1

AN INTRODUCTION TO DRUG DISCRIMINATION

A. GENERAL SCOPE AND INTRODUCTORY COMMENTS

Subjects (animals, including nonhuman and human primates) are considered able to *distinguish* or *discriminate* between two (or more) distinct stimuli if they can be trained to respond in a different manner when each stimulus is presented. The greater the difference between two stimuli, the more likely subjects are able to distinguish or discriminate between them. *Differentiation of* discriminable stimuli *is the basis for the drug discrimination method*. Discriminative stimulus control of behavior, a concept closely linked to operant conditioning, is a behavioral technique whereby a particular behavior (i.e., a particular response) is reinforced—at least during training. The drug

Drug Discrimination: Applications to Medicinal Chemistry and Drug Studies, First Edition.
Edited by Richard A. Glennon, Richard Young.

discrimination procedure—basically, a "*drug detection*" paradigm—uses a pharmacologically active agent as the discriminative stimulus. The technique has broad applicability both to the study of *animal behavior* and *investigations of drug action*. A closely related procedure, drug self-administration, utilizes relatively similar conditions to examine drugs as *reinforcers* (e.g., see Chapter 11 in Part II. by Negus and Banks). Whereas many investigators, particularly experimental psychologists, might utilize a drug as a "*discriminative stimulus*" or "*interoceptive cue*" (or, simply, "*cue*") to investigate animal behavior (i.e., the drug is held constant to investigate behavior), others, particularly pharmacologists and medicinal chemists, use the behavior to assess the actions of drugs (i.e., the behavioral component is held relatively constant to evaluate drug effects). The former approach has been addressed in psychology texts. With respect to the latter, there is no comprehensive text that describes the methods and approaches employed to study drug action. Those investigators trained in drug discrimination techniques ordinarily acquire their knowledge by serving as graduate students or postdoctoral fellows in laboratories where the technique is employed. Yet those trained in drug design are rarely schooled in drug discrimination. The purpose of this book is to bridge the gap and to focus on the drug discrimination procedure as it applies to the study of pharmacologically active substances. Here, emphasis is placed on the pharmacological and medicinal chemistry aspects of drug discrimination studies, including the role of stereochemistry, in examining structure–activity relationships and mechanisms of drug action, rather than on the use of the technique to investigate animal behavior.

Whereas the drug discrimination procedure is chiefly employed by those with training in psychology or pharmacology, those trained in drug design and drug development (e.g., medicinal chemists) typically have only a rudimentary grasp—at best—of the procedure. The drug discrimination paradigm, although somewhat labor intensive (and, hence, not particularly practical or suitable for the rapid screening of large series of agents), is of enormous applicability to the understanding of drug action. The present narrative will address the practical aspects of drug discrimination such as: What procedures can be used? How do the various procedures differ? How are drug discrimination studies conducted? What types of data can be obtained? How are data interpreted? Of what value are drug discrimination data? When are drug discrimination studies not applicable? And, what are the limitations of the drug discrimination procedure? One hopes that individuals involved in drug design and development who are not currently familiar with the drug discrimination technique will learn to appreciate the exquisite nature and power of this procedure and will become skilled at asking the types of questions that can be answered by those conducting drug discrimination studies. Whereas medicinal chemists should come to learn the types of information that drug discrimination studies can offer, pharmacologists might come to realize how medicinal chemists can apply the types of information that the paradigm routinely provides. As such, knowledge of more than one of the aforementioned disciplines should lead to a higher regard for the usefulness of the procedure. Indeed, a greater appreciation of the multidisciplinary perspectives of these disciplines may usher the contribution of even more intriguing scientific inquiries in the future. In addition, portions of this text will be of a very practical nature and will describe how such studies are conducted, their advan-

tages over certain other types of pharmacological evaluations, and their acknowledged limitations. Thus, this book is aimed at graduate students and both academic and industrial scientists, including pharmacologists, psychologists, psychiatrists, biologists, biochemists, chemists, medicinal chemists, and other investigators whose interests involve the design, development, and/or action of agents that act (primarily) at the level of the central nervous system.

The book is divided into two parts. Part I (Chapters 1–7) describes the drug discrimination paradigm, the various methods and techniques employed, and practical considerations, as well as examples of the general application of the methods utilized to investigate problems of interest. Part II (Chapters 8–16) consists of invited chapters from investigators who have published extensively in this area. They address specific topics or techniques that are of interest in drug evaluation and development.

As evidenced over the years, the drug discrimination paradigm is a robust and reliable technique that produces very reproducible results across laboratories. Many examples used in Part I of this book to illustrate the applicability of the drug discrimination paradigm to investigations of drug action are from studies conducted over the past 30+ years in our laboratories. The discussions are meant to be illustrative rather than comprehensive. That is, this volume is not intended to be a comprehensive review of the drug discrimination literature, or even a review of a specific drug or drug class. Indeed, many thousands of drug discrimination (i.e., stimulus generalization and antagonism) studies have been reported. What is presented in Part I is meant to serve as examples of the types of studies that can be conducted.

The chemical structures of some of the training drugs that have been employed in our laboratories, and that form the basis for a large part of the discussions in Part I, are shown in Figure 1-1. One reason for the focus on work from our laboratories is that our studies maintained relatively consistent methodologies and techniques and, consequently, have minimized the role of procedural or methodological differences. In general, there is excellent agreement between drug discrimination results from different laboratories regardless of animal species, schedule of reinforcement, and other factors. However, different training doses, pre-session injection intervals (PSIIs), animals (species or strain), routes of administration, schedules of reinforcement, and other factors can sometimes make it difficult to compare results between laboratories. For example, we have demonstrated that results of stimulus antagonism studies using 5-methoxy-*N*,*N*-dimethyltryptamine (5-OMe DMT; see Figure 1-1 for chemical structure), a relatively short-acting serotonergic-mediated hallucinogenic agent, as training drug differ dramatically depending upon the training dose employed [1]. That is, a 1.5 mg/kg training dose of 5-OMe DMT produces a discriminative stimulus that is quite different from that produced by a 3.0 mg/kg training dose, even when all other factors were held constant. This represents only a 2-fold change in training dose. Had these studies been conducted in two different laboratories, with one laboratory using the lower training dose and the other laboratory using the higher training dose, the results would have appeared inconsistent and in relative conflict with one another. Furthermore, had there been any methodological differences between the two laboratories, these differences might have been thought responsible for the inconsistencies observed. Likewise, Appel and co-workers [2] noted differences in stimulus generalization

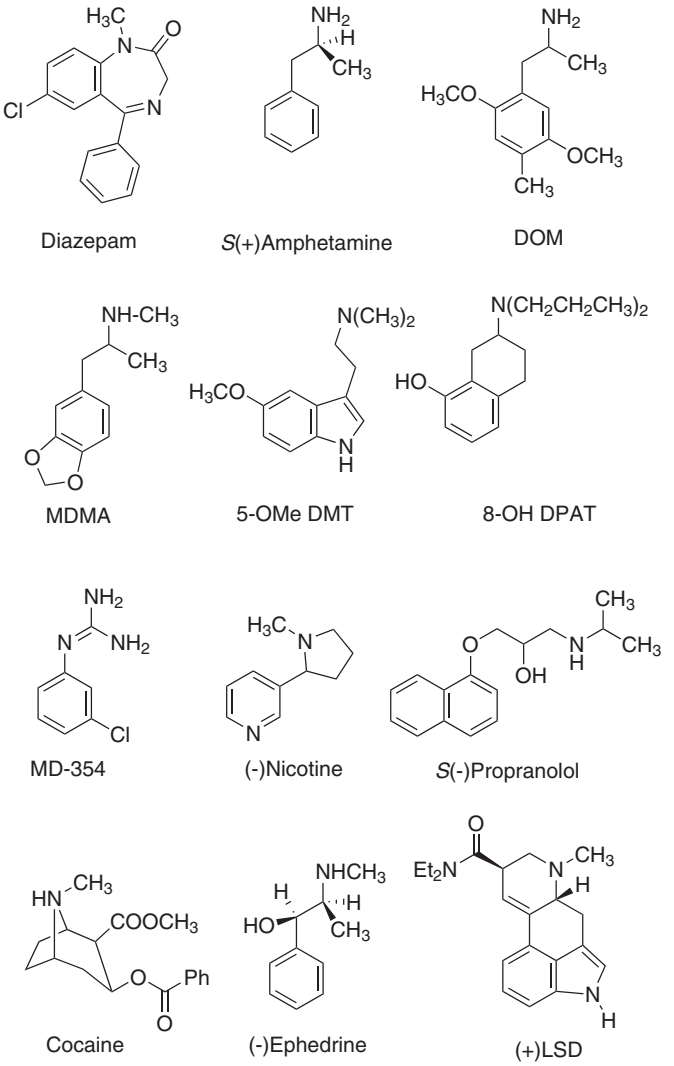

Figure 1-1. Chemical structures of some representative examples of agents that have been used as training drugs in our laboratories: diazepam, S(+)amphetamine, 1-(2,5-dimethoxy-4-methyl)phenyl-2-aminopropane (DOM), N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA), 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT), 8-hydroxy-2-(N,N-di-n-propylamino)tetralin, 3-chlorophenylguanidine (MD-354), (–)nicotine, S(–) propranolol, cocaine, (–)ephedrine, and (+)lysergic acid diethylamide.

(including stimulus generalization studies with 5-OMe DMT) and antagonism results employing (+)lysergic acid diethylamide (LSD) training doses of 0.02, 0.08, and 0.32 mg/kg. For further discussion of this issue see Chapter 3.

As a final note: much of the data from our laboratories was previously published in tabular rather than graphic form. These tabular data were used to prepare new graphical depictions for the present work. In a few instances, where data might have been previously presented in graphical form, graphs were replotted to abstract certain data from a published figure or to combine data published earlier in several different plots.

B. BACKGROUND AND UTILITY OF THE DRUG DISCRIMINATION PARADIGM

Humans have ingested and experienced the effects of psychoactive agents throughout history. In fact, the use of drugs can be traced through anthropological and archaeological evidence that dates back at least 5,000 to 10,000 years; for example, ancient Sumerians of 4000 B.C. referred to the poppy as the "joy plant" [e.g., 3]. "Psychoactive" drugs refer to chemical agents that exert an action upon the central nervous system (CNS), alter brain activity, and, consequently, produce a temporary change in an individual's mood, feeling, perception, and/or behavior. Such agents might be used for their religious or spiritual effects ("*entheogens*"), prescribed as therapeutic medications (e.g., opioids, anxiolytic agents, antidepressants, and antipsychotics), and/or are used (or abused) as recreational drugs (e.g., hallucinogens, stimulants, and related designer drugs). In each case, the subjective effects produced by such agents are generally not readily accessible to independent verification by an observer. However, methods were developed over 50 years ago whereby human subjects administered such drugs could self-rate their experiences on questionnaires [4]. Today, various subjective scales and behavioral inventories of the effects of drugs are often used and have become important tools for basic and clinical neuroscience research. For example, frequently used questionnaires include 1) scales of global drug effects, that rate the "overall strength," "liking," "good" or "bad" effects of an agent [e.g., see 5]; 2) the Addiction Research Center Inventory (ARCI) [6–8] that contains subscales of physical, emotional, subjective, and potential for abuse effects of a test agent in relation to those of standard drugs and/or drug groupings such as the Mar Scale (i.e., effects of marijuana as reference), Morphine-Benzedrine Group (MBG; index of euphoria), Pentobarbital-Chlorpromazine Group (PCAG; index of apathetic sedation), and Lysergic Acid Diethylamide Group (LSDG; index of dysphoria or somatic discomfort); 3) a Profile of Mood States (POMS) [9–11] that estimates the degree of similarity of a test agent to standard drugs (e.g., stimulants, sedatives, or anxiolytics) and identifies effects that might be aversive (e.g., tension-anxiety, depression-dejection, anger-hostility, fatigue, or confusion-bewilderment); and 4) the Drug-Class Questionnaire, which asks subjects to compare the effect(s) of a test drug to that of a list of drugs/drug classes [12, 13]. Generally, subjects furnish information about themselves through self-inventories and profiles are created of the perceptible effects and pharmacologic properties (e.g., potency and time course) of a drug; in practice, the effects of test agents are often compared to those of

known reference drugs. Scales and questionnaires are convenient because they do not usually require the services of a group of raters or interviewers. Their potential disadvantage might be that individuals do not completely comprehend the effect of the drug or their drug "experience" and, therefore, might not always give a report that is completely thorough or amenable to appropriate quantitative analysis, or open to definitive interpretation. Lastly, a newly synthesized agent is precluded, for obvious ethical and pragmatic reasons, from initial assessment in humans to determine whether its pharmacological action is similar to that of a known psychoactive agent. In such instances, animal protocols offer an alternative approach to characterize the pharmacological actions, mechanism of action, and safety of an agent. Common goals of such studies are to offer a possible mechanism of action and prediction of the pharmacological effects (and side effects) of an agent in humans.

The use of nonhuman animal subjects can be justified in such experiments on the basis of at least three criteria in that they 1) allow relatively precise control of extraneous variables; 2) are presumed to be simpler organisms that allow the study of drug action at a relatively elementary level but yet can form the foundation for deriving more complex aspects of drug action that are presumably reflected in human subjects; and 3) may be used to study the influence of certain drug effects that may (or could) not be studied with human subjects. As such, nonhuman animals could, and in some cases, be "more suitable" subjects for studying certain drugs than would humans. The rodent, for example, is not so "encumbered" with past experiences of drug effects and symbolic language-factors that might, perhaps, render the human subject as being "too complex" in certain evaluations of novel chemical entities.

The drug discrimination paradigm is an assay of, and relates to, the subjective effects of drugs in nonhuman or human animals. In a typical operant experiment, there are four basic components: 1) the subject and their "motivational condition," which increases the effectiveness of an event as reinforcement (e.g., an animal is often subjected to food restriction, which makes the presentation of food more effective as reinforcement); 2) the administration of a drug dose that exerts an effect on the subject, or its vehicle, and precedes a response by the subject; 3) an appropriate (or correct) response; and 4) presentation of reinforcement. *These elements may be termed the basic components of an operant analysis of drugs as discriminative stimuli:*

SUBJECT → DOSE of TRAINING DRUG (or VEHICLE) → RESPONSE → REINFORCEMENT

The drug or non-drug (i.e., vehicle) condition that leads to, or results in, a behavioral event (i.e., a particular response) and is followed by the presentation of reinforcement is called the *discriminative stimulus*. In laboratory subjects, discriminative control of behavior by (usually, but see Chapter 3) two treatments is established through the use of reinforcement (often referred to as *reward*). The treatments are used as antecedent "help" or "aid" events to control appropriate behavioral responses that are followed by reinforcement. Subjects are usually trained to distinguish the effects of a dose of drug (i.e., a dose of training drug) *versus* non-drug or vehicle (i.e., usually saline, a

0.9% sodium chloride solution that is often used as a solvent for many parenterally administered drugs) conditions, but subjects also have been trained to distinguish the effects of 1) a dose of drug *versus* another dose of the same drug; 2) a mixture of doses of drugs *versus* vehicle (termed "AND-discrimination); 3) a dose of one drug *versus* a dose of another drug (termed "OR-discrimination"); 4) a mixture of doses from two drugs *versus* each dose of each drug separately (termed "AND/OR-discrimination") (see Stolerman; Chapter 10 for an in-depth discussion); and 5) a dose of drug *versus* a dose of drug *versus* vehicle (i.e., termed a "3-condition or 3-lever method"; see Chapter 3). Some of the latter procedures are detailed in reports by Colpaert [14], Colpaert and Janssen [15], Stolerman et al., [16], Chapter 10 by Stolerman, and Chapter 16 by Colpaert. The most commonly employed procedure, however, is to conduct drug discrimination studies with a dose of drug *versus* vehicle (typically saline vehicle). For example, in a subject's course of training sessions in a two-lever operant conditioning task, a dose of training drug is administered (i.e., during the "*drug session*") and lever-presses on the drug-designated lever (for that subject) produce reinforcement. In other training sessions, vehicle is administered (i.e., during the "*vehicle session*") and responses on the (alternate or) vehicle-designated lever produce reinforcement. Historically, subjects in discrimination studies are linked by the assumption that their appropriate (i.e., "correct") responses following different treatments, on a consistent basis, are indicative that they are able to distinguish or discriminate between training-drug and vehicle (i.e., non-drug) conditions. As such, subjects' responses permit an experimenter to surmise that a drug effect has been "perceived" by the subject. A wide variety of centrally acting drugs can serve as discriminative stimuli (see below); some, but very few, peripherally acting agents also have been shown to exert stimulus control over behavior [e.g., 17]. The procedure is thus characterized as a highly sensitive and very specific drug detection method that provides both *qualitative* and *quantitative* data on the effect of a training drug in relation to the effect of a "*test*" (i.e., "challenge") agent. *Drug discrimination (as is true of any other pharmacological study) does not, however, provide the complete pharmacological characterization of an agent.* Nevertheless, the procedure can be used to investigate a wide array of pharmacological issues that relate to the stimulus properties of a drug: effect of route of administration, dose-response, time of onset and duration of action, degree of similarity of effect to other agents, stereochemistry, structure-activity relationships (SAR), activity of metabolites, and allows tests with a variety of receptor agonists and antagonists to establish putative mechanisms of action. The Drug Discrimination Bibliography (website: www.drugrefs.org), which contains >4,000 drug discrimination references published since 1951, was established by Dr. Ian P. Stolerman and is an excellent source of information on drug discrimination studies. The site is funded by the National Institute on Drug Abuse (NIDA) of the National Institutes of Health (NIH). The drug discrimination citations include journal articles, reviews, book chapters, and books. Unlike PubMed/MedLine, the database even cites abstracts from drug discrimination symposia. In addition, the website can be navigated to retrieve references selectively on particular drugs as training stimuli, drug classes, test drugs, authors, and method issues.

C. DRUG DISCRIMINATION: A SYNOPSIS OF THE APPROACH

In brief, the drug discrimination paradigm involves the training of animals (typically, but not limited to, rats) using (typically) a two-lever operant procedure, to "*recognize*" or "*discriminate*" the stimulus (i.e., "*cuing*") effects of a given dose of an agent (i.e., *the training drug*) under any one of several *schedules of reinforcement* (see Chapter 2). That is, administration of the training drug is normally paired with vehicle (i.e., the "*non-drug*" or "*default*" *condition*) and animals are trained, and learn, to make one response (e.g., to respond on the right-side lever in a two-lever operant chamber, or to turn in one direction in a T-maze) when administered the training dose of the training drug, and a different response (e.g., to respond on the opposite of two levers in a two-lever operant chamber, or to turn in the opposite direction in a T-maze) when administered vehicle, using a fixed *pre-session injection interval* (PSII). In a two-lever operant procedure, animals are trained for several weeks or, more commonly, months until they eventually, and consistently (over a period of several weeks), make ≥80% of their responses on the training-drug appropriate lever following administration of the training dose of the training drug, and ≤20% of their responses on the same lever following administration of vehicle. Once reliably trained, the animals can be administered lower doses of the training drug and they respond accordingly. That is, following administration of lower doses of training drug the animals will make fewer responses on the "*drug-appropriate lever*" in a two-lever operant procedure, and, at a very low dose of the training drug, the animals will respond as if they had been administered vehicle. In this manner, a *dose-response curve* can be constructed and an effective dose 50% (i.e., ED_{50} *dose*) can be calculated for the training drug. Keep in mind, however, a different training dose of the same training drug will most likely result in a different ED_{50} value. Hence, *when an* ED_{50} *dose is provided for the training drug, the training dose of the training drug* must *also be specified.*

Once animals are trained to discriminate a specific dose of training drug from vehicle, two general types of experiments can be performed: 1) tests of *stimulus generalization* ("*substitution*") and 2) tests of *stimulus antagonism* ("*blockade*"). Tests of stimulus generalization are employed to determine the similarity of the stimulus effects produced by a *challenge drug* (or "*test drug*") to those produced by the training drug. The challenge drug can be a different dose of the training drug or an entirely different agent. For example, when the challenge drug is the training drug, doses lower than the training dose of the training drug can be examined to generate a dose-response curve and an ED_{50} value can be calculated (as mentioned above and as more extensively described in Chapter 3), use of shorter pre-session injection intervals for the training dose of the training drug than that employed in training can identify the time-course for the onset of action of the training drug, or the use of longer pre-session injection intervals can be employed to determine the duration of action of the training dose of the training drug. These, and related studies, provide useful information about the training drug (time of onset? long-acting? short-acting?). Equally, or even more important with regard to understanding the actions between agents, is to administer novel *test* or *challenge* agents to the trained animals. Various doses of a non-training drug (i.e., *test* or *challenge* agent) can be administered to the trained animals to determine similarity

of stimulus effects. Doses of these *test* or *challenge* agents will cause the animals to divide their responses between the training-drug appropriate lever and the vehicle (or "*non-drug*," "default") lever. If administration of a given dose of test drug results in the animals making ≥80% of their (mean) percent responses on the training-drug-appropriate lever, it is assumed that the test drug and the challenge drug are producing similar (although not necessarily pharmacologically or mechanistically identical) stimulus effects. If all doses of a test agent produce ≤20% drug-appropriate responding, it is assumed that the test drug and the training drug produce dissimilar stimulus effects. This does not necessarily mean that the test drug is inactive; it simply means that the stimulus effects produced by the two drugs are different. For example, animals trained to discriminate morphine from vehicle do not recognize diazepam, and animals trained to discriminate diazepam from vehicle do not recognize morphine. In some instances, administration of a test drug will result in "*partial generalization*" (≥20%, but ≤80% drug-appropriate responding), which is acknowledged to be the most difficult type of result to interpret; this will be discussed in greater detail later (Chapter 3). Generally, doses of a challenge drug are administered until either stimulus generalization occurs, or until the animal's behavior is disrupted.

In tests of stimulus antagonism, doses of a recognized neurotransmitter receptor antagonist are administered in combination with the training drug to determine whether the stimulus effects of the training drug can be blocked. Alternatively, doses of new chemical entities (NCEs) can be examined in combination with a training drug of known mechanism of action to identify novel antagonists. This will be further discussed in chapters to follow.

A general outline of a few tests that can be conducted using the drug discrimination paradigm is shown in Figure 1-2. This is not by any means meant to be comprehensive and is provided only to serve as an introduction; much greater detail will be provided in ensuing chapters.

Indeed, using tests of stimulus generalization and antagonism, a number of questions regarding a novel, centrally acting agent can be answered (at least in part). For example, 1) Does Drug Y produce a stimulus effects similar to that of training Drug X? 2) What is the time of onset of action of Drug X? 3) What is the duration of action of the stimulus effects of Drug X? 4) Is Drug X a pro-drug, or is it active in its own right? 5) Are metabolites of Drug X active? 6) If metabolites of Drug X are active, what is their time of onset and their duration of action? 7) What is the mechanism of action of Drug X as a training drug? 8) If no antagonists are available for Drug X, how can antagonists be developed? 9) If Drugs X and Y produce similar stimulus effects, do they do so through a common or different mechanism of action? 10) What is the site of action of Drug X in the brain? These are just some of the types of questions that can be answered employing drug discrimination techniques.

D. DRUG DISCRIMINATION AND DRUGS OF ABUSE

The stimulus properties of many agents that are often viewed as drugs of abuse, such as cocaine, methamphetamine, morphine, heroin, ethanol, and (–)nicotine, have been

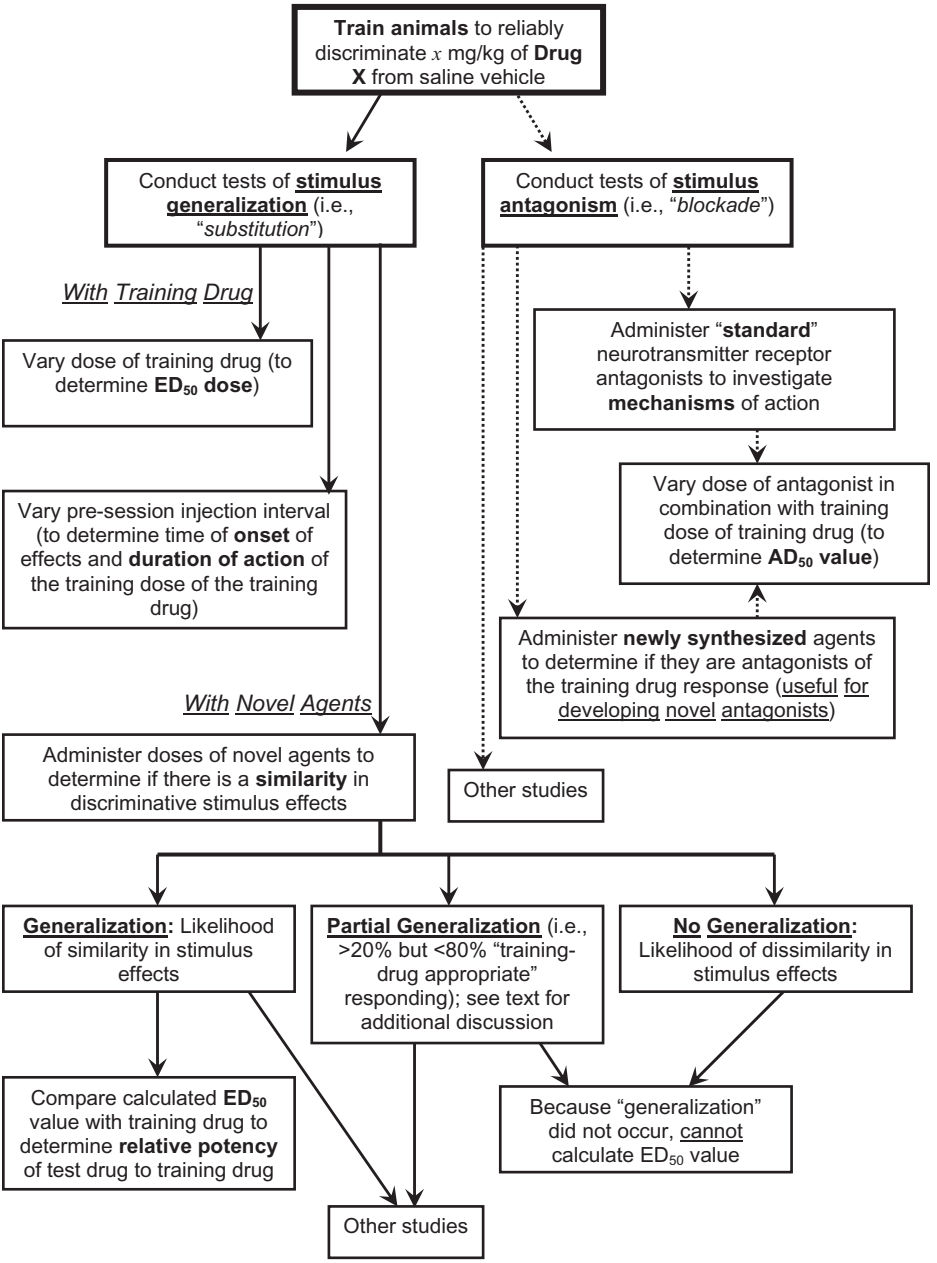

Figure 1-2. A simple schematic overview of some studies that can be conducted with animals trained to discriminate x mg/kg of a training drug, Drug X, from saline vehicle.

characterized in studies of drug discrimination. However, the discriminative stimulus effects of an agent should not be viewed as a first-line indicator of abuse potential (see also Chapter 6). That an agent can serve as a discriminative stimulus does not necessarily imply that it is (or might be) a drug of abuse. Although the stimulus effects of certain drugs might be related, to some degree, to their abuse potential, many agents that have been employed as training drugs (e.g., antipsychotics, most antidepressants, the β-adrenoceptor blocker propranolol, and the anxiolytic agent buspirone; see Table 3-1) have little or no liability for abuse. A more prudent approach to this issue is to view the results of drug discrimination studies in context with the results from assays that are thought to be more direct markers of potential for abuse such as self-administration (see Chapter 11 by Negus and Banks) and conditioned place preference, which investigate the various conditions under which drugs (as reinforcers) function to maintain behavior [18–20]. On the other hand, classical hallucinogens such as (+)lysergic acid diethylamide (LSD) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) are exceptions to that outlook because they are not readily self-administered by nonhuman animals but they do reliably serve as discriminative stimuli in animals, especially rodents, and more recently in nonhuman primates (see Chapter 13). Indeed, discrimination-derived data of various phenylalkylamine- and indolealkylamine-based hallucinogens, obtained from animals trained to discriminate the hallucinogen DOM from vehicle, have been shown to correlate highly with human (hallucinogenic) potencies for these agents [e.g., 21]. This is not to imply that drug discrimination procedures with hallucinogens serve as models or predictors of hallucinogenic activity/potency [22]. More likely, the method measures neurotransmitter activity and represents an assay of receptor-based mechanism of drug action (see Chapter 6).

On a related topic, it has been stated that the drug discrimination paradigm lacks psychiatric or psychopharmacological "*face validity*" because there is no reason to think that antianxiety agents, antipsychotics, or antidepressants will produce those effects in subjects who do not appear "anxious," "psychotic," or "depressed." This may be true. However, face validity refers to "what a test looks like it might reflect" as compared to "what it has been shown to reflect." As such, drug discrimination procedures do appear to simulate, to some degree, human investigation of drugs over time. In fact, the drug discrimination paradigm is one of a very few preclinical assays that actually has a counterpart procedure for humans. More importantly, however, the results from drug discrimination studies exhibit a robust degree of validity related to biological criteria. In particular, the assay functions superbly to determine 1) the degree of similarity of stimulus effects of a dose of training drug to those of other agents; 2) the importance of stereochemical factors; 3) in vivo structure–activity relationships that are based both on qualitative and quantitative data; 4) contribution of metabolites to drug action; 5) elucidation of possible mechanisms of drug action; and, lastly, but importantly; 6) correlations between data derived from drug discrimination experiments *versus* data from in vitro biochemical assays and/or data that relate to doses employed to produce particular pharmacological effects in humans. A reviewer of the literature would be hard-pressed to identify an alternative procedure that could boast such achievements.

E. ADVANTAGES OF THE DRUG DISCRIMINATION PROCEDURE

The drug discrimination procedure exhibits several advantages over other in vivo pharmacological assays that are utilized to study the effects and mechanism of action of drugs. For example, many behavioral pharmacology procedures measure the effects of drugs in relation to a subject's change in baseline activity level or response rate. As such, these assays are usually focused on increases, decreases, or other pharmacological effects of drugs on animal behavior. In contrast, drug discrimination studies are focused on whether subjects can "*detect*" the presence of stimulus effects of a dose of training drug in comparison to a vehicle or non-drug condition. Simply stated, *the drug discrimination paradigm can be summarized as a paradigm that allows subjects to identify the effects of a drug rather than being a procedure that studies the disruptive or excitatory effects of a drug*. In a typical drug discrimination study, subjects become behaviorally tolerant to any (initially) disruptive effects of a given dose of training drug on, for example, operant behavior, so that experimental results are not influenced by changes in rates of behavior. For a general discussion of this phenomenon, see Chapter 16 by Colpaert. Importantly, discriminative stimulus effects of a drug exhibit stability; tolerance, defined as a significant diminution in percentage drug-appropriate responding after repeated administration of the dose of training drug over long periods of time, does not readily occur to the stimulus effect. Thus, an investigator can study the semi-chronic effects of a drug treatment in the same experimental subject(s) over long periods of time. In fact, Schechter et al. [23], for example, trained rats to discriminate the stimulus effects of either 600 mg/kg of ethanol, 0.8 mg/kg of *S*(+)amphetamine, or 1.0 mg/kg of the 5-$HT_{1/2A}$ receptor agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP) from vehicle. Once each group of subjects was trained, and one year later, dose-response tests were conducted and ED_{50} values were calculated and compared. In each group, there was no marked change in the animals' sensitivity to the training dose of the training drug as indicated by similar dose-response functions and ED_{50} values. Retrospectively, we have observed a similar stability and consistency in the dose-response effects and ED_{50} values of rats trained to discriminate the stimulus effects of 1.0 mg/kg of *S*(+)amphetamine, 1.0 mg/kg of DOM, and 1.5 mg/kg of MDMA from vehicle, and have been continually amazed at how long (≥2 years) well-trained subjects can perform (at a high level) in drug discrimination studies (Young and Glennon, unpublished data).

Studies of drugs as discriminative stimuli also display specificity within a pharmacological class. For example, subjects trained to the stimulus effects of a CNS stimulant do not "*generalize*" (*transfer*, *substitute*, *recognize*—terms that are used interchangeably here, and in the general literature) to agents that belong to other pharmacological classes of agents (e.g., antianxiety agents, sedatives, or hallucinogens) as being similar to the training condition. Similarly, subjects trained to discriminate either ethanol, (+) lysergic acid diethylamide (LSD), diazepam, pentobarbital, or mescaline do not generalize to CNS stimulants. Indeed, investigators have studied many training drugs to determine whether drug-induced stimuli will generalize to agents within, or from different, pharmacological classes. The rationale of this approach is that subjects trained to discriminate a dose of a particular training drug from vehicle will exhibit stimulus

generalization only to test agents that share a similar stimulus effect, though not necessarily an identical mechanism of action (see Chapters 3 and 6). Thus, a training stimulus may generalize to a test agent to the extent that it contains pharmacological features that overlap with those produced by the training dose of training drug. Consequently, *the percent drug-appropriate responding that occurs to a test agent may be a reflection of the proportion of the pharmacological stimulus effects in that agent that resembles part of the set of pharmacological effects that are associated with reinforcement during discrimination training*. It should be recognized that structural similarity between agents does not guarantee stimulus generalization any more than does membership to a common pharmacological class of agents (e.g., anxiolytic agents) (see Chapters 3 and 6 for further discussion).

Lastly, drug discrimination studies have demonstrated remarkable *sensitivity* to the dose(s) of drugs that can serve as stimuli. In a number of cases, the effective training dose of a training drug has been shown to occur at a level that is much below the doses of that drug that affects other behaviors. For example, the discriminative stimulus effects of morphine in rats occurs at doses of ≤3.2 mg/kg (s.c.) versus vehicle, but such doses evoke only a slight effect in behavioral tests of analgesia such as in the tail-flick assay [e.g., 24–26]. In addition, the discriminative stimulus effects of a very low dose of a CNS-active agent *versus* vehicle may be obtained with prior training on an "easier" version of the same discrimination (i.e., a somewhat higher dose of that same drug *versus* vehicle). For example, Greenberg and co-workers [27] initially trained animals to discriminate 0.08 mg/kg of (+)LSD from vehicle. Once trained, the same animals were then "*retrained*" or "*faded*" to a "very low dose" of 0.01 mg/kg of (+)LSD and soon learned the new discrimination. Such techniques have been successfully utilized by other investigators to examine the stimulus effects of different doses of a variety of agents from many different drug classes [e.g., 28, 29]. This issue is important because few drugs exert only one pharmacological effect and different doses of an agent have been demonstrated to exert different discriminative stimulus effects (see Chapter 3).

REFERENCES

1. Young, R., Rosecrans, J.A., Glennon, R.A. (1983). Behavioral effects of 5-methoxy-N,N-dimethyltryptamine and dose-dependent antagonism by BC-105. *Psychopharmacology*, *80*, 156–160.
2. Appel, J.B., White, P.J., West, K.S., Holohean, A.M. (1982). Discriminative stimulus properties of ergot alkaloids. In: Colpaert, F.C. and Slangen, J.L., Eds. *Drug Discrimination: Applications to CNS Pharmacology*. Elsevier Biomedical Press, Amsterdam, pp 49–67.
3. Merlin, M.D. (2003). Archaeological evidence for the tradition of psychoactive plant use in the old world. *Economic Botany*, *57*, 295–323.
4. Beecher, H. K. (1959). *Measurement of Subjective Responses: Quantitative Effects of Drugs*. Oxford University Press, New York.
5. Jasinski, D.R., Johnson, R.E., Henningfield, J.E. (1984). Abuse liability assessment in human subjects. *Trends in Pharmacological Sciences*, *5*, 196–200.

6. Haertzen, C.A. (1965). Addiction Research Center Inventory (ARCI): development of a general drug estimation scale. *The Journal of Nervous and Mental Disease*, *141*, 300–307.
7. Haertzen, C. (1966). Development of scales based on patterns of drug effects, using the addicition research center inventory (ARCI). *Psychological Reports*, *18*, 163–194.
8. Haertzen, C.A., Hickey, J.E. (1987). Addiction Research Center Inventory (ARCI): measurement of euphoria and other drug effects. In: M.A. Bozarth, Ed. *Methods for Assessing the Reinforcing Properties of Abused Drugs*. Springer-Verlag, New York.
9. De Wit, H., Griffiths, R.R. (1991). Testing the abuse liability of anxiolytic and hypnotic drugs in humans. *Drug and Alcohol Dependence*, *28*, 83–111.
10. Foltin, R.W., Fischman, M.W. (1991). Assessment of abuse liability of stimulant drugs in humans: a methodological survey. *Drug and Alcohol Dependence*, *28*, 3–48.
11. McNair, D.M., Lorr, M., Droppleman, L.F. (1971). *Manual for the Profile of Mood States*. Educational and Industrial Testing Service, San Diego.
12. Fraser, H.F., Van Horn, G.D., Martin, W.R., Wolbach, A.B., Isbell, H. (1961). Methods for evaluating addiction liability. (A) "Attitude" of opiate addicts toward opiate-like drugs. (B) A short-term "direct" addiction test. *Journal of Pharmacology and Experimental Therapeutics*, *133*, 371–387.
13. Jasinski, D.R. (1977). Assessment of the abuse potential of morphine-like drugs (methods used in man). In: Martin W.R., Ed. *Drug Addiction I*. Springer-Verlag, Heidelberg.
14. Colpaert, F.C. (1982). Increased naloxone reversibility in fentanyl dose-dose discrimination. *Eurpean Journal of Pharmacology*, *84*, 229–231.
15. Colpaert, F.C., Janssen, P.A. (1982). OR discrimination: a new drug discrimination method. *European Journal of Pharmacology*, *78*, 141–144.
16. Stolerman, I.P., Mariathasan, E.A., White, J.A., Olufsen, K.S. (1999). Drug mixtures and ethanol as compound internal stimuli. *Pharmacology Biochemistry and Behavior*, *64*, 221–228.
17. Colpaert, F.C., Niemegeers, C.J., Janssen, P.A. (1975). Differential response control by isopropamide: a peripherally induced discriminative cue. *European Journal of Pharmacology*, *34*, 381–384.
18. Koob, G.F., Weiss, F. (1990). Pharmacology of drug self-administration. *Alcohol*, *7*, 193–197.
19. Bardo, M.T., Bevins, R.A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, *153*, 31–43.
20. Stolerman, I.P. (1993). Components of drug dependence: reinforcement, discrimination and adaptation. *Biochemical Society Symposium*, *59*, 1–12.
21. Glennon, R.A., Young, R., Benington, F., Morin, R.D. (1982). Behavioral and serotonin receptor properties of 4-substituted derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane. *Journal of Medicinal Chemistry*, *25*, 1163–1168.
22. Glennon, R.A. (1992). Animal models for assessing hallucinogenic agents. In: A., Boulton, G., Baker, P.H. Wu, Eds. *Models of Drug Addiction*. Humana Press, Totowa, pp 345–381.
23. Schechter, M.D., Signs, S.A., Boja, J.W. (1989). Stability of the stimulus properties of drugs over time. *Pharmacology, Biochemistry, and Behavior*, *32*, 361–364.
24. Gianutsos, G., Lal, H. (1976). Selective interaction of drugs with a discriminable stimulus associated with narcotic action. *Life Sciences*, *19*, 91–98.
25. Shannon, H.E., Holtzman, S.G. (1979). Morphine training dose: a determinant of stimulus generalization to narcotic antagonists in the rat. *Psychopharmacology*, *61*, 239–244.

26. Krynock, G.M., Rosecrans, J.A. (1979). Morphine as a discriminative stimulus: role of periaqueductal gray neurons. *Research Communications in Chemical Pathology and Pharmacology*, *23*, 49–60.
27. Greenberg, I., Kuhn, D.M., Appel, J.B. (1975). Behaviorally induced sensitivity to the discriminable properties of LSD. *Psychopharmacologia*, *43*, 229–232.
28. Overton, D.A. (1979). Drug discrimination training with progressively lowered doses. *Science*, *205*, 720–721.
29. White, F.J., Appel, J.B. (1982). Training dose as a factor in LSD-saline discrimination. *Psychopharmacology*, *76*, 20–25.