagonist (e.g., Holtzman 1985*a*; Valentino et al. 1983). Thus, responding on one of the levers is reinforced following antagonist administration, and responding on the other lever is reinforced following vehicle administration.

When a discrimination is trained based on drug versus vehicle, it has been well established that only some drugs will substitute for the training drug, and a significant correlation exists between drugs having stimulus properties comparable to the training stimulus and drugs that humans describe as having the same class of subjective effects (Schuster and Balster 1977). To date only discriminations based on precipitated withdrawal of the opiates and benzodiazepines have been reported using this method. We will review data showing that it is difficult to specify what stimulus is controlling behavior in these discriminations. Although experimenters attempt to establish withdrawal as the controlling stimulus, because the animals are maintained on a baseline of chronic drug, the discrimination may be based, at least in part, on the direct effects of the drug of dependence. To alleviate this problem we will describe data expanding the traditional two-lever discrimination to a three-lever choice procedure that includes the drug of dependence, the drug producing withdrawal, and saline.

TRAINING TO DETECT STIMULUS EFFECTS OF WITHDRAWAL

In a typical drug discrimination procedure, subjects are presented with a simple choice task in which they are differentially reinforced for responding in the

presence or absence of the drug. Either vehicle or a dose of drug is injected prior to training, and animals are allowed to respond while the drug is producing its effects. If the experimental contingencies are arranged so that one of the responses will be reinforced in the presence of the drug effect and the other response will be reinforced in the absence of the drug effect, then the drug can serve as a discriminative stimulus; i.e., its presence or absence will differentially control responding. Although two training conditions exist to the experimenter, drug versus vehicle, it is important to note that apparently it is only the presence of the drug stimulus that controls responding. The animal does not learn drug versus "vehicle" per se; instead, the discrimination appears to be based on "drug-specific stimuli" versus "all other stimuli." This distinction is apparent when testing animals that are trained in a two-choice task using a drug versus vehicle procedure. When tested with other drugs, only those known to produce similar subjective effects in humans will substitute for the training stimulus (Altman et al. 1977). For example, in animals trained to detect morphine, only *mu* agonists reliably produce full drug-lever selection, whereas *kappa* agonists, or drugs from other classes, produce vehicle-lever responding (Colpaert 1978; Holtzman 1985*b*; Lal et al. 1978). Since these other drugs can produce their own stimulus effects, this pattern of results is generally interpreted to mean that the training stimulus serves as a reference stimulus, and responding on the drug versus vehicle lever is controlled by how closely the test stimulus matches the stimulus produced by the training drug. This distinction may become particularly relevant when attempting to train the stimulus properties associated with withdrawal.

Initial attempts to train the stimulus properties of pure opiate antagonists were not successful. When given in nondependent rats these drugs (naloxone and naltrexone) either had low efficacy or did not function as discriminative stimuli (Lal et al. 1978; Overton 1982; Weissman 1978). In contrast, if animals were maintained on a chronic baseline of opioid dependence, the antagonists were more readily trained as discriminative stimuli (Gellert and Holtzman 1979; Hoitzman 1985*a*; Valentino et al. 1983). Discriminations based on this procedure have been reported for rats (Gellen and Holtzman 1979; Holtzman 1985*a*), pigeons (France and Woods 1987; Valentino et al. 1983), and monkeys (France and Woods 1989), and the data clearly indicate that the opioid antagonist given in this paradigm can function effectively as a discriminative stimulus. The critical question becomes, What is the nature of the stimulus that is controlling behavior?

Two-Choice Procedures

In the traditional "drug versus no-drug" discrimination procedure, subjects experience only the stimulus produced by the training drug, but in the opioid antagonist experiments this may not be the case. The problem arises because subjects are maintained on a chronic baseline of opioid drugs, which have been shown to produce discriminative stimuli of their own (Colpaert 1978; Herling et al. 1980; Holtzman 1985*b*). Although the experimenter intends that the discrimination training involve the effects associated with nattrexone as a reference stimulus, two other possibilities exist (table 1). The first is that the saline injection allows the opiate drug to exert a discriminative stimulus and the antagonist simply turns off the opioid stimulus. A second possibility is that the subject learns a distinctive stimulus associated with withdrawal and a second stimulus associated with the direct effects of the opiate. In this case, instead of training drug versus no-drug, it is possible that drug A versus drug B is trained.

Based on these alternatives, what would be acceptable evidence that precipitated withdrawal is actually the controlling stimulus? Perhaps the easiest way to answer this question is to terminate the chronic administration of the drug of dependence. Initially, physical signs of withdrawal should occur, and during this period vehicle should substitute for the antagonist. When spontaneous withdrawal has dissipated, if what the animals have learned is a precipitated-withdrawal stimulus, both the vehicle and the antagonist should now produce vehicle lever responding. Using this approach, Holtzman (1985*a*) has provided evidence that morphine withdrawal has indeed been trained as a discriminative stimulus.

More recently, France and Woods (1989) trained rhesus monkeys that were maintained on morphine (1.78 or 3.2 mg/kg daily) to discriminate the stimulus properties of naltrexone (0.01 mg/kg) from saline injections. When chronic morphine was terminated, monkeys switched their responding from the saline to the naltrexone lever between 8 and 27 hr post morphine injection, and naltrexone responding occurred when signs of opioid withdrawal were maximal. Another interesting aspect of this study was that doses of naltrexone that produced responding on the naltrexone lever also produced effects typically seen during opioid withdrawal, including salivation, miosis, occasional vocalizations, and irritability. These results indicate that dependence developed to the once daily morphine injections and that the naltrexone discriminative stimulus may have been related to the withdrawal-precipitating actions of naltrexone. Still, using this two-lever procedure it is unclear whether

dependence has developed and what the animals have actually learned. As noted by France and Woods (1989), “although the results of substitution studies support the notion that dependence has developed and monkeys were showing signs of withdrawal when the naltrexone lever was selected, these results might also be interpreted in a context of morphine (saline injection) vs. non-morphine (naltrexone injection) discrimination; the dose effects and time-effects are fully consistent with either hypothesis and neither can be conclusively rejected with the present paradigm” (p. 942). As described above, had these subjects been maintained free of morphine for an extended period of time, it may indeed have been possible to reject one of these hypotheses.

Using this procedure, in preliminary studies from this laboratory we have attempted to train rats to detect withdrawal from chlordiazepoxide (CDP) (Emmett-Oglesby et al. 1990). In these experiments, subjects were maintained on a baseline of 100 mg/kg of CDP per day, given in two doses of 50 mg/kg as part of a nutritionally balanced liquid diet (Idemudia and Lal 1989). They were trained with the benzodiazepine antagonist flumazenil (Ro 15-1788) 20 mg/kg, and saline on a two-lever choice task using food as a reinforcer, with daily training sessions occurring 6 hr after the morning feeding of CDP. The discrimination was rapidly acquired with error rates less than 5 percent within 30 training sessions. In tests with flumazenil, doses from 2.5 to 20 mg/kg all produced flumazenil-lever responding, with the dose eliciting 50 percent flumazenil selection (ED_{50}) of approximately 0.64 mg/kg. When chronic CDP was terminated, results from a variety of drug substitution tests suggested that stimulus control in this discrimination was actually maintained by both the direct effects of CDP and the effects of precipitated withdrawal by flumazenil. For example, during this drug-free period, CDP produced vehicle-lever responding, and PTZ produced flumazenil-lever responding, but both flumazenil and vehicle produced mixed responding on both levers. These results are consistent with the hypothesis that the stimulus trained was associated with both flumazenil and CDP, because the saline baseline failed to return to its prewithdrawal baseline.

Based on these preliminary studies, the parameters of the experiment were altered in an attempt to train subjects to detect withdrawal from dependence on benzodiazepines against a baseline of minimal CDP-like effects. The two major parameters that were altered included the dose of flumazenil used for training and the feeding schedule of chronic CDP. A much lower dose of flumazenil was used, 2.5 instead of 20 mg/kg, since the initial study revealed that these doses were essentially interchangeable, i.e., both produced full generalization to the

training dose of 20 mg/kg. Also, the morning dose was lowered to 25 mg/kg and the dose after testing was raised to 75 mg/kg. The rationale for this lower morning dose was to reduce the amount of CDP on the receptor at the time of training. During “vehicle” training, this change would presumably result in a decrease in the stimulus produced by CDP.

Following acquisition of this discrimination, flumazenil was substituted for the training stimulus in a dose-dependent manner (0.64-2.5 mg/kg), with an ED_{50} of 0.94 mg/kg. The animals were then taken off CDP, and in testing 8 days later, saline produced 67 percent flumazenil-lever selection, perhaps indicating similarity between spontaneous withdrawal at this time and the flumazenil stimulus that was trained under chronic CDP. In contrast, in testing 14 days after termination of CDP, the response to vehicle was not significantly different than before withdrawal of the vehicle. Thus, only flumazenil acting as an antagonist produced a controlling stimulus, rather than both flumazenil and CDP producing controlling stimuli.

After obtaining the time course data, these animals were placed back on chronic CDP and trained for an additional 3 months. Subsequently, the dose-effect curve for flumazenil was redetermined (figure 1); this curve (ED_{50} of approximately 0.64) was not significantly different than the initial dose-effect curve. CDP at doses of 5, 20, and 80 mg/kg did not substitute for the flumazenil stimulus. However, PTZ (5-40 mg/kg) did produce a dose-dependent substitution for flumazenil in these animals (figure 2). As described at the start of this review, in animals trained to discriminate PTZ, flumazenil substitutes for PTZ only after chronic administration of a benzodiazepine (Emmett-Oglesby et al. 1983, 1987). Thus, there is symmetrical generalization between these compounds that occurs only when flumazenil is tested in subjects maintained on a chronic baseline of benzodiazepine administration. Indeed, when subjects are trained to detect the effects of flumazenil directly (not while being administered a benzodiazepine), PTZ does not generalize to flumazenil (Rowan and Lucki 1989).

The data from this series of experiments suggest that subjects learn a stimulus that is associated with flumazenil, is PTZ-like in nature, and is similar to spontaneous withdrawal from the benzodiazepine CDP. Thus, these data are consistent with having trained precipitated withdrawal.

Although benzodiazepine and morphine withdrawals appear to have been trained using two-choice technology, these discriminations are intrinsically

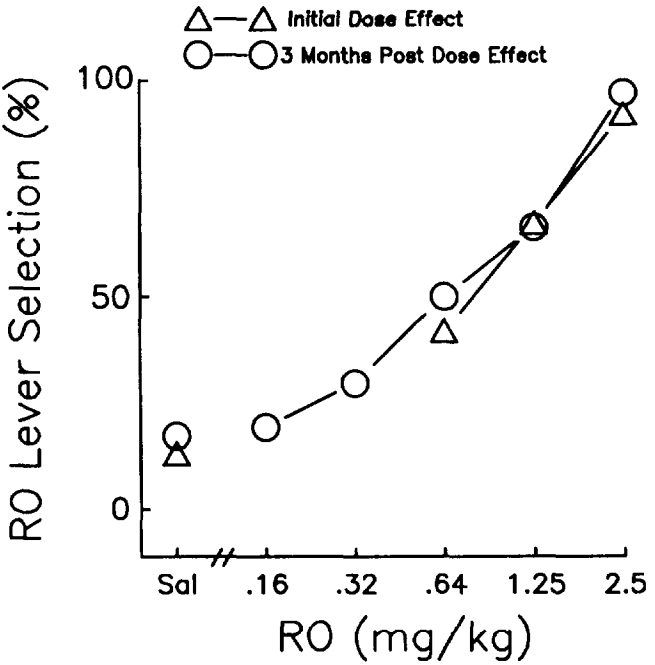

FIGURE 1. *Stability of flumazenil dose-effect curve. The dose-effect curve for flumazenil (Ro 15-1788 [RO]) was determined in rats (n = 12) trained to detect the stimulus properties of RO (2.5 mg/kg) while receiving chronic administration of chlordiazepoxide (CDP, 100 mg/kg/day) in the form of a nutritionally balanced liquid diet. On the ordinate, 100% RO lever selection would indicate that all animals selected the RO lever, while 0% RO lever selection would indicate that the animals selected the vehicle (saline) /ever. Rats were trained 6 hr after the morning CDP feeding (25 mg/kg) and received the remainder of the CDP dose (75 mg/kg) after training. The initial dose-effect curve produced an ED_{50} of approximately 0.64 mg/kg. When the animals were retested 3 months later, a similiar ED_{50} was obtained. These data indicate the stability of this discrimination across a 3-month training period.*

unsatisfactory. Proving that the stimulus produced by the antagonist is in fact related to withdrawal requires long periods when the subjects are drug free. Similarly, proving that the antagonist provides the only controlling stimulus also

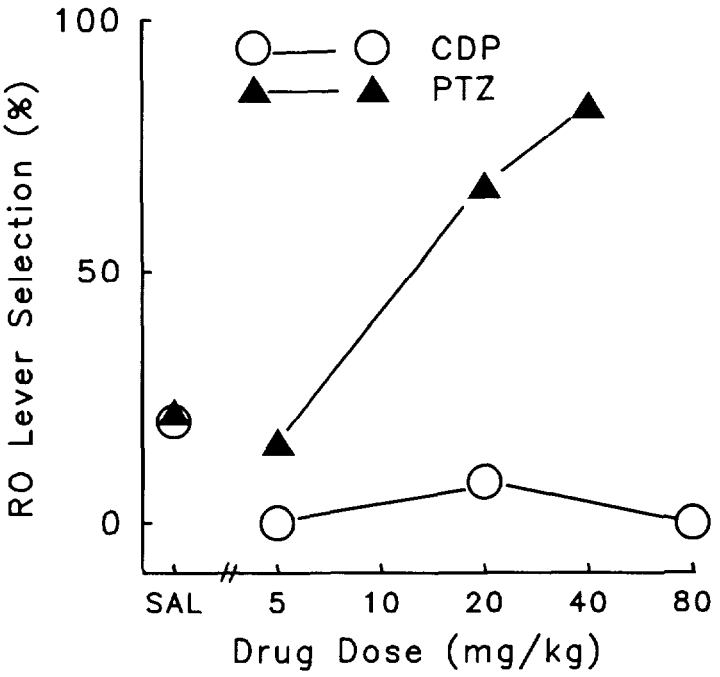

FIGURE 2. *Substitution of pentylenetefrazole for flumazenil. These animals (n = 12) were trained to detect the stimulus properties of flumazenil (RO, 2.5 mg/kg), while being maintained on chronic chlordiazepoxide (CDP, 100 mg/kg/day). They were then tested for substitution of COP (5, 20, and 80 mg/kg) and pentylenetetrazole (PTZ; 5, 20, and 40 mg/kg). CDP did not substitute for RO at any of the doses tested, while PTZ did produce a dose-dependent substitution for RO. These data support the hypothesis that withdrawal from benzodiazepines shares common stimulus properties with PTZ.*

requires this test. Moreover, few guidelines are available to decide how much of the drug of dependence should be given daily or how long after administration of the drug of dependence training should occur. Finally, several weeks of training must occur before the discrimination is learned, and only then can the critical drug-free test be conducted.

Introducing a Third Choice

Some of the problems that arise in a two-choice procedure may be readily resolved by the addition of a third choice that actively trains the drug of dependence as a controlling stimulus. There have been several reports of three-lever (rats) or three-key (pigeon) discriminations. These experiments usually attempt to train the animals to discriminate either the stimulus properties of two different drugs or different doses of the same drug versus saline (France and Woods 1987; Gauvin and Young 1989; White and Holtzman 1981, 1983). These experiments have demonstrated that the discriminative stimulus effects of drugs in a three-lever procedure can be very similar to training these compounds in a two-choice procedure. For example, this laboratory has been successful in training a three-lever discrimination between PTZ, midazolam, and saline. The acquisition data for this discrimination are illustrated in figure 3. As in a two-lever discrimination involving midazolam (Garcha et al. 1985; Woudenberg and Slangen 1989), diazepam and chlordiazepoxide produced midazolam-lever selection.

The particular advantage of this procedure in a drug of dependence study is that by training withdrawal versus the drug of dependence, the vehicle choice now can encompass all stimuli that are not similar to these two choices. Thus, the vehicle choice functions as a non-drug A or non-drug B choice but, interestingly, not necessarily as a "neutral" choice (Järbe and Swedberg 1982). Conceptually, it may be inconsequential whether any distinct stimulus is associated with the vehicle: the critical determinant is that the stimulus associated with this choice should be less intense than the other two stimuli trained. This assertion is made within the context of evidence from dose-dose discriminations that stimulus control resides entirely in the more intense of two stimuli. There are several experiments demonstrating that animals can learn to discriminate two doses of the same drug (Colpaert and Janssen 1982*a*, 1982*b*; DeVry and Slangen 1986; Young et al. 1989). In experiments such as these, when the magnitude of difference between the two doses trained is large, the discrimination approximates a drug versus saline discrimination. Hence, in a three-choice discrimination involving drug dependence, the nature of the stimulus associated with the vehicle may not be important as long as it is less intense than the other two stimuli being trained.

There are three likely configurations that could be trained in a three-choice discrimination involving a drug of dependence, its antagonist, and saline. Based on the stimulus condition associated with vehicle administration, these

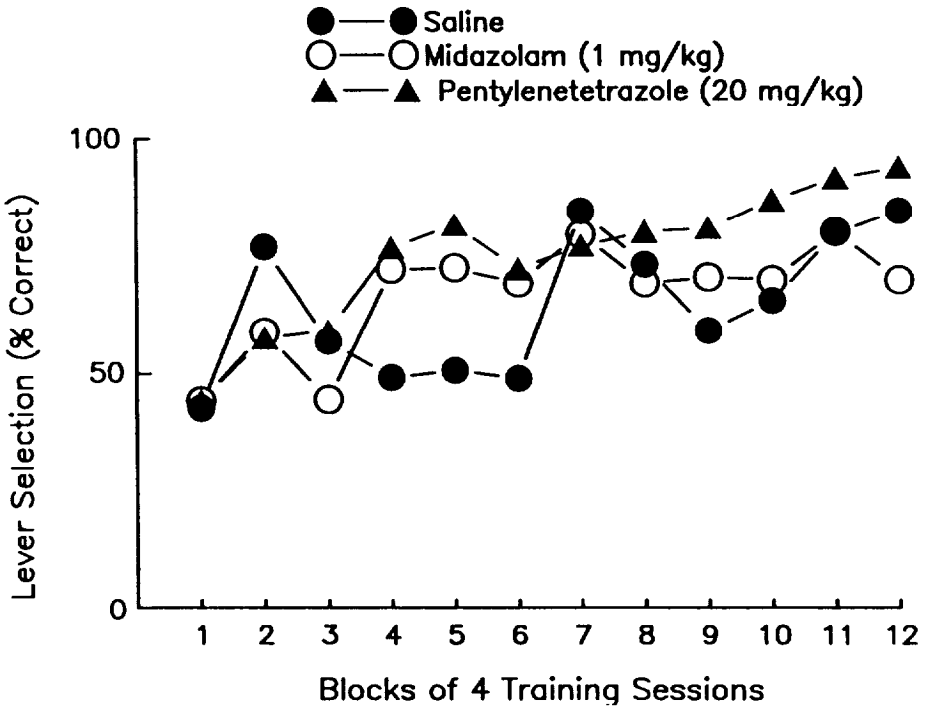

FIGURE 3. *Acquisition of three-choice discrimination in non-dependent animals. The animals were trained to detect the stimulus properties of pentylenefetrazole (20 mg/kg), midazolam (1.0 mg/kg), and saline. The acquisition of the discrimination is presented in terms of percent correct lever responding for the three compounds. The discrimination developed differentially for the three choices, as determined by animals reaching a 75% correct-lever selection criterion.*

possibilities can be conceptualized in the following ways (see table 1): antagonist versus vehicle (neutral) versus agonist; antagonist versus vehicle (low-dose agonist effect) versus agonist (highdose agonist effect); antagonist (intense withdrawal) versus vehicle (mild withdrawal) versus agonist. An experiment that illustrates the difficulties of specifying which of these cases occurs involved training naltrexone, morphine, and saline in morphine-dependent pigeons. France and Woods (1987) attempted to show

TABLE 1. *Possible Controlling Stimuli: 2-Choice Discrimination*[1]

Withdrawal	Neutral
Neutral	Drug of dependence
Withdrawal	Drug of dependence

Possible Controlling Stimuli: 3-Choice Discrimination?

Withdrawal	Neutral	Drug of dependence
Withdrawal	Drug of dependence L	Drug of dependence H
Withdrawal H	Withdrawal L	Drug of dependence

[1]Stimuli in the left panel arise following antagonist treatment whereas stimuli in the right panel follow vehicle administration. The first theoretical possibility for the stimuli controlling the two-lever discrimination is the antagonist versus vehide. A second possibility is that the animals simply learn the onset and offset of the agonist treatment. In the third case, during vehicle training the animals learn to discriminate the stimulus properties of the antagonist versus the agonist (drug of dependence).

[2]Stimuli in the left panel arise following antagonist treatment; stimuli in the center panel arise following vehicle administration; and stimuli in the right panel arise following administration of the chronic agonist. The first possibility trains the drug of withdrawal (antagonist) versus a neutral stimulus (vehicle) versus the stimulus properties of the drug of dependence. A second possibility is that during vehicle training the animals learn to discriminate the stimulus properties of the lower dose of the drug of dependence than is trained on the third choice lever (Drug of dependence H). In the third case, the animals simply learn the withdrawal stimulus in a high and low form versus the drug of dependence.

that naltrexone produced a withdrawal-related stimulus in this three-key discrimination. When morphine administration was terminated and subjects were retested at various intervals, responding occurred on the morphine key at 1 hr, the saline key at 6 hr, and the naltrexone key at times up to 30 hr. Unfortunately, no test results beyond this time were reported, and thus it is impossible to know if these subjects would have eventually arrived at a point in time where naltrexone produced saline-key responding. France has examined this problem (personal communication) and notes that naltrexone did produce saline responding, but the baseline appeared to be unstable. Hence, it is possible that these animals have learned a discrimination in which withdrawal is one of the controlling elements, but the degree of control is difficult to specify.

Based on these experiments we attempted to expand the two-lever flumazenil (2.5 mg/kg) experiment to include another lever for CDP. Animals were maintained on chronic CDP, 100 mg/kg/day, fed in divided doses, 25 mg/kg in the a.m. and 75 mg/kg in the p.m, as described for the two-lever discrimination above. The animals were trained to detect the stimulus properties of flumazenil (2.5 mg/kg), CDP (20 mg/kg), and saline. The acquisition of the discrimination developed differentially for the three compounds, as determined by reaching a 75 percent correct-lever selection criterion. The discrimination of flumazenil developed in approximately 12 trials, while the CDP and saline discrimination took closer to 36 and 46 trials, respectively (figure 4). The animals also showed dose-dependent generalization for both flumazenil and CDP (figures 5 and 6). In the flumazenil generalization, there was no responding on the CDP lever. As the dose of flumazenil increased, the animals switched their responding from the saline lever to the flumazenil lever. It is also interesting to note that the generalization curve for flumazenil is very similar to that obtained in the two-lever flumazenil discrimination. In the CDP generalization curve the animals demonstrated dose-dependent increases in CDP-lever selection as the dose of CDP increased. The animals switched from saline to CDP-lever responding as the dose increased with little flumazenil-lever selection.

Problems With Three-Choice Procedures for Studying Withdrawal

A three-choice discrimination involving a baseline of chronic drug administration presents several technical problems. Many of these difficulties are shared with the two-choice discriminations reviewed above. For example, what dose of the antagonist should be used for training, and how frequently and in what dose should the drug of dependence be administered each day? Also, how long after a maintenance injection of the drug of dependence should training occur? The answers to these questions are generally arrived at empirically and are based on the goals described previously for two-choice discriminations.

We suggest that subjects be trained on as large a dose of the drug of dependence as possible. There are three reasons for this suggestion. First, bigger doses of drugs are easier to learn and provide better discriminative control than smaller doses (Colpaert and Janssen 1982*b*; Overton 1982, 1987); thus, the discrimination training conditions should be readily distinguishable for the subject.

A second reason for using large doses of drugs arises from the unknown nature of the stimulus baseline induced by chronic administration of the drug of

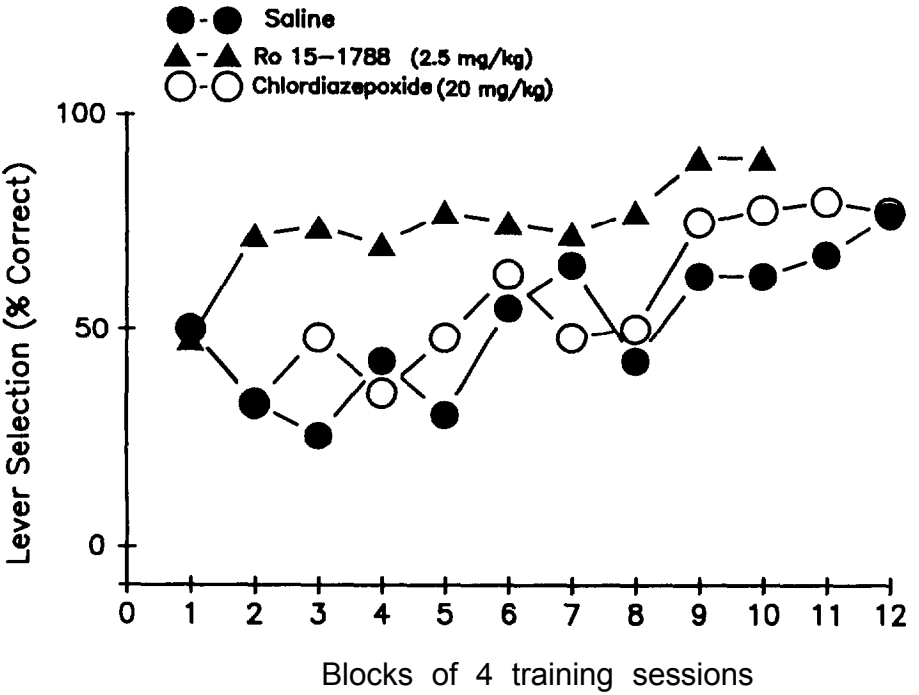

FIGURE 4. *Acquisition of three-choice discrimination in animals dependent on CDP. The animals were trained to detect the stimulus properties of flumazenil (RO, 2.5 mg/kg), chloroiazepoxide (CDP, 20 mg/kg), and saline, while maintained on a liquid diet containing CDP (100 mg/kg/day). Acquisition of the discrimination is presented in terms of percent correct lever responding for the three compounds. The discrimination developed differentially for the three choices, as determined by animals reaching a 75% correct lever selection criterion. The RO discrimination developed in approximately 12 trials, while the CDP and saline took closer to 36 and 46 trials, respectively.*

dependence. Several scenarios are possible, at least one of which requires large training doses of the drug of dependence. For example, suppose that subjects are trained at 6 hr after administration of chronic drug. If the drug has not completely cleared the body (which is presumably the case if withdrawal is to be precipitated when an antagonist is trained), then it is likely that this drug is

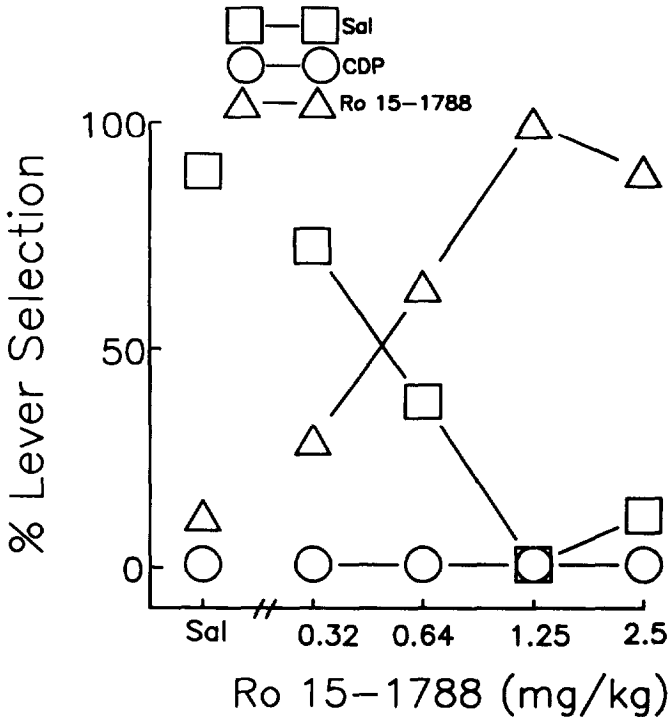

FIGURE 5. *Generalization of flumazenil in a three-choice discriminatrion. The dose-effect curve for flumazenil (Ro 15-1788 [RO]) was determined by testing independent doses in rats (n = 9) trained to detect RO (2.5 mg/kg), chlordiazepoxide (CDP, 20 mg/kg), and saline in a three-lever choke task. Animals were maintained chronically on CDP 100 mg/kg/day. On the ordinate, 100% lever selection indicates that all animals initially responded on the lever shown by the symbol to a fixed ratio of 10 lever presses. As percent selection of the RO lever increased in a dose-dependent manner, saline-lever selection decreased. No CDP-lever selection was evident during the test.*

exerting a stimulus effect. Thus, the problem for the subject will be to differentiate between the stimulus effects of chronic drug versus the stimulus effects of this same drug given in a larger dose for training purposes. The closer these two stimuli are in magnitude, the more difficult this discrimination will be (Colpaert and Janssen 1982*a*, 1982*b*). Consequently, when administered for

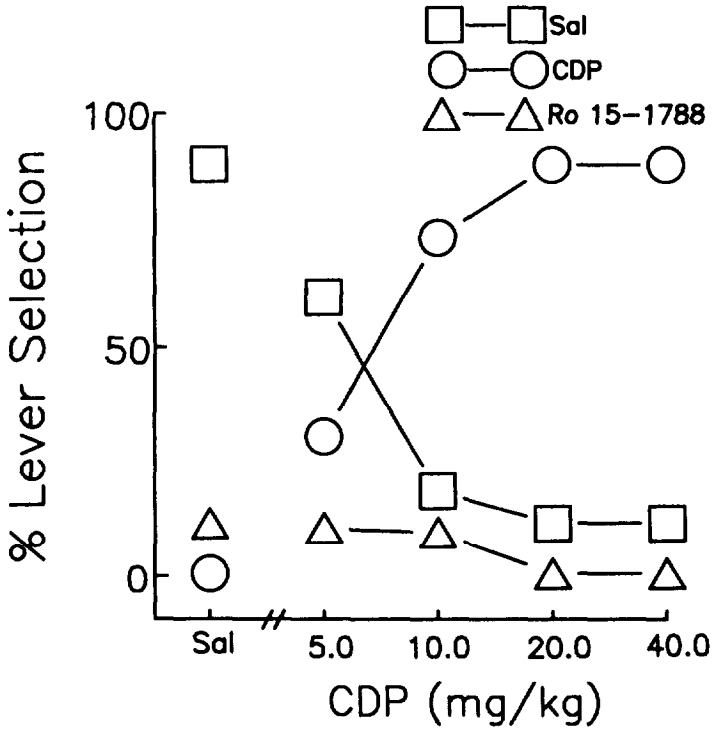

FIGURE 6. *Generalization of chlordiazepoxide in a three-choice discrimination. Dose-effect curve for chlordiazepoxide (CDP) was determined by testing independent doses in rats (n = 9) trained to detect flumazenil (Ro 15-1788 [RO], 2.5 mg/kg), chlordiazepoxide (CDP, 20 mg/kg), and saline in a three-lever choice task. Animals were maintained chronically on CDP, 100 mg/kg/day. On the ordinate, 100% lever selection indicates that all animals initially responded on the lever shown by the symbol to a fixed ratio of 10 lever presses. As percent selection of the CDP lever increased in a dose-dependent manner, saline-lever selection decreased. Little RO-lever selection was evident during any test.*

training purposes, the dose of the drug of dependence should be large enough to maximize the difference between these two stimuli.

A third reason for using large doses is based on experiments demonstrating tolerance to the stimulus effects of drugs. When subjects are trained to detect drugs such as morphine, cocaine, or midazolam, if the training drug is administered several times daily in doses significantly higher than the training dose, tolerance is seen. Typically the dose-effect curve for the detection of the training drug shifts two- to fourfold to the right. (For a review of this literature, see Young and Sannerud 1989.) This degree of tolerance is usually seen following periods of drug administration lasting up to 10 days, and we are unaware of any studies that have assessed whether a greater degree of tolerance develops if chronic administration is continued for the extended periods that drug-withdrawal studies entail.

This type of tolerance is likely to be a significant problem in training the drug of dependence. For example, when our subjects that are trained to detect the benzodiazepine midazolam are tested acutely with CDP, it substituted for midazolam with an ED_{50} of approximately 2.25 mg/kg. In contrast, when training on the discrimination was hatted and rats were injected with CDP, 20 mg/kg every 8 hr for 7 days, the ED_{50} for CDP substitution increased to approximately 5.0 mg/kg (unpublished observations). Thus, as little as 60 mg/kg/day of CDP can produce greater than twofold tolerance to the benzodiazepine training stimulus, and this finding suggests that in our drug withdrawal studies in which subjects receive CDP, 100 mg/kg/day, the degree of tolerance should be at least this profound. Similarly, when rats are used as subjects in a morphine-withdrawal discrimination, at least twofold tolerance is likely to develop to the stimulus effects of opioids if the dose of morphine used chronically to produce dependence is on the order of 15 mg/kg/day or greater (Emmett-Oglesby et al. 1988; Shannon and Holtzman 1976).

The combination of factors, including dosedose discriminations and tolerance, is likely to produce significant difficulties in learning a three-choice discrimination based on drug withdrawal. If the difference between vehicle (residual drug effects from chronic administration) and the training dose is to be distinguished, a training dose may be necessary that is at least twofold, and perhaps as much as fourfold, higher than that necessary for training in the absence of chronic drug administration.

Duration and Timing of Training

As described above, a critical test in a drug-withdrawal discrimination occurs when subjects are taken off the chronic regimen and allowed to remain drug

free for several days or weeks. When spontaneous withdrawal has subsided, tests with both the antagonist and its vehicle should produce vehicle-choice responding. This test has the disadvantage of being carried out after long periods during which animals are not trained on the discrimination, and, given the other problems with maintaining stimulus control in a three-choice discrimination, it seems likely that this particular test may be even more difficult to run than in two-choice discriminations.

One procedure that may help to minimize this difficulty is to overtrain the animals on the three-choice discrimination. Suppose a “learning” criterion is adopted such that animals must initially choose the correct lever before receiving a reinforcer for 9 of 10 consecutive sessions. Data from experiments with exteroceptive stimuli show that overtraining results in sharper generalization gradients and a longer duration of stimulus control (Mackintosh 1974). Since this latter feature is what is desired in a drug-withdrawal experiment, it seems likely that either setting a very stringent criterion or training for many sessions beyond the minimum criterion will result in sufficient stimulus control to test during prolonged periods when training is not possible.

How frequently should the drug of dependence be given chronically, and how long after administration should discrimination training occur? Data are not available to answer either question definitively, but the literature supports the following suggestions. Regarding frequency of dosing, the general observation in drug-dependence studies is that maximum dependence occurs when high concentrations of the drug are given frequently (e.g., Kalant et al. 1971). The limiting case appears to occur with constant drug infusion (for discrimination training this might be achieved with pumps or pellet implants), and there are reasons to believe that this would facilitate learning a three-choice discrimination. Among other points, a constant drug infusion would minimize the stimulus produced during chronic treatment with the drug of dependence (no peaks would occur in plasma drug concentration) and would prevent spontaneous withdrawal (no valleys would occur in plasma drug concentration). Although this approach suffers from not modeling human drug taking behavior, it is attractive because it provides stable baselines for both dependence and the training of discriminative stimuli.

If drug is absorbed constantly, the question of how long after chronic administration to wait before training becomes moot. If chronic drug is given intermittently, the answer to this question depends on the pharmacokinetics of the drug of dependence. At the time training occurs, enough of the drug should

be on the receptor that an antagonist will precipitate withdrawal, but not so much drug should be present that the vehicle condition is not sufficiently different from the drug of dependence condition. We suggest that it is perhaps better to err on the side of too much drug still present than on the side of too little. In the latter case, withdrawal will be difficult to train because the antagonist cannot precipitate as intense a stimulus, and because the antagonist stimulus may be difficult to differentiate from spontaneous withdrawal occurring under the vehicle condition.

CONCLUSIONS

Three-choice drug discriminations are conceptually interesting and offer more potential for studying withdrawal phenomena than their two-choice counterparts. Unfortunately, little information is available concerning fundamental parameters of three-choice learning. Moreover, this technology has been used only recently to study drug withdrawal. As more data accumulate from these experiments, it should be possible to specify optimal parameters for training the discrimination. More importantly, it should be possible to unequivocally determine whether withdrawal-related stimuli can be studied using drug discrimination methodology.

One caveat should perhaps be added. The withdrawal-related stimuli of most interest are probably those arising from spontaneous withdrawal, for it is this type of withdrawal that humans usually experience. Whether a three-choice discrimination can be extended to encompass training of spontaneous withdrawal is unknown, but such a discrimination would probably be even more difficult to establish than one using antagonists. On the other hand, in other tests spontaneous and precipitated withdrawal are usually seen to differ only in their magnitude and duration of signs and symptoms. Thus, it is likely that data acquired from studies of precipitated withdrawal will have direct application to human phenomena. In addition, it should be possible to test to what extent spontaneous withdrawal substitutes for the antagonist in these discriminations, which should also serve as a proof that similar events are being measured.

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Schedule-Induced Self-Injection of Drugs

George Singer

INTRODUCTION

In our laboratories we have combined a self-injection technique with a food delivery schedule and developed the method of schedule-induced self-injections (SISI). We have been able to classify drugs according to acquisition patterns and have shown that the stimulus complex necessary for self-injection to occur varies with the type of drug presented. The presence of a food delivery schedule and the state of nutrition interact with the drug in the acquisition and maintenance of drug-intake behaviors.

This model can be used in traditional pharmacological studies to investigate specific and general blockers of drug intake. Other studies have shown the importance of intact dopaminergic neurons in the nucleus accumbens septum for the acquisition and maintenance of drug intake behavior. Biochemical studies with the SISI model have shown that an increase in corticosteroid levels is associated with schedule-induced behaviors. The theoretical implications as well as implications for therapeutic programs are included in the following discussion.

REINFORCEMENT VERSUS PHYSICAL DEPENDENCE

Drug and alcohol research and treatment programs are usually based on one of two assumptions. The first is the psychological concept of “reinforcement” and its major role in the acquisition and maintenance of drug intake behavior, and the second is the pharmacological concept of physical dependence.

Those who subscribe to the notion of reinforcement believe that a drug is either initially reinforcing or acquires reinforcement value after repeated exposure. The facts that most naive organisms generally reject drugs initially because of

This paper is an update of a paper presented as the Fifty-first Beattie Smith Lecture in the Department of Psychiatry at the University of Melbourne in 1985.

aversive taste or aversive after-effects, and that, even in the later stages of drug intake, aversive after-effects remain are ignored. In addition, it is often postulated that drugs create a new “need state” and that the satisfaction of this need is reinforcing. Because this need state cannot be measured independently from the drug intake behavior, it is not a useful concept.

Those who subscribe to the notion of physical dependence regard physical dependence as synonymous with withdrawal and believe that organisms take drugs and become dependent on them in order to relieve the discomfort of withdrawal. Experimental evidence does not confirm this thesis and shows that relief of withdrawal is neither a necessary nor a sufficient condition for either the acquisition or the maintenance of drug intake.

This is not an attempt to deny that physiological changes occur in the central nervous system (CNS). Rather, it is the role of these changes as a major driving force to take drugs that is questioned. These remarks do not apply to drug discrimination and should be confined to theory and experimentation concerned with voluntary drug intake.

The purpose of this paper is to review recent research, including 10 years of drug research in our laboratories, in order to provide guidance for research and treatment programs.

METHOD

Using a combination of self-injection techniques and the scheduled delivery of food pellets to animals with reduced body weight (schedule-induced self-injection), we have produced a classification of psychoactive drugs based on behavioral factors shown in table 1.

The scheduled food delivery, in which one pellet is delivered every 60 sec to a chronically hungry animal, causes frustration and stress, which are reflected in increased plasma corticosterone levels. This is shown in table 2. These data suggest that the initiation of drug intake behavior is dependent on the interaction of the following three factors: (1) the presence of a drug molecule in the injection solution; (2) reduced body weight; and (3) the use of a food delivery schedule. In common parlance, these factors are drug, nutrition, and stress. These factors form a stimulus complex that is necessary for the initiation of drug intake behavior in animals. (The effect of the combination of factors, however, varies with the type of drug.)

TABLE 1. *A classification of drugs according to the acquisition of intake patterns*[a]

Group 1	Group 2	Group 3	Group 4
Alcohol Some opiates (e.g., heroin)	Amphetamine Cocaine Phentermine	Δ^9-THC[b] Nicotine Methadone Acetaldehyde Benzodiazepines Barbiturates	Haloperidol Saline
Self-injections occur without the schedule but are enhancedby reduced body weight and the schedule	Self-injections are greatly enhanced with reduced body weight	Self-injections occur in the presence of the schedule plus reduced body weight	Currently unsuccessful in inducing self-injection

[a]From Singer and Wallace 1984*a*.
[b]Delta-9-tetrahydrocannabinol.

TABLE 2. *Mean plasma corticosterone levels (µg/100 mL after schedule-induced) drinking behavior*[a]

	Mean Plasma Corticosterone Levels	
Day	Scheduled Animals	Nonscheduled Animals
2	274	134
10	265	111
20	270	125

[a]From Finlay and Wallace 1981.

In human drug intake behavior the variety and complexity of factors that interact are probably much greater, and the total stimulus complex is more difficult to specify. One observation from this research is that the interaction of reduced body weight and CNS stimulants, which are also anorectics, leads to increased

stimulant drug intake. This phenomenon has also been noted by other researchers (Papasava and Singer 1985; Papasava et al. 1981, 1985*a*, 1985*b*; Takahashi et al. 1978). Thus, when anorectic drugs are effective in producing weight loss, their addiction potential increases (Papasava et al. 1985*a*, 1985*b*). Cur research also shows that stress enhances the intake of most, but not all, drugs of abuse-a point sociologists have made for some time. In another series of experiments we have shown that the factors that are important in the initiation of drug intake are not always essential for its maintenance if a relatively stable pattern of intake has developed. Some of this work is shown in table 3.

Of particular relevance in the present context are the findings of an experiment conducted by Madden et al. 1979. In this experiment, it was shown that after 20 days of heroin self-injection, rats will self-inject saline at high rates, a behavior that does not normally occur. Moreover, it was shown that rats that were allowed to self-inject saline after self-injecting heroin had fewer withdrawal symptoms than rats that were withdrawn from heroin without the opportunity to self-inject saline. This finding supports earlier conclusions that drug dependence involves many factors and shows that this is also true for withdrawal symptoms.

Kalant (1982) has recently shown that the occurrence and degree of tolerance can be conditioned to the environment. However, this phenomenon, which has been called behaviorally conditioned tolerance, becomes less environment-dependent as the drug dose is increased.

In an elegant presentation at the conference Dr. Siegel has also argued that tolerance is dependent on the environment.

STIMULUS COMPLEX INTERACTION MODEL

Collectively, these data show that dependence, withdrawal, and tolerance result from the interaction between environment and pharmacological factors and that all these phenomena can be explained in terms of a stimulus complex interaction model. The hypothesis on which this model is based raises a number of interesting questions about treatment programs. First and foremost is the efficacy of combining pharmacological and psychological therapies. Although the use of Antabuse and methadone in conjunction with environmental or psychological treatments is an application of this principle, this combination is rare in regard to drug and alcohol treatment programs. The

TABLE 3. *Maintenance patterns of drug self-injection*[a]

Drug	Intake and Response
Schedule removal	
Heroin	Intake drops
Nicotine	After 5 days of schedule, intake drops. After 14 days, intake maintained. Will initiate intake without schedule if body weight is 80%, but acquisition is very slow (>22 days).
Cocaine	Intake high if body weight low
Alcohol	Intake drops
Body weight restitution	
Nicotine	Intake maintained if recovery period occurs with nicotine intake under SISI.[b] Intake maintained if only saline available during recovery.
Cocaine	Intake drops immediately
Δ^9-THC[c]	Intake drops
Substitution of saline (vehicle) for drug	
Heroin	Responding maintained
Antagonism of drug effect	
Heroin and naloxone pretreatment	Responding for drug drops to saline levels

[a]See review by Singer and Wallace 1984*a* for individual references.
[b]Schedule-induced self injection.
[c]Delta-Stetrahydrocannabinol.

question is whether it is better to totally change the stimulus complex in treatment procedures or to remove one component of the stimulus complex and keep everything else constant.

For example, a total change in the stimulus complex occurred when drug-addicted Vietnam War veterans were discharged and returned to their home towns. Everything changed, including friends, job, home conditions, and food and reports indicate that most of these veterans were able to withdraw from drugs (Jaffe 1980).

The alternative to a total environment change is removing the drug gradually. We have observed outpatients on a methadone maintenance program in which all features of the stimulus complex were maintained except that methadone was substituted for heroin. This procedure was based on Madden's (1983) laboratory work and has shown some early promise in a clinical setting. However, further clinical research is needed to clarify these findings. Table 4 shows this experimental paradigm.

The experiments that follow outline some of the research that was designed to specify more precisely the relationship between environment, drug intake behavior, neurochemical substrates, and neuroanatomical loci. First, we conducted traditional pharmacological experiments using the schedule-induced self-injection procedure. These experiments were directed toward elucidating the neurochemical substrates that mediate the intake of specific drugs. For instance, phentermine has both dopaminergic and noradrenergic effects. The question is whether one or both of them are involved in phentermine intake. The method used was to let animals self-inject the drug until a stable baseline was established. When this occurred, animals were injected with various blocking agents known to interfere with neurotransmitter function. It should be noted that conditions that control for the effect of activity changes due to treatment compounds are essential in these experiments.

Table 5 shows the data from a range of pharmacological blocking studies. It can be seen that while phentermine has both noradrenergic and dopaminergic effects, the dopaminergic effects maintain self-injection. An example is shown in figure 1.

A further step in this research is to identify specific neuroanatomical loci involved in self-injection. This has been achieved by introducing neurotoxins directly into specific brain areas.

Table 1 indicated that nicotine self-injection is contingent on the presence of the schedule. Furthermore, the schedule increases the level of plasma corticosterone. When dopaminergic neurons in the nucleus accumbens septum

TABLE 4. *Experimental paradigm*

Phase 1	Phase 2	Results
Methadone is given once daily in an orange juice vehicle to heroin-free outpatients.	Orange juice administration is continued without methadone.	Patients are unable to estimate methadone content of orange juice and show few withdrawal symptoms.

TABLE 5. *Traditional pharmacological experiments*[a]

Drug Self-Injected	Pretreatment	Effect
Phentermine	Alpha blockers	None
	Beta blockers	Stops self-injection
	Haloperidol (dopamine blocker)	
Alcohol	Naloxone	Stops self-injection
	Buprenorphine	
Benzodiazapines	Naloxone	None
	GABA blockers	Stops self-inection
	Benzodiazapine blocker	Stops self-injection

[a]See review by Singer and Wallace 1984*a*.

are depleted with the neurotoxin 6-hydroxydopamine, much nicotine self-injection behavior is abolished (Singer et al. 1982), as are schedule-induced increases in plasma corticosteroids (Wallace et al. 1983) (See table 6).

Similar findings occur in the case of heroin self-injection (Singer and Wallace 1984*b*), which is also enhanced by the stress-producing schedule. These examples show that both the stress factor and drug intake are regulated by dopamine neurons and suggest that stress management, which leads to a reduction in cortisol levels, could be a promising component in drug treatment programs for some drugs, e.g., nicotine, alcohol, and opiates.

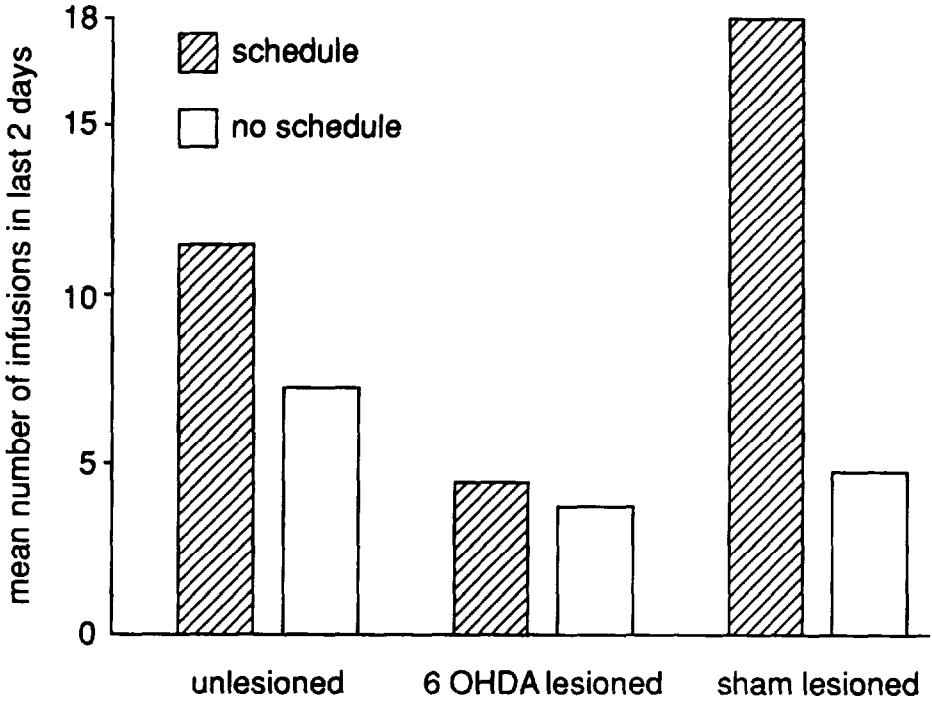

FIGURE 1.

TABLE 6. *Mean plasma corticosterone levels for 6-OHDA-lesioned and sham-lesioned rats*[a]

Lesion	Schedule	No Schedule
6-OHDA	12.34	19.19
	(1.99)	(3.64)
Sham	26.55	13.59
	(2.37)	(2.25)

[a]Measured after 10 daily 1-hr sessions of scheduled food or food delivered in one presentation. Corticosterone levels are µg/100 mL (±SEM).

CONCLUSION

The acquisition of drug self-administration is related to nutritional state and also to neuroendocrine factors. These variables interact with different drug molecules in different ways. Some of these environmental factors have been neglected by those working in the field of drug discrimination. We have identified an involvement of corticosteroids and dopaminergic neurons in the nucleus accumbens septum. Some of our findings should be useful in the interpretation of data and in the design of experiments in drug discrimination.

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Use of Drug Discrimination in Drug Abuse Research

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INTRODUCTION

Because drug discrimination (DD) requires that organisms attend to the effects of drugs to obtain reinforcement (contingent on the occurrence of an appropriate response), it has been a reliable, sensitive, and, most important, pharmacologically specific assay (Appel et al. 1982; Cunningham and Appel 1988). This quality has enabled investigators to classify and differentiate closely related compounds (Barry 1974) and gather important information concerning their mechanisms of action in vivo (Appel et al. 1978). The assay has been especially useful in the study of abused drugs because the subjective effects of most, if not all, such compounds are characterized by dramatic changes in state (e.g., euphoria, dysphoria) that are readily discriminated by both humans and other animals; indeed, drugs may well be abused because of the speed and potency with which they cause such changes to occur.

Like previous international symposia, this meeting has been concerned with important basic issues such as the mechanisms by which complex drug cues are transduced (Philips 1990; Stolerman 1990), the laws governing the development of tolerance to drug cues (Siegel 1990; Young 1990), and the relationship between DD and related state-dependent phenomena (Overlon 1990). A relatively new focus was on how DD, which has involved primarily animal research, can be used to further our understanding of important clinical aspects of substance abuse in humans; in this regard, the papers on cocaine

craving (Ehrman 1990) and withdrawal from benzodiazepines and opiates (Emmett-Oglesby 1990) were of particular interest.

However, it is impossible to comment more than superficially on all the uses of DD reviewed in the past 2 days. Therefore, we will try to amplify three of them-differentiation of neuronal mechanisms, receptor interactions, and stereospecificity of discriminative stimulus effects-with data involving hallucinogens, stimulants, and related compounds gathered over many years in our laboratory.

NEURONAL MECHANISMS

DD has contributed to our understanding of the mechanisms subserving the effects of (±) 2,5-dimethoxy-4-methylamphetamine (DOM), (+) lysergic acid diethylamide (LSD), and related indoleamine and phenylethylamine hallucinogens in a variety of ways. For example, Glennon (1990) reports that, by training rats to discriminate DOM from saline, it has been possible to (1) classify which agents produce DOM-like effects, (2) study the actions of DOM metabolites, (3) formulate in vivo structure-activity relationships, (4) demonstrate a correlation between stimulus generalization potencies and human hallucinogenic potencies, and (5) investigate mechanisms of action.

We began using DD in the early 1970s to analyze the involvement of serotonin (5HT) in the behavioral effects of LSD in animals, in part because other assays (animal models) then in use had been yielding interesting but variable results that often depended more on species, level of motivation or incentive, schedule of reinforcement, and baseline response rate than on the particular dose of the drug being given in a particular test situation (Appel 1968).

At that time, it was generally held that most, if not all, hallucinogens act presynaptically by directly suppressing the characteristically slow, regular activity (“firing rate”) of an inhibitory 5-HT system, the cell bodies of which are located in the dorsal raphe (Aghajanian et al. 1968). However, evidence was beginning to accumulate in our own (Appel et al. 1970) and other laboratories (Trulson et al. 1976, 1981) that was at variance with this hypothesis and suggested that hallucinogens act postsynaptically by both inhibiting and exciting 5-HT target neurons (Jacobs 1983). A somewhat later and particularly compelling result was that low doses (0.005 mg/kg) of lisuride hydrogen maleate (LHM), a structural congener of LSD that is not known to be hallucinogenic in humans, was found to be even more potent than LSD in

suppressing raphe unit activity (White 1986). It thus became important to differentiate the subjective effects of LSD and LHM in animals, a task that proved to be difficult but possible with DD, in conjunction with extensive antagonism testing and selective drug vs. drug training procedures (White and Appel 1982*a*, 1982*b*, 1982*c*) and impossible in less sensitive assays such as the limb flick (White et al. 1981).

Recently, we used an even more discriminating, three-lever drug vs. drug vs. saline procedure similar to those described by Bigelow (1990) and Emmett-Oglesby (1990) to reexamine the stimulus effects of LSD (0.08 mg/kg) and LHM (0.04 mg/kg); we were again able to show that, in spite of their structural similarities, these compounds are functionally quite different and, more important, have different mechanisms of action.

Figure 1 shows that rats can discriminate quantitatively similar doses of LSD and LHM with little confusion (Callahan and Appel 1990). That is, when given substitution (dose-response) tests with LSD (0.02-0.16 mg/kg), animals respond in a dose-related manner on either the LSD- or the saline-appropriate lever; they never respond on the LHM lever (left panel). Similar effects occur when the same animals are tested with LHM (0.005-0.08 mg/kg); that is, responding occurs on either the LHM- or saline-appropriate lever but not on the LSD lever (right panel).

One example of how the mechanisms underlying the effects of LSD and LHM differ is suggested by the results of a generalization experiment (figure 2); when tested with the dopamine (DA) agonist apomorphine, animals responded on either the LHM- or the saline-appropriate lever (left panel); however, when given the 5-HT_2 agonist quipazine, they responded on the LSD-appropriate lever (middle panel) and, following the convulsant pentylenetetrazol (which does not act primarily through either DA or 5-HT systems) they pressed the saline-appropriate lever (right panel). Thus, LHM appears to be considerably more dopaminergic than LSD (White 1986), a result also seen during extensive combination testing with DA and 5-HT antagonists (White et al. 1982*b*, 1982*c*).

Indeed, we have never been convinced that DA plays a significant role in the stimulus effects of LSD. Although it is true that apomotphine sometimes generalizes at least partially to LSD (in two-lever, apomorphine-vs.-saline discriminations), we have never found that apomotphine substitutes for LSD in three-lever (LSD-LHM-saline) discriminations or that the LSD cue generalizes more than 40 percent to apomorphine in animals trained to discriminate LSD

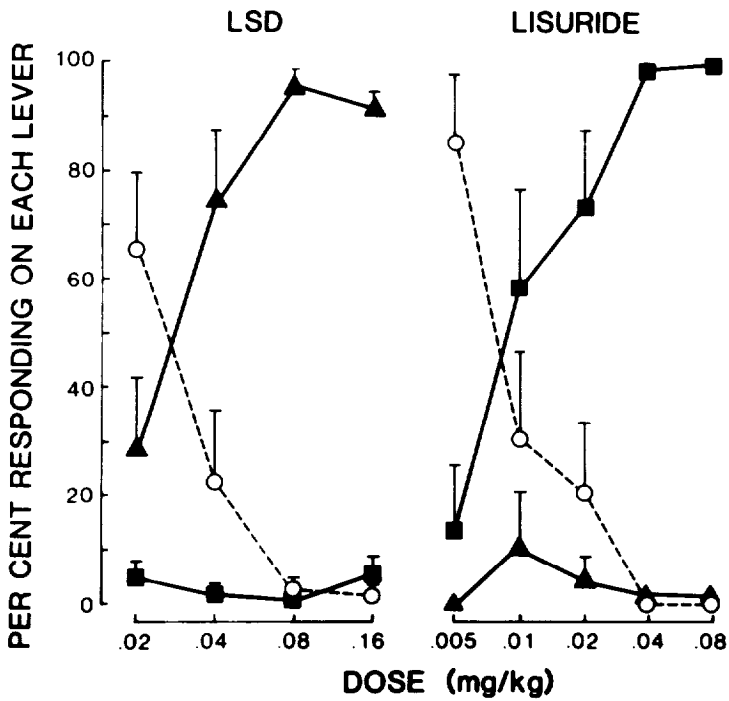

FIGURE 1. *Results of dose-response tests with LSD (left panel) and lisuride (LHM; right panel) in rats (N = 9) trained to discriminate LSD (0.08 mg/kg) from LHM (0.04 mg/kg) from vehicle (saline). Solid lines with closed triangles denote percent responding on the LSD-appropriate lever; solid lines with closed squares denote responding on the LHM-appropriate lever; broken lines with open circles denote responding on the saline-appropriate lever. All points are means ± SEM of eight or nine subjects that completed at least 20 responses on any one of the three levers. (Reprinted from Callahan and Appel (1990), with permission.)*

from saline; moreover, this small amount of “generalization” is unrelated to dose (Appel et al. 1978). More important, in experiments currently in progress, the effects of other DA agonists including (+)amphetamine, cocaine, quinpirole (LY 171555) and SCH 2390 do not appear to resemble those of LSD. In addition, no DA antagonist yet tested in combination with hallucinogens blocks the effects of either LSD or mescaline (table 1).

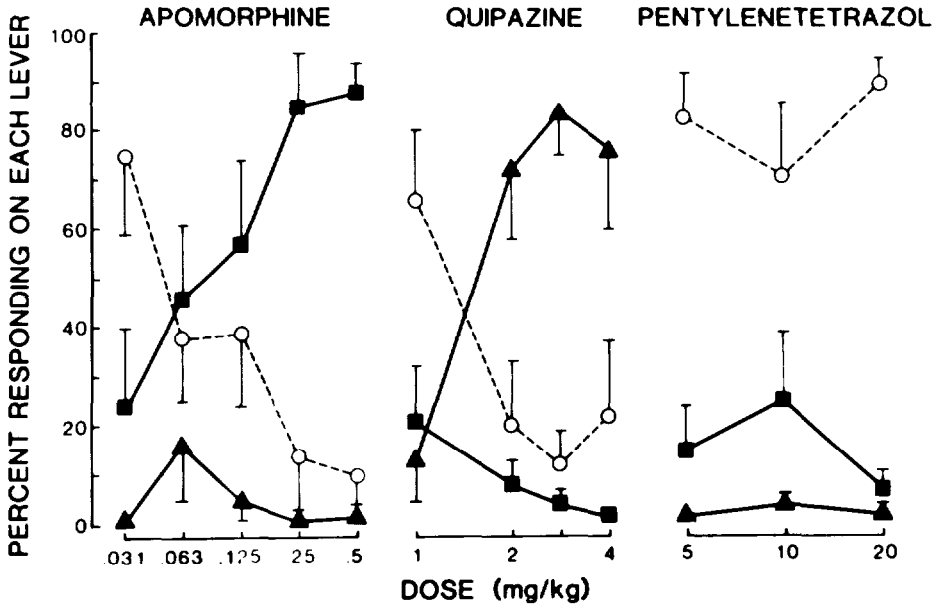

FIGURE 2. *Results of substitution (generalization) tests with apomorphine (left panel), quipazine (center panel), and pentylenetetrazol (right panel) in rats trained to discriminate LSD (0.08 mg/kg) from LHM (0.04 mg/kg) from saline. Symbols as in figure 1. (Reprinted from Callahan and Appel (1990), with permission.)*

RECEPTOR INTERACTIONS

DD has also provided evidence that the same receptors and receptor subtypes that mediate other physiological and biochemical effects of abused substances are involved in their subjective properties and, hence, their abuse potential. This point seems to be true with central nervous system (CNS) stimulants (Goudie 1990; Woolverton 1990) and other DA agonists (Appel et al. 1988) cannabinoids (Järbe 1990), nicotine (Rosecrans and Villanueva 1990) sedative-hypnotics (Barry 1990), opiates (Woods et al. 1990), and phencyclidine-like agents (Balster 1990). The same point has been particularly important in the case of hallucinogens, because the effects of these substances have been so difficult to categorize with other procedures (Appel 1988).

TABLE 1. *Results of combination (antagonism) tests in animals trained to discriminate LSD (L) or mescaline (M) from saline*

Training Drugs(s)	Putative Antagonist	Reference
Complete Antagonism (≤ 40%):		
(L) (M)	BC-105	Colpaert et al. (1982) Cunningham and Appel (1987) Holohean et al. (1982) Nielsen et al. (1985) Winter (1978)
(L)	Bromo-LSD	Cunningham and Appel (1987)
(M)	Cinanserin	Browne (1978) Browne and Ho (1975) Winter (1975, 1978)
(L) (M)	Cyproheptadine	Browne (1978) Browne and Ho (1975) Kuhn et al. (1978) White and Appel (1982*c*) Appel and Callahan (1989)
(L) (M)	Ketanserin	Cunningham and Appel (1987) Nielsen et al. (1985) Appel and Callahan (1989)
(L) (W)	LY-53857	Cunningham and Appel (1987) Appel and Callahan (1989)
(L) (M)	Metergoline	Cunningham and Appel (1987)
(L)	Methiothepin	Kuhn et al. (1978)
(L)	Methysergide	Kuhn et al. (1978)
(L) (M)	Pirenpirone	Colpaert et al. (1982) Appel and Callahan (1989) Cunningham and Appel (1987) Nielsen et al. (1985)
(L)	Ritanserin	Colpaert et al. (1985)
(L)	Trazodone	Cunningham and Appel (1987)

TABLE 1. *Continued*

Training Drugs(s)	Putative Antagonist	Reference
Partial Antagonism (41-79%):		
(L)	Bromo-LSD	Colpaert et al. (1982) Kuhn et al. (1978)
(L)	Cinanserin	Colpaert at al. (1982) Kuhn et al. (1978)
(L)	Cyproheptadine	Colpaert et al. (1982) Kuhn et al. (1978)
(L) (M)	Metergoline	Browne (1978) Colpaert et al. (1982)
(L) (M)	Methysergide	Browne and Ho (1975) Colpaert et al. (1982) Hirschhorn and Rosecrans (1974)
(L)	Metitepine	Colpaert et at. (1982)
(L)	Methiothepin	White and Appel (1982c)
(L)	Mianserin	Colpaert et al. (1982)
(L)	Naloxone	Hirschhorn and Rosecrans (1974)
No Antagonism (≳ 80%):		
(L) (M)	Atropine	Browne and Ho (1975) Hirschhorn and Rosecrans (1974) Kuhn et al. (1978)
(L)	(+) Butaclamol	Kuhn et al. (1978)
(L)	Chlorimipramine	Kuhn et al. (1978)
(L)	Cyprcheptadine	Hirschhorn and Rosecrans (1974)
(L)	Fluoxetine	Kuhn et al. (1978)
(L)	Fluphenazine	Kuhn et al. (1978)
(L)	(Alpha)-Fluphenthixol	Kuhn et al. (1978) Appel and Callahan (1989)

TABLE 1. *Continued*

Training Drugs(s)	Putative Antagonist	Reference
(L) (M)	Haloperidol	Colpaert et al. (1982) Holohean et al. (1982) Kuhn et al. (1978) Nielsen et al. (1985) White and Appel (1982*c*)
(L)	8-OHDPAT	Cunningham and Appel (1987)
(L)	Naloxone	Colpaert et al. (1982)
(L)	Phenoxybenzamine	Colpaert et al. (1982)
(L) (W)	Phentolamine	Browne and Ho (1975) Kuhn et al. (1978)
(M)	Pimozide	Browne and Ho (1975)
(L)	Promethazine	Kuhn et al. (1978)
(M) (L)	Propranolol	Browne and Ho (1975) Colpaert et al. (1982) Kuhn et al. (1978)
(L)	Pyrilamine	Colpaert et al. (1982)
(L)	R-56413	Colpaert et al. (1985)
(M)	Sch-23390	Appel and Callahan (1989)
(L)	Spiperone	Colpaert et al. (1982) Cunningham and Appel (1987)
(L)	Trifluoperazine	Kuhn et al. (1978)
(L)	L-tryptophan	Kuhn et al. (1978) Appel and Callahan (1989)
(L) (M)	Xylamidine	Browne (1978) Browne and Ho (1975) White and Appel (1982*c*)

Hallucinogens and Serotonin

Although they may not be as convincing as the correlations with receptor binding reported by Glennon (1990) and are certainly incomplete (because the catalog of 5-HT receptor subtypes never seems to stop growing), results from our laboratory suggest that the stimulus effects of both LSD (Cunningham and Appel 1987) and mescaline (Appel and Callahan 1989) are, like those of DOM, mediated primarily, if not exclusively, by 5-HT_2 receptors. For example, in one series of experiments, the effects of LSD were compared systematically to those of 5-HT_1 agonists.

Figure 3 shows that the stimulus effects of 0.08 mg/kg of LSD generalize to the 5-HT_2 agonist quipazine as well as a sufficiently high dose of LSD but not to compounds that act primarily at 5-HT_{1A} or 5-HT_{1B} receptor sites-namely, 8-hydroxy-2(di-n-propylamino)tetralin (8-OHDPAT) and 1-(m-triiluoromethylphenyl)piperazine (TFMPP), respectively (Cunningham and Appel 1988). Moreover, LSD does not substitute either for 8-OHDPAT (Cunningham et al. 1987) in animals trained to discriminate 0.4 mg/kg of this 5-HT_{1A} agonist from saline (figure 4), or TFMPP (Cunningham and Appel 1986) in animals trained to discriminate 0.8 mg/kg of this 5-HT_{1B} agonist from saline (figure 5).

Similarly, figures 6 and 7 show that the effects of mescaline generalize to other hallucinogens (Callahan and Appel 1988) and, like those of LSD, are blocked potently and completely by centrally acting, nonselective 5-HT and selective 5-HT_2 antagonists (Appel and Callahan 1989) but not by either peripheral 5-HT or DA antagonists (table 1).

Amphetamine, Cocaine, and Dopamine

Although DD has been less successful in delineating the mechanisms underlying the subjective effects of CNS stimulants than those of hallucinogens and other 5-HT receptor agonists, the papers of Goudie (1990) and Woolverton (1990) indicate that this situation is changing rapidly. Studies involving this assay are beginning to suggest, as we have argued elsewhere (Broadbent and Appel 1990), that all stimulants may not be as similar as hitherto supposed and that (+) amphetamine and cocaine may act differently at DA (Goudie 1990) as well as other (5-HT?) receptor sites (Broadbent and Appel 1990).

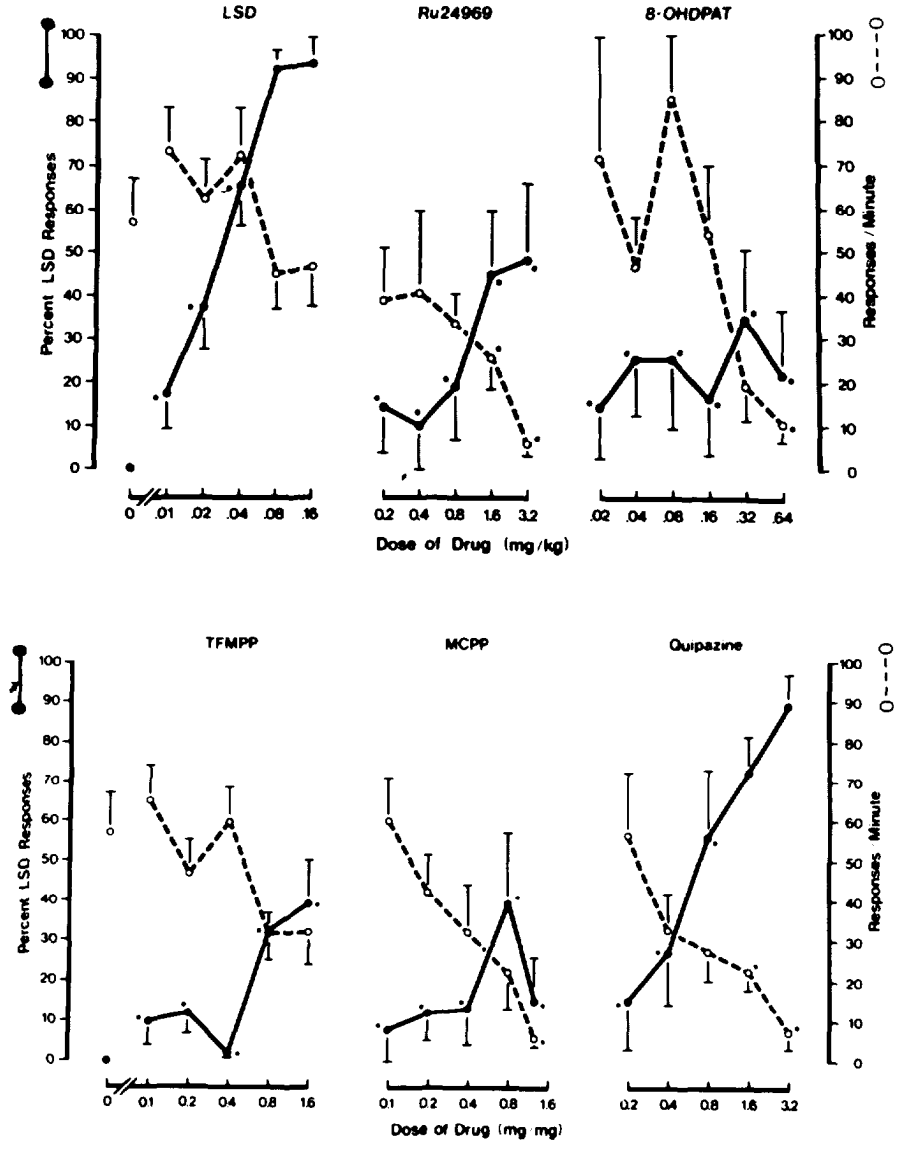

FIGURE 3. *Results of dose-response and substitution tests with putatively selective 5-HT agonists in rats trained to discriminate LSD (0.08 mg/kg) from saline. Dots = performances during test sessions that were significantly different from previous LSD training session. (Reprinted from Cunningham and Appel (1987), with permission.)*

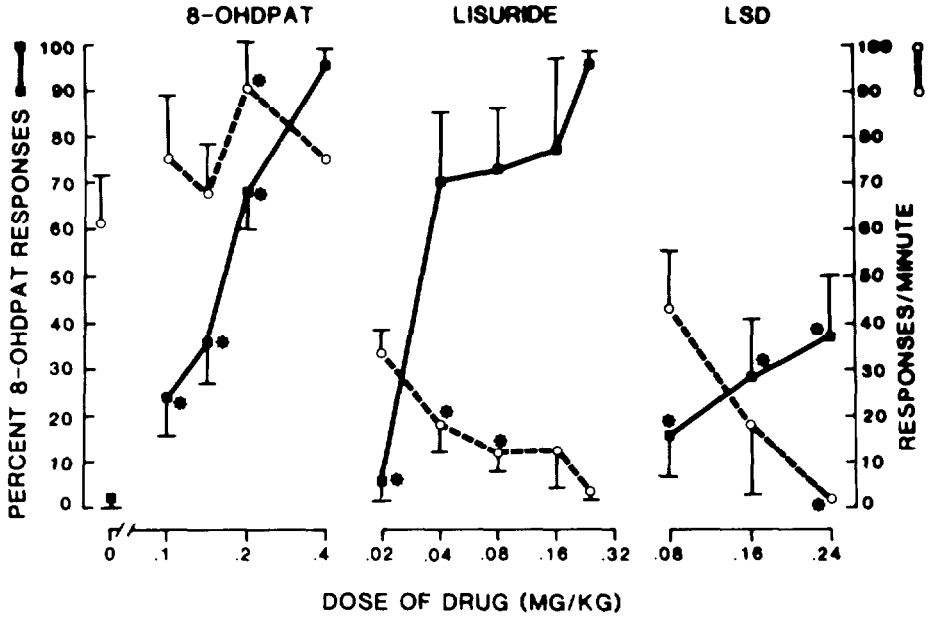

FIGURE 4. *Results of dose-response and substitution tests with the ergot derivatives lisuride and LSD in animals trained to discriminate 8-OHDPAT (0.4 mg/kg) from saline. * = performances during test sessions that were significantly different from the previous 8-OHDPAT training session. (Reprinted from Cunningham et al. (1987), with permission.)*

Our own work with (+) amphetamine has not been extensive. We showed some time ago that, in amphetamine-trained animals, the effects of this CNS stimulant are not at all like those of LSD and other hallucinogens (Kuhn et al. 1974) and, more recently, replicated this result in rats trained to discriminate 0.08 mg/kg of LSD from saline (above). We have also found, in agreement with Goudie (1990) that D_1 and, especially, D_2 receptors are involved in the behaviorally relevant actions of (+) amphetamine (Callahan et al. 1991; Kuhn et al. 1974). In addition, and in response to Goudie, we have seen no evidence that the stimulus effects of (+) amphetamine are altered significantly by compounds that act (either presynaptically or postsynaptically) at noradrenergic (NE) receptor sites.

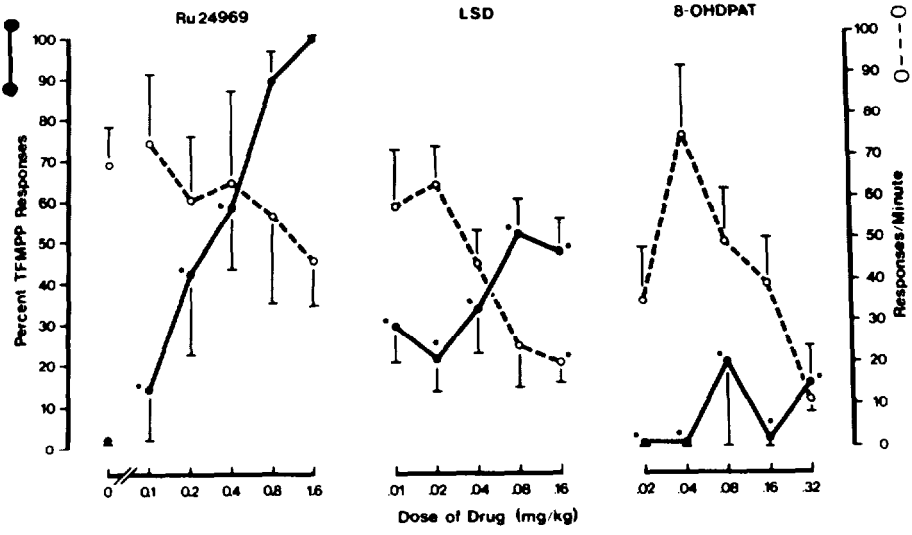

FIGURE 5. *Results of dose-response and substitution tests with Ru 24969, LSD, and 8-OHDPAT in rats trained to discriminate 1-(m-trifluoromethylphenyl) piperazine (TFMPP) from saline. Dots = performances during test sessions that were significantly different from previous TFMPP training session. (Reprinted from Cunningham et al. (1986), with permission.)*

We have been more interested in the mechanisms underlying the stimulus effects of cocaine and, like Woolverton (1990), have found that they involve DA (especially DA uptake inhibition) in important and complex ways. Although some directly acting DA agonists, particularly those that act at D_2 receptors (LY 171555), appear to substitute at least partially for cocaine (Barrett and Appel 1989*a*; Callahan et al. 1991) other clinically effective dopaminergic compounds, including bromocriptine and amantadine, apparently do not (table 2).

Unfortunately, we have had great difficulty in blocking- the stimulus effects of cocaine with both D_1 and D_2 antagonists (figure 8), although more potent, nonselective DA antagonists such as cis-flupenthixol may be effective in this regard.

As Woolverton (1990) points out, the stimulus effects of cocaine generalize readily to DA uptake inhibitors such as bupropion, nomifensine, and GBR

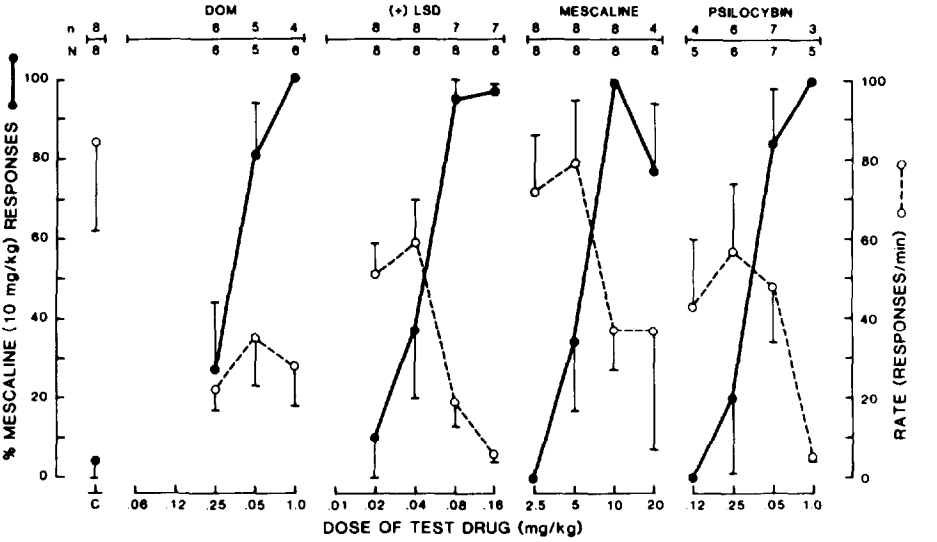

FIGURE 6. *Results of dose-response and substitution tests with other hallucinogens in rats trained to discriminate mescaline (10 mg/kg) from saline. Compounds tested, number of animals responding (n), and number of animals tested (N) at each dose are shown at top of figure. (Reprinted from Callahan and Appel (1988), with permission.)*

12909 (Broadbent et al. 1989*b*); we have also found that this generalization can be blocked at least partially by cis-flupenthixol.

Further evidence that DA mediates the subjective effects of cocaine is provided by results such as those illustrated in figure 9; in this experiment, the ability of three DA agents to substitute for cocaine was potentiated significantly by a subthreshold (nondiscriminable) dose of cocaine, which nevertheless inhibits DA uptake (Barrett and Appel 1989*b*).

STEREOSPECIFICITY: DIFFERENTIATION OF THE EFFECTS OF (+) AND (-) MDA

DD has provided important information about designer drugs such as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), although the mechanisms

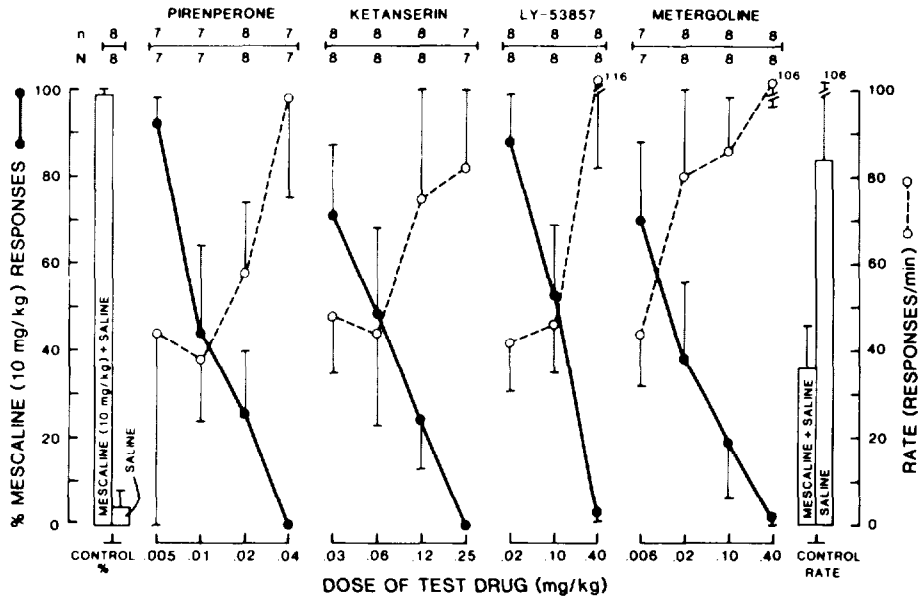

FIGURE 7. *Results of tests with 5-HT_2 antagonists given 60 min prior to experimental sessions to rats treated with mescaline (10 mg/kg) 15 min prior to testing. Compounds tested, number of animals responding (n), and number of animals tested (N) at each dose are shown at top of figure. (Reprinted from Appel and Callahan (1989), with permission.)*

mediating the effects of these abused neurotoxins (Insel et al. 1989; Johnson et al. 1986; Schmidt 1987) continue to evade precise delineation. For example, the optical isomers of MDA have been reported to have different, stimulant-like or hallucinogen-like effects on the basis of findings that (-) MDA mimics DOM but not (+) amphetamine, while (+) MDA mimics amphetamine but not DOM (Glennon and Young 1984; Glennon et al. 1982).

We have been able to partially replicate and extend these results by training animals to discriminate equivalent intraperitoneal doses (1.25 mg/kg) of the enantiomers of MDA from saline (Appel et al. 1990). Figure 10 shows that the stimulus effects of the (-) isomer generalize completely to those of LSD as well as DOM, partially to mescaline, and not at all to stimulants such as (+)amphetamine and cocaine.

TABLE 2. *Drugs that do not substitute completely for cocaine (10 mg/kg, IP)*

Drug	Dose (mg/kg)	Percent (±SEM)	Rate (±SEM)	N
Dopaminergic agents				
Amantadine (60 min)				
NaCl	—	3 ± 2	51 ± 11	9
Cocaine	10.0	100 ± 28	28 ± 8	9
Amantadine	12.5	33 ± 15	38 ± 9	9
	25.0	31 ± 14	19 ± 8	8
	37.5	23 ± 17	5 ± 2	5
Bromocriptine (30 min)				
NaCl	—	2 ± 1	84 ± 6	9
Cocaine	10.0	99 ± 1	28 ± 8	9
Bromocriptine	2.5	11 ± 6	48 ± 9	9
	5.0	8 ± 7	46 ± 7	9
	7.5	21 ± 14	36 ± 10	7
NE and 5-HT uptake inhibitors				
Desipramine (15 min)				
NaCl	—	3 ± 1	67 ± 12	19
Cocaine	10.0	99 ± 1	30 ± 6	19
Desipramine	2.5	52 ± 10	22 ± 5	19
	5.0	38 ± 9	12 ± 3	19
	7.5	55 ± 13	5 ± 2	12
Desipramine (30 min)				
NaCl	—	3 ± 1	78 ± 11	10
Cocaine	10.0	99 ± 1	28 ± 8	10
Desipramine	5.0	13 ± 8	12 ± 4	10
	7.5	30 ± 10	11 ± 4	10
	10.0	21 ± 16	34 ± 22	6
Imipramine (30 min)				
NaCl	—	5 ± 1	64 ± 8	13
Cocaine	10.0	100 ± 0	32 ± 8	13
Imipramine	5.0	24 ± 10	39 ± 13	13
	10.0	12 ± 6	16 ± 4	12
	15.0	10 ± 5	15 ± 6	7
	20.0	26 ± 20	9 ± 4	4

TABLE 2. *Continued*

Drug	Dose (mg/kg)	Percent (±SEM)	Rate (±SEM)	N
Nisoxetine (45 min)				
NaCl	-	10 ± 8	46 ± 6	12
Cocaine	10.0	100 ± 0	32 ± 9	12
Nisoxetine	4.5	9 ± 7	35 ± 7	12
	9.0	12 ± 8	14 ± 2	12
	13.5	19 ± 8	23 ± 14	12
	18.0	44 ± 14	20 ± 5	8

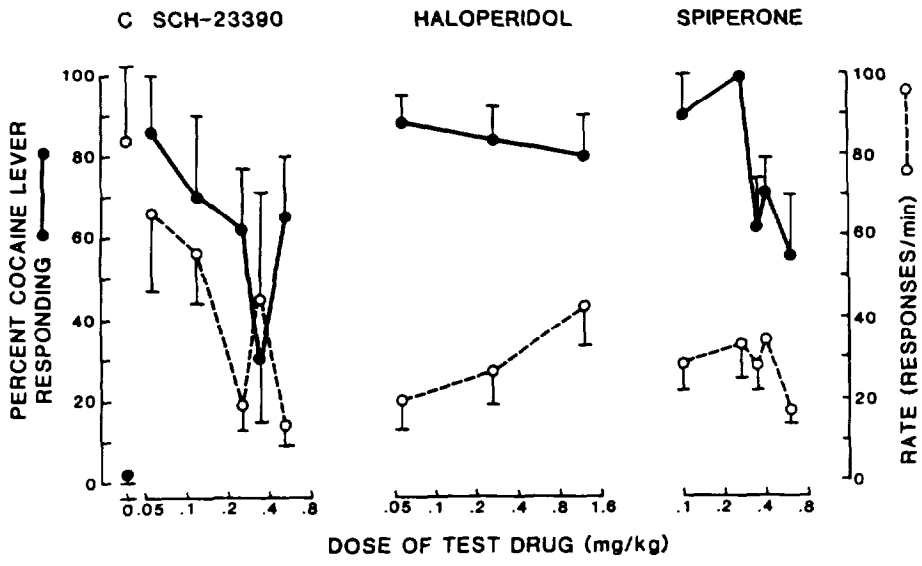

FIGURE 8. *Results of combination tests with three DA antagonists in animals trained to discriminate cocaine (10 mg/kg) from saline. (Reprinted from Barrett and Appel (1989a), with permission.)*

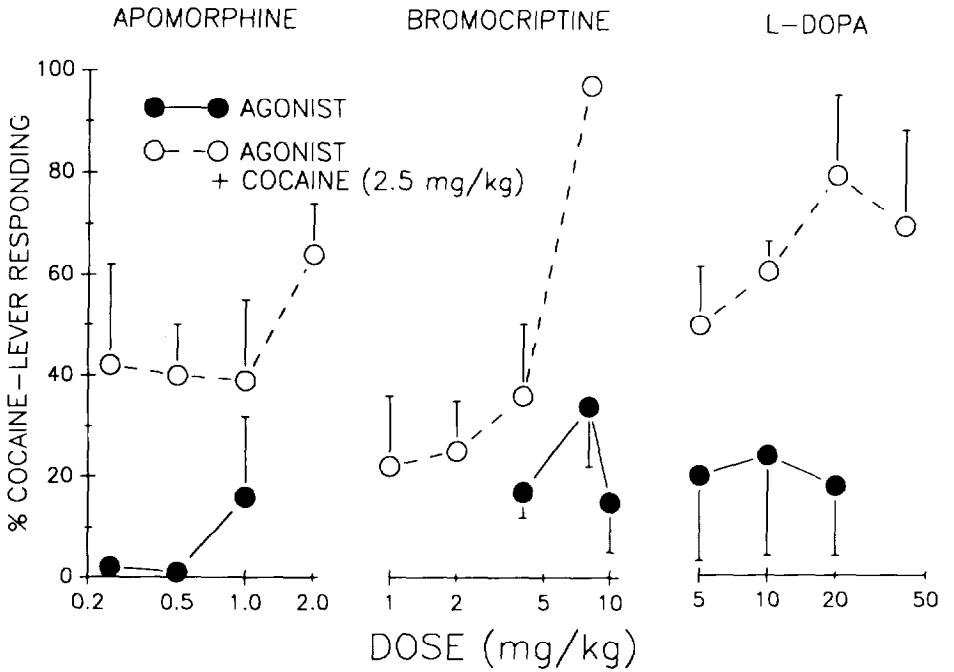

FIGURE 9. *Potentiation of the cocaine-like effects of apomorphine, bromocriptine, and L-DOPA by a low dose of cocaine (2.5 mg/kg).*

The discriminative stimulus properties of the (+) isomer of MDA are less clear than those of the (-) isomer in that they generalize completely to LSD and, surprisingly, cocaine and only generalize partially to DOM, mescaline, and (+)amphetamine (Fig. 11).

Moreover, the (-) MDA, but not the (+) MDA cue can be blocked by 5-HT_2 antagonists such as pirenpirone, suggesting that, at least at the dose tested (1.25 mg/kg), the (-) isomer is more serotonergic as well as LSD-like than the (+) isomer (Appel et al. 1990).

CONCLUSIONS

Historically, the most important contribution DD has made to drug abuse research (as well as to other aspects of psychopharmacology) has been in the area of behavioral pharmacodynamics (Appel et al. 1978), which is concerned

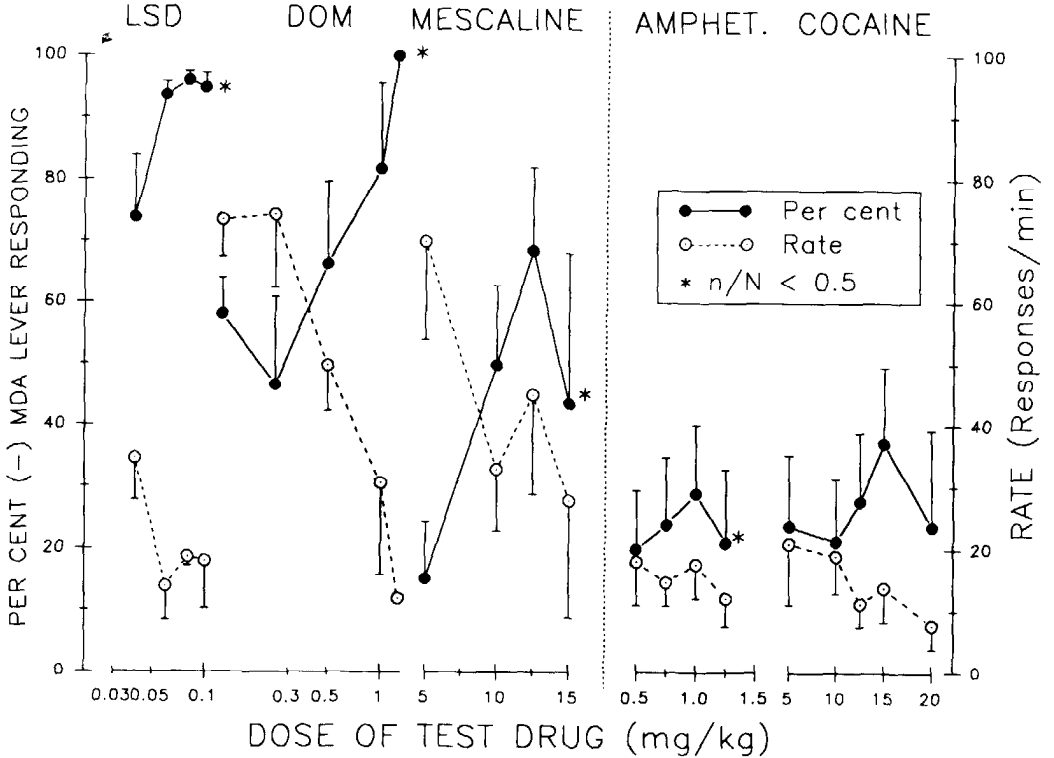

FIGURE 10 *Results of substitution tests with hallucinogens (LSD, DOM, mescaline) and stimulants ((+) amphetamine, cocaine) in rats trained to discriminate (-) MDA (1.25 mg/kg) from saline. n/N < 0.5 means that ratio of number of animals responding (n) to the number of animals tested (N) was less than 0.5 (50%).*

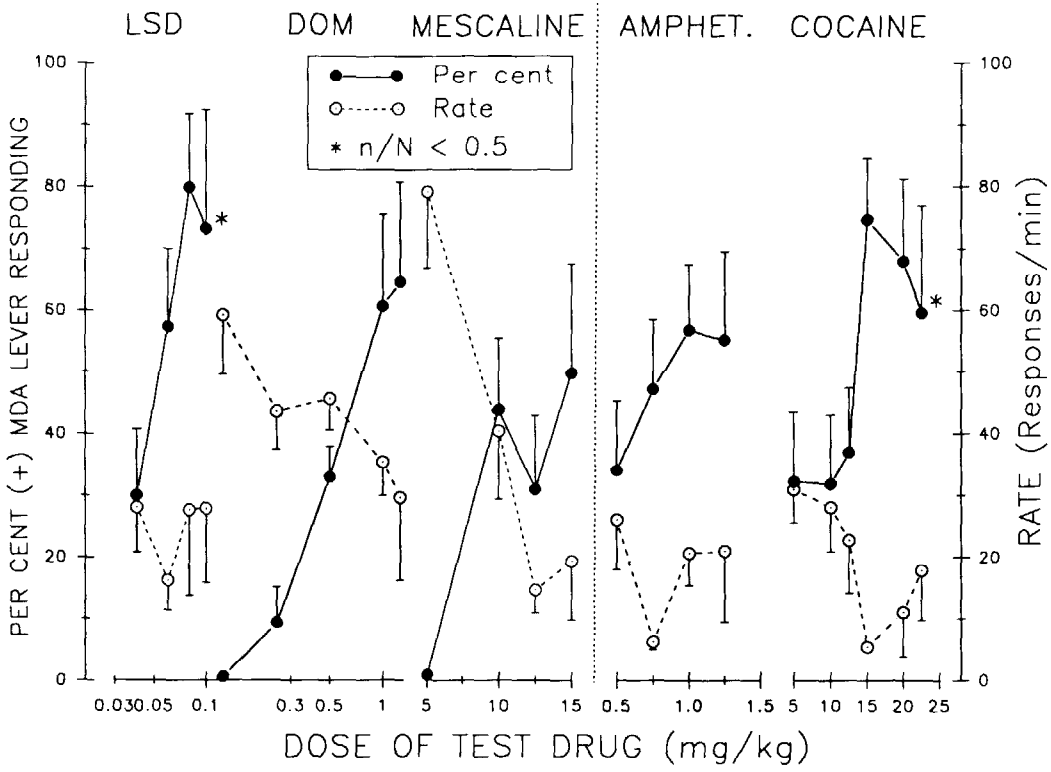

FIGURE 11. *Results of substitution tests with hallucinogens (LSD, DOM, mescaline) and stimulants ((+) amphetamine, cocaine) in rats trained to discriminate (+) MDA (1.25 mg/kg) from saline. n/N < 0.5 means that ratio of number of animals responding (n) to number of animals tested(N) was less than 0.5 (50%).*

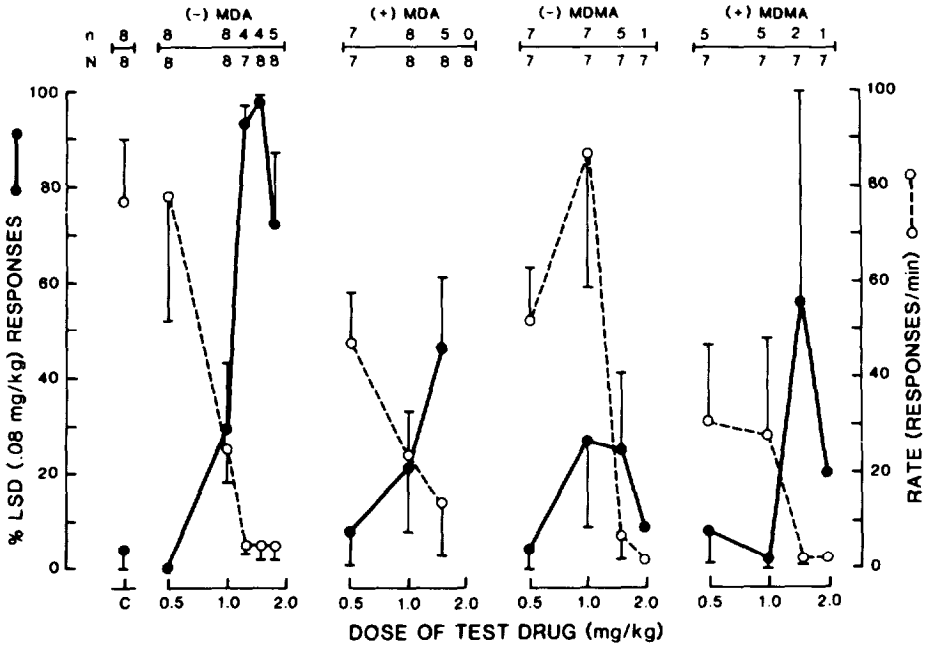

FIGURE 12. *Results of substitution tests with the (+) and (-) enantiomers of MDA and MDMA in rats trained to discriminate LSD (0.08 mg/kg) from saline. Compounds tested, number of animals responding (n). and number of animals tested (N) at each dose are shown at top of figure. (Reprinted from Callahan and Appel (1988), with permission.)*

with delineating the neuronal and receptor mechanisms most relevant to the overt and covert (subjective) effects of drugs in intact, behaving organisms. With regard to the substances with which this review has been primarily concerned (indoleamine and phenylethylamine hallucinogens, CNS stimulants, and compounds that may have both hallucinogen-like and stimulant-like effects), DD has (1) indicated that the most likely neuronal mechanism through which DOM, LSD, and mescaline act in vivo is direct stimulation of postsynaptic serotonin (5-HT_2) receptors (although research still needs to be done with substances that affect 5-HT_{1C}, 5-HT_3, and 5-HT_4 mechanisms); (2)demonstrated that CNS stimulants such as amphetamine and cocaine may have different subjective effects that involve different DA mechanisms; and (3) provided clues to the nature of the states induced by the optical isomers of

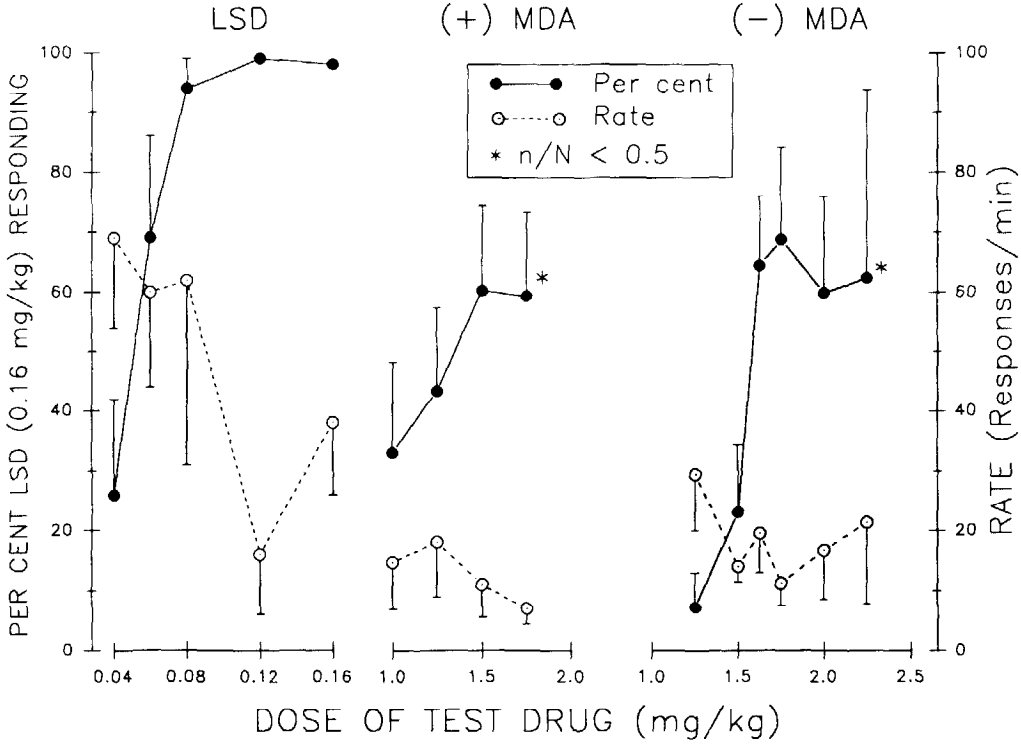

FIGURE 13. *Results of substitution tests with (+) and (-) enantiomers of MDA and MDMA in rats trained to discriminate LSD (0.16 mg/kg) from saline. n/N < 0.5 means that ratio of number of animals responding (n) to number of animals tested (N) was less than 0.5 (50%).*

designer drugs and, hence, why these dangerous, neurotoxic substances continue to be abused (Downing 1986; Peroutka 1987).

However, DD can also be useful in other aspects of both preclinical and clinical pharmacology. For example, if they do not have other side effects that preclude this possibility, the fact that 5-HT_2 antagonists block the discriminable effects of DOM, LSD, and mescaline suggests that drugs like pirenpirone, ketanserin, ritanserin, and especially risperidone (Meert 1990) ought to be useful in the treatment of the bad trips induced by indoleamine and phenylethylamine hallucinogens. The results of DD experiments also indicate that DA uptake inhibitors (bupropion, nomifensine) ought to be more effective than NE uptake inhibitors (desipramine, imipramine) in attenuating cocaine craving and relieving the symptoms of cocaine withdrawal.

Finally, because of its correlation with many other diverse effects of abused drugs ranging from receptor binding to reports of hallucinations, DD has been of value in both the analysis of structure-activity relationships (Glennon 1990) and the design and development of potential therapeutic agents (Balster 1990; Meert 1990).

However, no pharmacological assay is free of problems, and DD certainly is no exception. The technique is difficult and time-consuming to implement properly; it therefore should not be used by investigators who have had little experience conducting long-term behavioral experiments or who are interested in screening large numbers of compounds for a given effect in a short period of time.

Another problem is that the assay involves the psychological process of stimulus generalization, the interpretation of which is fraught with difficulties. That is, the extent to which any stimulus generalizes to any other stimulus obviously depends on stimulus or dimensional “similarity”-however that concept may be defined (Herrnstein 1984)-but also on many other, less obvious factors, including the organism’s experience, training history, and sensory capacity (Domjan and Burkhard 1986). Thus, it should not be surprising to learn that the results of DD experiments (e.g., the extent of generalization of a compound to other, related agents) depend critically on stimulus (drug) parameters such as training dose and nonstimulus events such as the sensitivity of the particular training procedure used before testing begins (Järbe 1989).

As an example of how important one of these variables, training dose, can be, we have shown that the effects of 0.08 mg/kg of LSD generalize to (-) MDA to a much greater extent than they do to other designer drugs (figure 12)-a result that supports those of other investigators (Nichols et al. 1986). However, this effect does not always occur; for instance, it is not at all clear that the stimulus properties of 0.16 mg/kg of LSD generalize any more than partially to the two isomers of MDA (figure 13). Thus, it is premature to conclude from these data that any isomer of any designer drug has or does not have LSD-like effects although other results suggest that the effects of (-) MDA (1.25 mg/kg) generalize convincingly to LSD (Appel et al. 1990). Similar effects occur with other drugs of abuse including amphetamines (Stolerman and D'Mello 1981) and cocaine (Broadbent et al. 1989*a*).

For all these reasons, the results of DD research should not be used casually-for example, to provide quick answers to deceptively simple questions such as whether compound X is a narcotic. Assuming we know what, if anything, the term "narcotic" means pharmacologically, the best the assay can do is (1) identify the extent to which the effects of a given range of doses of X resemble those of prototypic members of a given class of opiates, e.g., mu or *kappa* agonists, in a given species; and (2) suggest one or more likely mechanisms whereby X might act *in vivo:* Are its subjective effects blocked by naloxone?

In addition, as in any assay, the results of DD experiments depend on the specificity (or, too often, lack of specificity) of the agents used by the investigative team as pharmacological tools. Is SCH 23390 a selective D_1 antagonist, and, if so, is the ability of this substance to partially block the effects of cocaine (Woolverton 1990) due to this aspect of its neuropharmacology?

Finally, a number of technical problems continue to plague DD research. While none of them may be of crucial importance to the success or failure of the assay (which is still better than most others for all the reasons we have discussed), debate about them continues to consume time that could probably be spent more profitably in other ways. For example, there is still little agreement on whether test data should be analyzed quantally or quantitatively (Stolerman et al. 1990) and on what the criteria for substitution (generalization) or antagonism ought to be: Should these concepts be defined statistically or by some fixed percentage, as in psychophysics? A few of these problems might be solved or at least diminished if some degree of standardization existed in the field. To this end, it might be interesting to ask a select committee of members

of the Society for the Stimulus Properties of Drugs or the European Behavioral Pharmacology Society to meet in Geneva to develop a set of standards to guide all DD research. However, even if such standards could be established, they would probably be ignored; how often do organic chemists, pharmacologists, or, for that matter, politicians pay attention to the results of Geneva conventions?

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Symposium Critique

L.S. Harris

The charge I was given by Dr. Glennon was to sum up and critique the meeting from the point of view of an elder statesman who has a strong interest in the effects of chemicals on the central nervous system (CNS) yet is not directly involved in drug discrimination (DD) research. This role has been thrust on me more and more lately and makes me feel like the chairman of the board who can sit through a long, complex board meeting and brilliantly sum up and point to new directions but 2 weeks later can barely remember what went on.

I was very appreciative of Drs. Schuster and Overton for providing a fine historical perspective. After listening for the past 2 days, I am again impressed by the breadth and depth of the progress in the DD field in the past few years. The technique itself has much to commend it. However, many of its virtues have led and may continue to lead to problems and difficulties. For instance, although DD is the best animal procedure we have for predicting subjective effects in humans, it leads, in many instances, to overinterpreting the data. The problems of false positives and false negatives, which were alluded to by Drs. Goudie and Bigelow, are not generally recognized. That the field is greatly in need of additional human studies to provide validity for the animal studies is beginning to emerge, as demonstrated by the presentations of Drs. Johanson and Bigelow and other published reports. More, however, are needed.

Among the beauties of the DD technique are good selectivity, relative operational ease, and reproducibility from laboratory to laboratory. These qualities are also among its dangers, however. Testing for potential side effects during drug development seems to be becoming a screening technique. Standardization may lead to formalization of the problem of false positives and false negatives and, in the long run, actually stifle originality in drug development. An example from my own experience is the use of analgesic tests. Over several decades we developed test procedures, such as the hot plate and tail flick tests, that were highly predictive of analgesic activity in humans. Unfortunately, they were also highly correlative with dependence

liability and thus were not very useful alone in developing new analgesics with less or no abuse potential. We did have a clue from serendipitous studies in humans with the opiate antagonist nalorphine, which had little or no analgesic activity in the hot plate and tail flick tests. It antagonized morphine in these procedures, yet in humans it proved to be equipotent with morphine as an analgesic. In addition, it had little or no dependence potential in animals or humans. Of course, we now know that nalorphine is a mixed agonist-antagonist. In our development of other mixed agonist-antagonist analgesics we discarded compounds that had strong activity in the tail flick and hot plate tests. We developed tests to quantify opioid antagonism and selected compounds for further evaluation on this basis. Thus pentazocine, cyclazocine, and so on were sent out for clinical evaluation as analgesics with little evidence for their efficacy in laboratory animals. Thus, from my experience, new advances are more likely to be made by pursuing leads rather than perseverating in formerly useful screening procedures.

On this note, let me point out that DD is not a test for dependence or abuse liability. It does have the ability to classify drugs pharmacologically and, thus, put in place one of the findings necessary for regulatory action. I would strongly oppose the use of DD alone in making decisions about scheduling drugs under either national or international control. Indeed, many people forget that under the Psychotropic Convention, a substance must have pharmacological properties similar to an already controlled substance *and*-I emphasize and-have similar abuse potential *and* produce public health and social harm. These effects cannot be predicted from discrimination data alone.

I was pleased to see the great attention being paid to the use of DD in mechanistic studies. This is a very positive direction for the field to take. However, along with Dr. Schuster, I would like to see more emphasis on the role of behavioral and environmental factors in DD. For instance, has enough been done to explore various schedules of reinforcement? I wonder whether the theories of rate dependence would influence DD designs. Would schedules engendering high output of behavior be more effective in revealing the discriminative properties of CNS depressants and vice versa? It has been my experience that, although behavioral techniques are useful in studying drugs, we probably learn more by using drugs to study behavior.

Although dose-response studies are becoming standard in DD experiments, not enough attention is being paid to biodispositional and pharmacokinetic parameters. This lack of attention often leads to misinterpretation of results.

A word now about correlations, which continue to be used frequently in this field. Correlations do not prove causal relationships. They are only suggestive. When correlations are constructed, all data on each parameter-positive, negative, and inconclusive-must be included. If they are not, your data are likely to be misinterpreted.

Finally, a word about the development of medications for the treatment of the chemical dependencies. I see DD techniques as playing a key role in this development. Both animal and human data generated using discriminative stimulus techniques will play an essential role in developing truly unique new treatments for this massive worldwide public health problem.

I trust I have not stepped on too many toes and apologize for any feathers ruffled. If I erred in any of my remarks, it was because of my lack of hands-on experience in the field, and I hope you will forgive me.

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